Mismatch Repair Incompatibilities in Diverse Yeast Populations

Duyen T. Bui,^{*,1} Anne Friedrich,[†] Najla Al-Sweel,^{*} Gianni Liti,[‡] Joseph Schacherer,[†] Charles F. Aquadro,^{*} and Eric Alani^{*,2}

^{*}Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853-2703, [†]Université de Strasbourg, Centre National de la Recherche Scientifique, Génétique Moléculaire, Génomique, Microbiologie, Unité Mixte de Recherche, 7156, F-67000 Strasbourg, France, and [‡]Institute for Research on Cancer and Ageing of Nice, 06107 Nice, France
ORCID ID: 0000-0002-5011-9339 (E.A.)

ABSTRACT An elevated mutation rate can provide cells with a source of mutations to adapt to changing environments. We identified a negative epistatic interaction involving naturally occurring variants in the *MLH1* and *PMS1* mismatch repair (MMR) genes of *Saccharomyces cerevisiae*. We hypothesized that this MMR incompatibility, created through mating between divergent *S. cerevisiae*, yields mutator progeny that can rapidly but transiently adapt to an environmental stress. Here we analyzed the *MLH1* and *PMS1* genes across 1010 *S. cerevisiae* natural isolates spanning a wide range of ecological sources (tree exudates, *Drosophila*, fruits, and various fermentation and clinical isolates) and geographical sources (Europe, America, Africa, and Asia). We identified one homozygous clinical isolate and 18 heterozygous isolates containing the incompatible MMR genotype. The *MLH1*–*PMS1* gene combination isolated from the homozygous clinical isolate conferred a mutator phenotype when expressed in the S288c laboratory background. Using a novel reporter to measure mutation rates, we showed that the overall mutation rate in the homozygous incompatible background was similar to that seen in compatible strains, indicating the presence of suppressor mutations in the clinical isolate that lowered its mutation rate. This observation and the identification of 18 heterozygous isolates, which can lead to MMR incompatible genotypes in the offspring, are consistent with an elevated mutation rate rapidly but transiently facilitating adaptation. To avoid long-term fitness costs, the incompatibility is apparently buffered by mating or by acquiring suppressors. These observations highlight effective strategies in eukaryotes to avoid long-term fitness costs associated with elevated mutation rates.

KEYWORDS DNA mismatch repair; genetic incompatibility; mutator phenotype; natural yeast isolates; experimental evolution; adaptation; *Saccharomyces cerevisiae*

MOST spontaneous mutations that occur in natural populations are neutral or deleterious (Kimura 1967; Eyre-Walker and Keightley 2007). However, in changing environments, bacteria and yeast can display mutator phenotypes, often through the loss of DNA mismatch repair (MMR) function, that provide a competitive advantage by increasing the chance of obtaining the first adaptive mutation(s) (Chao and Cox 1983; LeClerc *et al.* 1996; Taddei *et al.* 1997; Boe

et al. 2000; Denamur *et al.* 2000; Giraud *et al.* 2001; Tanaka *et al.* 2003; Townsend *et al.* 2003; Bui *et al.* 2015). Ultimately, such benefits are lost due to long-term fitness costs caused by the accumulation of deleterious mutations. However, in the case of MMR defective bacteria, once adapted to an environment, cells can recover MMR functions through horizontal gene transfer (Denamur *et al.* 2000). In support of this idea, Taddei *et al.* (1997) suggested that mutators are likely to be common in natural asexual populations, but then disappear to avoid long-term fitness costs once favorable mutations reach fixation (Taddei *et al.* 1997; Giraud *et al.* 2001; Wielgoss *et al.* 2013).

While it might be common for a eukaryotic organism such as yeast to have lost MMR functions (Thompson *et al.* 2006; Raynes *et al.* 2012), there is little evidence that these functions can be recovered through horizontal gene transfer (Liti and Louis 2005). One approach that yeast could employ to

Copyright © 2017 by the Genetics Society of America

doi: <https://doi.org/10.1534/genetics.116.199513>

Manuscript received December 22, 2016; accepted for publication February 10, 2017; published Early Online February 13, 2017.

Supplemental material is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.199513/-/DC1.

¹Present address: Department of Microbiology and Immunology, University of California, San Francisco, CA 94143-2200.

²Corresponding author: Department of Molecular Biology and Genetics, Cornell University, 459 Biotechnology Bldg., Ithaca, NY 14853-2703. E-mail: eea3@cornell.edu

avoid long-term fitness effects associated with a mutator phenotype is mating to a nonmutator strain, followed by sporulation and segregation of genotypes so that the mutator locus and a beneficial mutation are no longer linked. Such a strategy is considered effective because outcrossing has the potential to generate new genotypes at a much higher frequency than spontaneous mutation (Ruderfer *et al.* 2006). This strategy predicts that a hybrid diploid in which the mutator phenotype is recessive would be observed in nature.

We developed a model to explain how a genetic incompatibility involving the DNA mismatch repair genes *MLH1* and *PMS1* could arise in yeast (Figure 1; Heck *et al.* 2006; Demogines *et al.* 2008b; Bui *et al.* 2015). We define incompatibility as a negative epistatic interaction between *MLH1* and *PMS1* genes that results in an elevated mutation rate. In this model, based on ideas first proposed by Dobzhansky and Muller as a mechanism for speciation (Dobzhansky 1936; Muller 1939; Muller and Pontecorvo 1940; Orr 1995), a common ancestral state gives rise to derived strains (S288c, SK1 in our case) that acquire neutral or beneficial mutations in the *MLH1* and *PMS1* MMR genes. Mating between these divergent populations would create a hybrid genotype. In laboratory experiments MMR incompatible spore progeny of the hybrid combination were shown to confer a higher mutation rate and provide a fitness advantage to adapt to high salt (Heck *et al.* 2006; Bui *et al.* 2015). These studies suggested that such an advantage would be transient because the mutator genotype would result in a long-term fitness cost (Heck *et al.* 2006). Consistent with a fitness cost, none of the 65 wild, lab, and clinical strains/isolates examined displayed the incompatible genotype (Demogines *et al.* 2008b). However, the number of strains/isolates examined was too small to determine whether their absence in the incompatibility group was lower than expected by chance if there were no epistatic interactions involving *MLH1* and *PMS1* genes. An attractive explanation for this observation is that incompatible isolates exist transiently to provide an adaptive advantage, but mechanisms such as outcrossing restore the compatible genotype. Consistent with this idea is a sequence analysis of a 32-kb genomic region of yeast (Chr. XIV, 445–477 kb), which provided evidence for recombination between the S288c and SK1 groups (Steinmetz *et al.* 2002; Heck *et al.* 2006; Demogines *et al.* 2008b).

A challenge to the above model is that several studies have estimated that the number of clonal generations that two yeast strains would have experienced prior to outcrossing is very high, one outcrossing event per 12,500–62,500 generations (Ruderfer *et al.* 2006; Magwene *et al.* 2011). While such observations suggest that yeast has primarily an asexual lifestyle, random mating between natural strains can be achieved at high rates in the lab and can be elevated in different environments, such as in the gut of the fruit fly (Reuter *et al.* 2007; Murphy and Zeyl 2010). It is also worth noting that population structure analyses of domestic and wild yeasts showed evidence for many different natural recombinant/mosaic genomes (Liti *et al.* 2009; Strobe *et al.* 2015),

and outcrossing rate is likely to be different depending on the ecological origin of the isolates and their stress conditions; e.g., a high outcrossing rate was observed for domestic isolates (reviewed in Marsit and Dequin 2015). Thus isolates heterozygous for MMR genotypes could in stress conditions undergo sporulation and yield incompatible haploid progeny.

Previously, we showed that incompatible strains display a transient fitness advantage (Bui *et al.* 2015). In the present study, we looked to see whether evolved incompatible strains maintain fitness in nonstress and stress conditions. Our work showed that such strains rapidly displayed fitness defects in nonstress conditions. This observation encouraged us to survey 1010 *Saccharomyces cerevisiae* wild, clinical, industrial, and lab strains from worldwide collections for their *MLH1* and *PMS1* genotypes. We identified and carefully characterized one clinical isolate (YJM523) that displayed the homozygous incompatible *MLH1*–*PMS1* genotype. Long-term fitness costs appeared to have been avoided in this strain through extragenic mutations that suppressed its mutation rate. Based on the above, we hypothesize that in natural populations, a mutator state can be created to transiently promote adaptive evolution, but can then be suppressed once beneficial mutations go to fixation.

Materials and Methods

Strains and media

S. cerevisiae spore clones from the S288c strain background were analyzed in competition assays (Figure 2; Supplemental Material, Table S1 in File S3; Winston *et al.* 1995). These strains were grown in YPD (yeast extract, peptone, dextrose) and YPD + 1.2 M NaCl (Rose *et al.* 1990). Natural and S288c-derived strains transformed with pEAA613 (*ARS*–*CEN* *NatMX*, *kanMX::insE-A₁₄*; Table S1 and Table S2 in File S3) were maintained in YPD media containing 50 µg/ml nourseothricin. Yeast transformations were performed as described (Gietz and Schiestl 2007).

EAY1365 (*MATa*, *ura3-52*, *leu2Δ1*, *trp1Δ63*, *his3Δ200*, *lys2::insE-A14*, *mlh1Δ::KanMX4*, *pms1Δ::KanMX4*), an S288c-derived strain, was used to measure reversion to Lys⁺. This strain was transformed with *ARS*–*CEN*, *LEU2*, *cMLH1* (pEAA213 and derivatives) and *ARS*–*CEN*, *HIS3*, *cPMS1* (pEAA238 and derivatives) plasmids, and were grown in minimal selective media lacking histidine and leucine (Table S1 in File S3; Rose *et al.* 1990). In this paper genes derived from the S288c background are designated with a “c” (e.g., *cMLH1*) and those derived from SK1 with a “k” (e.g., *kMLH1*). YJM555 is a homozygous diploid strain derived from the natural isolate YJM523 (see details in Strobe *et al.* 2015). YJM523 and YJM555 were obtained from the Fungal Genetics Stock Center collection (<http://www.fgsc.net/>).

Plasmids

pEAA213 (*cMLH1*, *ARSH4 CEN6*, and *LEU2*) and pEAA214 (*kMLH1*, *ARSH4 CEN6*, and *LEU2*) were described previously

Figure 1 A model proposing that an incompatibility involving the MMR genes *MLH1* and *PMS1* drives adaptive evolution. In this model (A), an ancestral isolate bearing *MLH1* Gly 761 and *PMS1* Arg 818/822 alleles acquire neutral or beneficial mutations that lead to the derived S288c (purple, Asp 761 and Arg 818/822) and SK1 (green, Gly 761 and Lys 818/822) group isolates. Mating between the derived isolates can yield an allele combination (*MLH1* Asp 761 and *PMS1* Lys 818/822) that displays negative epistasis and thus a mutator phenotype. Previous work suggested that recombination has occurred between the two derived classes, leading to exchange of genetic information and a hybrid mutator genotype that can also recombine to reconstruct derived or ancestral genotypes (Heck *et al.* 2006). As described in the text, sequences of *MLH1* and *PMS1* genes from 1010 *S. cerevisiae* from worldwide collection genomes were grouped according to their amino acid residues 761 (G or D) in *MLH1* and 818 (R or K) in *PMS1* (File S1 and File S2). One isolate maps to the predicted hybrid mutator category. Of the 1010 isolates, 904 are homozygous for the *MLH1* 761 and *PMS1* 818/822 genotypes. The distribution in each genotype is shown here and a list of isolates in the S288c and SK1 derived classes is shown in Table S2 in File S3. (B) A total of 106 isolates are heterozygous for the *MLH1* 761 and/or *PMS1* 818/822 genotypes, with the distribution shown. On the left are the genotypes that can lead to both mutator and nonmutator progeny (see Table 1 and text). On the right are genotypes that can lead only to nonmutator progeny.

(Argueso *et al.* 2003; Heck *et al.* 2006; Table S3 in File S3). *MLH1* expression is driven in both plasmids by the S288c *MLH1* promoter. *MLH1* from YJM555 was cloned into pEAA213 by amplifying *MLH1* from genomic DNA (Hoffman and Winston 1987; Rose *et al.* 1990) using high-fidelity polymerase (Roche, Indianapolis, IN) and primers AO324 and AO821 (Table S4 in File S3). The PCR-amplified product containing the entire *MLH1* open reading frame (ORF) was digested with *Bam*1H and *Nhe*I and inserted into the corresponding sites of pEAA213. The entire PCR fragment was DNA sequenced. All of the resulting constructs expressed *MLH1* via the S288c *MLH1* promoter.

pEAA238 (*cPMS1*, *ARSH4*, *CEN6*, and *HIS3*) and pEAA239 (*kPMS1*, *ARSH4*, *CEN6*, and *HIS3*) were described previously (Table S3 in File S3; Argueso *et al.* 2003; Heck *et al.* 2006). In both plasmids, *PMS1* expression is driven by the S288c *PMS1* promoter. *PMS1* from YJM555 was cloned into pEAA238 by amplifying *PMS1* from genomic DNA using Roche high-fidelity polymerase and primers AO548 and AO481 (Table S4 in File S3). The PCR-amplified product containing the entire *PMS1* ORF was digested with *Aat*II- and *Mlu*I and inserted into the corresponding sites of pEAA238. The entire PCR fragment was DNA sequenced. All of the resulting constructs expressed *PMS1* via the S288c *PMS1* promoter.

pEAA613 contains the *URA3* promoter-*kanMX::insE-A₁₄* reversion reporter constructed using overlap PCR (Ho *et al.* 1989). Briefly, this reporter is expressed via the *URA3* promoter (−402 to the ATG start site). A 55-bp sequence containing a +1 frameshift in the 14-bp homopolymeric A run (*insE-A₁₄*; Tran *et al.* 1997) was inserted immediately after

the *URA3* ATG, followed by codons 18–269 of the *KanMX* ORF derived from pFA6-*KanMX*, and 159 bp of *KanMX* downstream sequence that contains a transcription termination sequence (also derived from pFA6-*KanMX*; Goldstein and McCusker 1999). This reporter construct was inserted into pLZ259 (*ARS-CEN*, *NatMX*, kindly provided by Dr. L. Zhu). Derivatives of pEAA613 that contain homopolymeric tracts of 10 A (in frame, pEAA611) and 11 A residues (+1 out of frame, pEAA612) residues were also constructed. Finally, a set of reporter constructs (pEAA614–616) were built in which the *kanMX::insE-A₁₄* reporter was expressed via the *LEU2* promoter (−308 to the ATG start site of the *LEU2* promoter fused to the *KanMX* ORF).

Spore clone competitions

Clones derived from incompatible strains (*cMLH1::KanMX*, *kPMS1::HIS3*, *pmr1*) evolved in high salt for 70 generations (EAY3688, EAY3689, and EAY3690 containing *pmr1* mutations *T459A*, *T412C*, and *T2G*, respectively; Bui *et al.* 2015) were mated with the unevolved and compatible strain EAY3241 (*kPMS1::HIS3*, *kMLH1::NatMX*, *PMR1*; Table S1 in File S3). Diploids were selected on YPD plates containing clonNAT (100 μg/ml) and genecitin (200 μg/ml) and then sporulated on 2% agar media containing 1% potassium acetate. The resulting spore clones were genotyped for *PMR1* by isolating chromosomal DNA (Holm *et al.* 1986) from individual clones and sequencing the PCR amplified *PMR1* locus. These clones were genotyped for *MLH1* by testing for resistance to genecitin and clonNAT and for the *MAT* locus by mating to tester strains.

Figure 2 Competition experiments of spore clones from postadaptation strains. Clones derived from incompatible strains evolved in high salt for 70 generations (EAY3688-*pmr1-T459A*, EAY3689-*pmr1-T412C*, and EAY3690-*pmr1-T2G*; Bui *et al.* 2015) were mated with the unevolved and compatible strain EAY3241 (Table S1 in File S3). The resulting diploids were sporulated and genotyped for *PMR1*, *MLH1*, *PMS1*, and mating type. Spore clones of the same mating type and of the four possible genotypes (*kMLH1-kPMS1*(compatible)-*PMR1*, *kMLH1-kPMS1*(compatible)-*pmr1*, *cMLH1-kPMS1*(incompatible)-*PMR1*, and *cMLH1-kPMS1*(incompatible)-*pmr1*) were grown overnight in YPD and

then mixed in equal proportions (transfer 0). Four, three, and three spore clones of each of the genotypes (12–16 in total per competition) were competed from the matings involving *pmr1-T459A*, *pmr1-T412C*, and *pmr1-T2G*, respectively. The 2×10^7 cells of the initial mixed culture (transfer 0) were transferred into 6 ml of YPD or YPD + 1.2 M NaCl and then grown for 24 hr (approximately seven generations of growth). The same amount of cells was used in subsequent transfers. At the indicated transfers, cells were genotyped for compatibility (*kPMS1::HIS3* and *kMLH1::NatMX*) and incompatibility (*kPMS1::HIS3* and *cMLH1::KanMX*) on YPD plates containing geneticin or nourseothricin.

Spore clones of the same mating type and of the four possible genotypes (*kMLH1-kPMS1*(compatible)-*PMR1*, *kMLH1-kPMS1*(compatible)-*pmr1*, *cMLH1-kPMS1*(incompatible)-*PMR1*, and *cMLH1-kPMS1*(incompatible)-*pmr1*) were grown overnight in YPD and then mixed in equal proportions (transfer 0). Four, three, and three spore clones of each genotype were competed from the matings involving *pmr1-T459A*, *pmr1-T412C*, *pmr1-T2G*, respectively. Ten competitions in total were performed, and different spore clones obtained from different tetrads were pooled to minimize effects of background mutations that might arise in evolved populations. The 2×10^7 cells of the initial mixed culture (transfer 0) were transferred into 6 ml of YPD or YPD + 1.2 M NaCl and then grown for 24 hr at 30° (approximately seven generations of growth). The same amount of cells (to achieve an initial OD₆₀₀ of 0.1, Shimadzu UV-1201 spectrophotometer) was used in subsequent transfers, with cells grown under the same conditions. At transfer numbers indicated in Figure 3, cells in the culture were genotyped for compatibility (*kPMS1::HIS3*, *kMLH1::NatMX*) and incompatibility (*kPMS1::HIS3*, *cMLH1::KanMX*) on YPD plates containing geneticin (200 µg/ml) or clonNAT (100 µg/ml).

DNA sequencing analysis

A total of 1010 *S. cerevisiae* isolates were investigated in the context of the 1002 Yeast Genomes Project (File S1 and File S2). Illumina reads were mapped against the *S. cerevisiae* 288c reference genome R64-1-1 with Burrows-Wheeler Aligner 0.7.4-r385 and the sequence of the *MLH1* and *PMS1* genes were inferred for all isolates with GATK (FastAlternateReferenceMaker).

The 2.3 kb *MLH1* and 2.6 kb *PMS1* ORFs from YJM521 and YJM523 (Table 1 and Table S1 and Table S2 in File S3) were determined by sequencing PCR-amplified DNA from chromosomal DNA (Hoffman *et al.* 1987) using Expand High Fidelity Polymerase (Roche Life Sciences). Primers AO324 and AO821 were used to amplify the *MLH1* ORF and primers AO481 and AO548 were used to amplify the *PMS1* ORF (Table S4 in File S3). PCR products were sequenced by the Sanger method in the Cornell BioResource Center. Duplicate clones from the strain stocks were sequenced for confirmation purposes. To further show that YJM523 was not heterozygous for sequences

at the *Mlh1* (amino acid position 761) and *Pms1* (amino acid position 818) incompatibility loci, 20 different PCR products, derived from unique PCR primer combinations, were sequenced using primer AO328 for *MLH1* and primer AO485 for *PMS1* (Table S4 in File S3). In no case was heterozygosity detected at the *MLH1* or *PMS1* incompatibility loci.

Flow cytometry

Cells from isolates shown in Table 1 were fixed overnight in cold ethanol (70% final), washed, and resuspended at 1.2×10^7 cells/ml in 50 mM sodium citrate (pH 7). They were then treated for 1 hr with RNase A at a final concentration of 1 mg/ml, after which they were stained with propidium iodide at a final concentration of 40 µg/ml. Flow cytometry was performed on a Cyflow Space, Partec.

Sporulation test

Isolates shown in Table 1 were sporulated for 2–3 days on sporulation medium (10 g/liter potassium acetate, 20 g/liter agar) at 30°.

lys2-A₁₄ reversion assays

Independent colonies of EAY1365 (relevant genotype *lys2-A₁₄*) containing the *ARS-CEN*, *MLH1* and *ARS-CEN*, *PMS1* plasmids presented in Table S3 in File S3 were inoculated YPD overnight and then plated onto LYS, HIS, LEU dropout and HIS, LEU dropout synthetic plates. These strains were analyzed for reversion to Lys⁺ as described previously (Tran *et al.* 1997; Bui *et al.* 2015). The 95% confidence intervals were determined as described by Dixon and Massey (1969). The Mann–Whitney *U*-test (Mann and Whitney 1947) was performed to determine the significance of the differences in median reversion rates.

kanMX::insE-A_{11–14} reversion assays

EAY1369 (*cMLH1-cPMS1*, compatible), EAY1370 (*cMLH1-kPMS1*, incompatible), EAY1372 (*msh2Δ*), and YJM555 were transformed with pEAA613 or pEAA616 and grown on YPD media containing clonNAT (100 µg/ml). Independent transformants were subsequently grown overnight in YPD + clonNAT and then plated on to YPD + clonNAT (50 µg/ml) and

Figure 3 Fitness of postadaptation incompatible and compatible strains in YPD and YPD + 1.2 M NaCl. Fitness (w) was determined in cultures genotyped for *MLH1*–*PMS1* compatible and incompatible clones in YPD and YPD + 1.2 M NaCl as described in the *Materials and Methods* and Figure 2 and Table 2. Ten same mating-type competitions (competitions 1–4 for *pmr1*–*T459A* evolved, 5–8 for *pmr1*–*T412C* evolved, and 9–10 for *pmr1*–*T2G* evolved) are shown. For the YPD transfer experiments, 14 incompatible and 14 compatible clones at transfer 24 (6, 4, 4, each from *pmr1*–*T459A*, *pmr1*–*T412C*, and *pmr1*–*T2G*, respectively) were genotyped for *PMR1*. For the YPD + 1.2 M NaCl transfer experiments, 12 incompatible and 12 compatible clones at transfer 24 (4 each from the *pmr1*–*T459A*, *pmr1*–*T412C*, and *pmr1*–*T2G*) were genotyped for *PMR1*.

YPD + clonNAT (50 μ g/ml), genecitin (300 μ g/ml). These strains were analyzed for reversion to resistance to genecitin using methods described previously (Tran *et al.* 1997; Bui *et al.* 2015). The 95% confidence intervals were determined as described by Dixon and Massey (1969). Pairwise Kruskal–Wallis tests were performed to determine the significance of the differences in median reversion rates. Mann–Whitney *U*-tests were performed to determine the significance of the differences in median reversion rates (Wilcoxon 1945; Mann and Whitney 1947).

As controls, we measured resistance to genecitin (G418) for EAY1369 and YJM555 lacking the *kanMX::insE-A_{11–14}* reporter plasmids. Ten independent overnight cultures were analyzed for each strain. We did not observe resistant colonies in any of the cultures and thus estimate median frequencies of resistance to be $<9.7 \times 10^{-10}$ for EAY1369, and $<6.9 \times 10^{-10}$ for YJM555; these values correspond to mutation rates that are $<2 \times 10^{-10}$, indicating that spontaneous reversion to G418^r would not interfere with the detection of G418^r using the *kanMX::insE-A_{11–14}* reporter plasmids.

YJM $\times$ EAY3235 cross

To determine whether a single locus is present in YJM555 that suppressed *MLH1*–*PMS1* incompatibility, we mated meiotic spores of YJM555 to the incompatible strain EAY3235 (*MATa*, *lys2-A₁₄*, *ura3*, *trp1*, *leu2*, *MLH1*_{S288c}::*KanMX*, *PMS1*_{S288c}–*R818K::HIS3*). Four independent Ura⁺ G418^r colonies were sporulated and tetrad dissected. The spore clones from four viable spore tetrads all displayed 2:2 segregation for *lys2-A₁₄* / *LYS2*, *trp1* / *TRP1*, and *ura3* / *URA3*. The sporulation viability of the diploid was 82%, with 62% of tetrads displaying four viable spores (79 tetrads dissected).

A total of 42 *lys2-A₁₄* spore clones (one Lys[–] spore clone chosen per tetrad) were tested using a patch assay for reversion to Lys⁺. The 42 spore clones all displayed ~1:1 ratios for *MLH1*_{S288c}::*KanMX* / *MLH1*_{YJM555} segregation (the ORFs for *MLH1*_{YJM555} and *MLH1*_{S288c} are identical; Figure 4), *PMS1*_{YJM555} / *PMS1*_{S288c}–*R818K::HIS3* (genotyped using PCR

primers that distinguished between the presence or absence of the *HIS3* insertion), *trp1* / *TRP1*, *leu2* / *LEU2*, and *ura3* / *URA3* (Table S4 in File S3). To confirm phenotype testing, the *PMS1* gene was PCR amplified from three *PMS1*_{YJM555} and two *PMS1*_{S288c}–*R818K::HIS3* spore clones. A total of 700 bp of the *PMS1* gene surrounding the incompatibility site at amino acid 818 were sequenced; all five spore clones displayed DNA sequences that corresponded to the assigned phenotype. To determine mutator phenotypes, each of the 42 *lys2-A₁₄* spore clones was struck to single colonies on YPD media. Five ~2 mm in diameter colonies from each spore clone were then patched onto 1.5 cm $\times$ 1.5 cm squares on lysine dropout plates. It is important to note that colony morphology and size varied somewhat between spore clones, and for four spore clones, the colonies grew to ~1 mm in diameter. Plates were scored for Lys⁺ reversion colonies after 3 days of growth at 30°, with the median number of colonies recorded per patch.

Data availability

Strains and plasmids are available upon request, and the DNA sequences of the *MLH1* and *PMS1* genes from 1010 isolates are present in File S1 and File S2. Supporting information contains all detailed descriptions of all supplemental files.

Results

Incompatibility confers a fitness cost in evolved strains in nonstress conditions

In Bui *et al.* (2015), we showed that haploid yeast containing the incompatible S288c *MLH1*–*SK1* *PMS1* genotype (abbreviated as *cMLH1*–*kPMS1*) displayed a transient growth advantage in high salt that resulted from mutations in the *PMR1* gene. This observation encouraged us to determine the time frame in which incompatible strains that display an initial transient adaptive advantage can maintain fitness in non-stress and stress conditions. To answer this, we mated three different high-salt evolved clones, constructed to contain the incompatible *cMLH1*–*kPMS1* combination, to the unevolved

Table 1 Nineteen natural isolates that contain incompatible *MLH1*–*PMS1* combinations

Isolate name	Genotype <i>MLH1</i>	<i>PMS1</i>	Ploidy (<i>n</i>)	Origin (ecological, geographical)
CLIB324_2	SK1/S288c	SK1/S288c	2	Bakery, Vietnam
CBS4455	S288c	SK1/S288c	3	Beer, South Africa
CECT1462	SK1/S288c	SK1/S288c	4	Beer, United Kingdom
NCYC_2780	SK1/S288c	SK1/S288c	4	Human clinical, Belgium
2680	SK1/S288c	SK1/S288c	4	Human clinical, Spain
YS8(E)	SK1/S288c	SK1/S288c	4	Bakery, unknown
SD-15	SK1/S288c	SK1/S288c	4	Bakery, Italy
WLP001	SK1/S288c	SK1/S288c	4	Beer, USA
WLP013	SK1/S288c	SK1	4	Beer, United Kingdom
WLP006	SK1/S288c	SK1	4	Beer, unknown
UCD_06-645	SK1/S288c	SK1/S288c	4	Fruit, Davis, California
Win-8B	SK1/S288c	SK1	2	Beer, United Kingdom
Ponton 11	SK1/S288c	SK1/S288c	2	Human clinical, Spain
CLI_16	SK1/S288c	SK1/S288c	4	Human clinical, France
CLI_19	SK1/S288c	SK1/S288c	2	Human clinical, France
CLI_23	SK1/S288c	SK1/S288c	4	Human clinical, France
CLI_26	SK1/S288c	SK1/S288c	4	Human clinical, France
YJM521	SK1/S288c	SK1/S288c	NT	Human clinical, California
YJM523	S288c	SK1	NT	Human clinical, California

The 1010 isolates were genotyped at amino acid 761 for *MLH1* and amino acid 818 in *PMS1*. Isolates were genotyped as S288c if they contained the S288c amino acids (D761 in *MLH1* and R818 in *PMS1*), and SK1 if they contained the SK1 amino acids (G761 in *MLH1* and K822 in *PMS1*). SK1/S288c indicates that both SK1 and S288c alleles were detected in the strain at the indicated locus. We analyzed the yeast genomes of the 1010 isolates by placing them into clades. We then computed the mean, median, SD, and SE for the number of singletons within these clades and compared the number of singletons of the 19 isolates to their associated clades. We did not observe an excess of singletons for any of the isolates (1002 Yeast Genomes Project; <http://1002genomes.u-strasbg.fr/>). Ploidy was determined by FACS, and all but CLIB324_2, WLP001, and Win-8B are capable of sporulating (*Materials and Methods*). NT, not tested.

compatible strain EAY3241. These incompatible strains, EAY3688 (*pmr1*-T459A), EAY3689 (*pmr1*-T412C), and EAY3690 (*pmr1*-T2G), were evolved for 70 generations in high salt (Bui *et al.* 2015; Table S1 in File S3). This window was chosen because incompatible strains displayed an adaptive advantage over compatible strains when evolved for 70 generations that was lost after 120 generations (Bui *et al.* 2015). The diploids were then sporulated and the resulting spores genotyped for incompatibility, mating type, and *PMR1* (Figure 2). We then created for each *pmr1* allele mixed cultures that contained equal proportions of the same mating type spore clones that were *PMR1* compatible, *pmr1* compatible, *PMR1* incompatible, or *pmr1* incompatible, and grew them overnight, followed by subsequent transfers, in YPD or YPD + 1.2 M NaCl (roughly seven generations of growth per transfer; *Materials and Methods*). Our goal in these studies was to determine whether the incompatible strains would ultimately display a fitness defect in rich media due to the accumulation of recessive mutations, as predicted by Heck *et al.* (2006). We also tested whether the rapidly evolved incompatible strains would continue to show an adaptive advantage in high salt media.

We measured in rich media fitness differences between incompatible and compatible strains. As shown in Figure 3, Table 2, and Table S5 in File S3, we observed a gradual decrease in fitness of incompatible cells. This observation supports the idea that being incompatible is not favorable shortly after adaptation (Bui *et al.* 2015). At the end of the transfer experiment, most competitions resulted in a higher proportion of compatible cells and all 28 independent clones (half compatible, half incompatible) isolated from transfer 24 were

PMR1, indicating that all three *pmr1* mutations were deleterious in rich media.

For competitions in YPD + 1.2 M NaCl, we observed a general decline in the fitness of incompatible strains compared to compatible strains in the first 50 generations of competition. In the next 100 generations, some incompatible strains displayed increased fitness (Figure 3 and Table 2 and Table S5 in File S3). Sequencing of 24 clones (12 compatible, 12 incompatible clones) at transfer 24 showed that all retained their initial adaptive *pmr1* mutations. These results showed that further adaptive advantages beyond the initial transient one were likely countered by the accumulation of deleterious mutations.

Natural isolates were identified that display the incompatible genotype

Previously Heck *et al.* (2006) identified one site each in *MLH1* [amino acid 761, Asp (D) in S288c, Gly (G) in SK1] and *PMS1* [amino acid 818/822, Arg (R) in S288c, Lys (K) in SK1] that accounted for the MMR incompatibility seen in progeny derived from crossing S288c and SK1 strains, with only *MLH1*-D761/*PMS1*-K818 conferring a mutator phenotype (Figure 1; Heck *et al.* 2006; Demogines *et al.* 2008b). We took advantage of a recent whole-genome sequencing analysis of a worldwide collection of 1010 *S. cerevisiae* isolates to determine whether the incompatible combination exists in nature (File S1 and File S2; full genomes and their analyses will be presented in the 1002 Yeast Genomes Project; <http://1002genomes.u-strasbg.fr/>). All 1010 isolates displayed D and/or G at amino acid position 761 in *MLH1* and R and/or K at position 818/822 in *PMS1*.

Figure 4 Polymorphisms observed in the closely related YJM555 and YJM320 *S. cerevisiae* strains. (A) DNA sequence, as shown by chromatogram traces, of the *MLH1* and *PMS1* incompatibility sites in YJM521 and YJM523. DNA sequencing reactions are shown for two *MLH1* and *PMS1* sequences that were independently PCR amplified (using primers AO324, AO821 for *MLH1* and AO548, AO481 for *PMS1*; Table S4 in File S3) from YJM521. Sequencing reactions were performed on 10 *MLH1* and *PMS1* DNA fragments independently PCR amplified from YJM523; one such reaction is shown for *MLH1* and *PMS1* (see *Materials and Methods* and Table S4 in File S3 for details). Amino acids in the *MLH1* and *PMS1* ORFs are shown that correspond to the presented DNA sequences. These are Gly (G) or Asp (D) at amino acid 761 in *MLH1*, Arg (R), or Lys (K) at amino acid 818/822 in *PMS1*. Homo, homozygous for genetic information at an incompatibility site; het, heterozygous. (B) Haplotype structure of *MLH1* (11 polymorphic sites) and *PMS1* (32 polymorphic sites)

based on the genome sequence of S288c, SK1, YJM320, and YJM523. YJM555 is a strain derived from the isolate YJM523 (Strope *et al.* 2015), and YJM523 and YJM555 contain identical and homozygous *MLH1* and *PMS1* sequences. Sequences identical to S288c (purple font) and SK1 (green font) are indicated. Synonymous sites are shown as blue asterisks. Sites in *MLH1* and *PMS1* that contribute to MMR incompatibility are shown by the red "i." Sequences in black font were not found in S288c or SK1; those in black and underlined were not seen in 1007 of the other 1008 yeast isolates that were sequenced (they were seen in the closely related strain YJM554) (Strope *et al.* 2015). The 12-bp insertion seen in the SK1 *PMS1* ORF (Table 3) is indicated by a +. For *MLH1* nucleotide position of each polymorphic site in the open reading, from left to right, are: 486, 720, 812, 834, 997, 1044, 1237, 1875, 2032, 2108, and 2282. For *PMS1* the positions are: 122, 177, 210, 213, 258, 333, 335, 465, 552, 558, 708, 711, 810, 855, 858, 939, 1150, 1175, 1191, 1199, 1201, 1249 (insertion), 1329, 1538, 1575, 1691, 1782, 1821, 2303, 2322, 2364, and 2453.

In total, 904 of the 1010 isolates are homozygous for polymorphisms in *MLH1* and *PMS1*. As shown in Figure 1 and Table S2 in File S3, 693 of these isolates (*MLH1*–G761/*PMS1*–R818) mapped to the ancestral group, 182 to the S288c group (*MLH1*–D761/*PMS1*–R818), 28 to the SK1 group (*MLH1*–G761/*PMS1*–K818), and one (the YJM523 clinical isolate) to the incompatible group (*MLH1*–D761/*PMS1*–K818). The isolates in both the S288c and SK1 groups are not closely related, suggesting that the incompatible genotype could arise independently or are different combinations of mosaic genomes derived from a common event (Schacherer *et al.* 2009; Strope *et al.* 2015). A Fisher's exact test of the observed number of isolates in each category showed that the number of isolates in the incompatible group was lower than expected by chance if there were no negative epistatic interactions involving this combination of *MLH1*–*PMS1* ($P = 0.018$). These data suggest that there may be active selection against the incompatible genotype. We hypothesize that because of the long-term fitness cost associated with the incompatibility, there would be few if any natural isolates maintained as incompatible.

Among the 1010 isolates, 106 are heterozygous for the *MLH1* 761 and/or *PMS1* 818/822 genotypes. Previously we showed in experiments where both incompatible and compatible genotypes were present that the incompatible genotype is recessive (Argueso *et al.* 2003). In total, 18 of the 106 isolates have the potential to generate incompatible progeny from meiotic spores, assuming that they are diploids competent to undergo meiosis and form haploid spores (Table 1). Of the 18, 12 are tetraploid, 4 are diploid, and 1 is triploid, with most capable of sporulating. Eight were

obtained from human clinical samples, 6 from breweries, 3 from bakeries, and 1 from fruit.

A total of 14 of the 18 isolates display heterozygous S288c/SK1 *MLH1* (amino acid 761) and S288c/SK1 *PMS1* (amino acid 818/822) genotypes, suggesting that they could have been created through matings between homozygous strains containing S288c and SK1 *MLH1*–*PMS1* genotypes. Sporulation of diploid isolates with these genotypes would yield meiotic progeny in which one-quarter would have the incompatible genotype (see YJM521 in Figure 4A). One isolate (CBS4455) displays an S288c *MLH1*–S288c/SK1 *PMS1* genotype and three an S288c/SK1 *MLH1*–SK1 *PMS1* genotype, possibly generated through crosses between compatible and incompatible strains. If sporulated, half of the meiotic progeny from these four isolates would display the incompatible genotype. These results suggest that the potential to generate incompatible progeny is present in compatible natural isolates and this potential may facilitate adaptive evolution by providing a brief increase in the mutation rate.

The above results encouraged us to look more closely at YJM523, which is the only one of 1010 isolates homozygous for the incompatible genotype (Figure 1, Figure 4, File S1, and File S2). YJM523 is a clinical isolate sampled from Stanford University Hospital (Stanford, CA; Strope *et al.* 2015; deposited in the Phaff Yeast Culture Collection, University of California, Davis, CA). A previous genomewide phylogenetic analysis of 93 *S. cerevisiae* strains showed that a spore clone derivative of YJM523, YJM555, is closely related to the compatible strain YJM320 (Strope *et al.* 2015; Figure 4B and Table 3). We speculate that YJM523 and YJM320 are recently derived from the same origin, perhaps created by a

Table 2 Fitness of evolved incompatible and compatible strains as a function of transfer in YPD and YPD + NaCl media

Transfer	Fitness, $w \pm \text{SEM}$			<i>n</i>
	YPD	YPD + NaCl		
3	0.98 $\pm$ 0.01	0.98 $\pm$ 0.01		10
4	0.97 $\pm$ 0.02	0.95 $\pm$ 0.02		6
8	0.95 $\pm$ 0.01	0.93 $\pm$ 0.03		10
12	0.93 $\pm$ 0.03	1.06 $\pm$ 0.09		6
14	0.92 $\pm$ 0.02	1.06 $\pm$ 0.05		10
16	0.92 $\pm$ 0.02	1.05 $\pm$ 0.06		10
20	0.91 $\pm$ 0.02	1.06 $\pm$ 0.06		10
24	0.90 $\pm$ 0.03	1.09 $\pm$ 0.05		10

The proportion of compatible and incompatible genotypes was determined after the indicated number of transfers for spore clone competitions performed in YPD and YPD + 1.2 M NaCl (Figure 3). Fitness (w) values were calculated as $w = ((p_t/q_t)/(p_0/q_0))^{1/t}$, where t equals the number of generations after T transfers (seven generations per transfer; Hartl and Clark 2007), p_0 and q_0 are the number of incompatible and compatible cells, respectively at transfer 0, and p_t and q_t are the number of incompatible and compatible cells, respectively, at the indicated transfer. n is the number of unique competitions performed for each data set. In this table the average fitness of the three *pmr1* genotypes is presented. In Table S5 in File S3, the fitness values for each *pmr1* genotype are shown.

single mating event between S288c and SK1 group isolates (see below and Discussion). It is important to note that at the genomewide level, it does not appear that YJM523 is the result of a “direct” cross between S288c and SK1. If this was the case, then the YJM523 genome would show a mosaic of large regions identical to S288c interspersed with regions of high sequence divergence corresponding to SK1, but this was not observed (A. Friedrich and J. Schacherer, 1002 Yeast Genomes Project; <http://1002genomes.u-strasbg.fr/>).

Previously we showed that the distribution of sequence polymorphisms among 10 *S. cerevisiae* strains for a 32-kb region indicated evolutionarily recent recombination among S288c and SK1 class strains, with YJM320 showing extensive evidence of recombination with the S288c group (Heck *et al.* 2006). At that time we speculated that the incompatible genotype was generated through interstrain crosses but was not observed in our earlier sampling due to fitness costs. The phylogenetic and single nucleotide polymorphism (SNP) data presented in Strobe *et al.* (2015), Figure 4 and Table 3 strengthen this model, suggesting that YJM523 resulted from a cross between S288c and SK1 group isolates that yielded the incompatible genotype. Consistent with this hypothesis is the finding that YJM523 displayed a *PMS1* SNP pattern consistent with an SK1 class origin (16-nt positions identical to the SK1 sequence vs. six identical to S288c), and an *MLH1* SNP pattern consistent with an S288c class origin (all 11 sites identical to S288c sequence). YJM320 is consistent with an SK1 class origin for both *PMS1* (16-nt positions identical to the SK1 sequence vs. six identical to S288c) and *MLH1* (eight sites identical to the SK1 sequence vs. three identical to the S288c sequence). That the pattern for synonymous sites matches the nonsynonymous sites gives us confidence that it reflects evolutionary history and not selective convergence of amino acid residues.

Table 3 Nonsynonymous substitutions in *MLH1* and *PMS1*

Strain	Amino acid position in <i>MLH1</i>						Amino acid position in <i>PMS1</i>							
	240	271 ^a	333	678	703	761								
S288c	S	L	E	D	P	D								
SK1	R	P	K	N	L	G								
YJM523	S	L	E	D	P	D								
YJM320	S	P	E	N	L	G								
	Amino acid position in <i>PMS1</i>						41	112	384	392	400	401	416 ^b	513 564 768 818
S288c	N	I	F	E	T	A	T	Y	A	K	R			
SK1	S	T	V	E	S	A	TCEGT	F	A	K	K			
YJM523	N	I	V	V	S	S	TCEGT	Y	V	R	K			
YJM320	N	I	V	V	S	S	TCEGT	Y	V	R	K			

All substitutions that result in amino acid changes are shown relative to the S288c strain. S288c, SK1, and YJM320 are compatible for the *MLH1*–*PMS1* genotype; YJM523 is incompatible.

^a Proline residue at *MLH1*-271 partially suppresses the *cMLH1*–*kPMS1* incompatibility (Demogines *et al.* 2008b).

^b Compared to S288c, SK1, YJM523, and YJM320b strains/isolates contain a four amino acid insertion (CEGT) after T416 in *PMS1*.

The *MLH1*–*PMS1* incompatible combination derived from a natural isolate confers a mutator phenotype in the S288c strain background

We used the highly sensitive *lys2*-*A*₁₄ reversion assay to assess the mutator phenotype of the *MLH1* and *PMS1* gene combinations from YJM523. The *lys2*-*A*₁₄ allele contains a homopolymeric run of 14 A residues inserted out of frame within the *LYS2* gene. In this assay wild-type and MMR null strains display an ~10,000-fold difference in the rate of reversion to Lys⁺ (Tran *et al.* 1997). The *MLH1* and *PMS1* genes from YJM523 were cloned into *ARS*–*CEN* vectors and transformed into an *mlh1Δ pms1Δ* S288c strain that was used previously to characterize *MLH1*–*PMS1* combinations from 65 natural isolates (Demogines *et al.* 2008b). The YJM523 *MLH1*–*PMS1* combination confers a 196-fold higher mutation rate compared to the *cMLH1*–*cPMS1* combination (Table 4). This mutation rate is higher than that seen for the *cMLH1*–*kPMS1* incompatibility (75-fold), indicating that there are likely to be other polymorphisms present in the two genes that enhance the incompatibility (Demogines *et al.* 2008b). Together these data indicate that the *MLH1*–*PMS1* combination from YJM523 displays a strong incompatibility in the S288c strain background.

Strain containing the *MLH1*–*PMS1* incompatible combination displays a low mutation rate

Table 4 shows that the YJM523/555 *MLH1*–*PMS1* gene combination confers a mutator phenotype in the S288c strain background. However, neighbor-joining analysis of the genomes of this and other strains presented by Strobe *et al.* (2015) shows that the branch on the tree leading to YJM555, a spore clone derivative of YJM523, is not unusually long, and is similar to that seen for compatible strains, suggesting that YJM523 may not have been a mutator for a long evolutionary period. One way to reconcile this observation is that the *MLH1*–*PMS1* incompatibility formed in YJM523 facilitated a rapid increase in mutation rate that was then

Table 4 Mutation rates in an S288c strain containing *MLH1* and *PMS1* genes derived from S288c, SK1, and YJM523

<i>MLH1</i> – <i>PMS1</i> genotype	Lys ⁺ reversion rate (10 ^{−7}), [95% C.I. (10 ^{−7})]	Relative rate	<i>n</i>
S288c–S288c, compatible	4.1 (1.7–13.8)	1	13
S288c–SK1, incompatible	311 (111–919) ^a	75	16
YJM523/555–YJM523/555	808 (566–4,450) ^a	196	39
<i>mlh1</i> Δ, <i>pms1</i> Δ	45,300 (13,170–126,800) ^a	10,970	10

EAY1365 (relevant genotype *mlh1*Δ::*KanMX4*, *pms1*Δ::*KanMX4*) was transformed with *ARS*–*CEN* plasmids containing the *MLH1* and *PMS1* genes obtained from the indicated strain or isolate (Table S1 and Table S2 in File S3). Independent cultures (*n*) were examined for reversion to Lys⁺. Median mutation rates are presented with 95% confidence intervals, and relative mutation rates compared to the wild-type strain are shown.

^a Significantly different from S288c–S288c (*P* < 0.001, Mann–Whitney test).

returned to normal by the occurrence of suppressor mutations that restored normal MMR functions. To further test this idea, we constructed a set of vectors (Figure 5 and Table S3 in File S3; *Materials and Methods*) that can be transformed into natural yeast strains to serve as a proxy to estimate genome mutation rate. We introduced homopolymeric A sequences into the *KanMX* ORF immediately after its methionine 17 codon, with the goal of creating a sensitive mutation detection assay. Previous work showed that homopolymeric runs undergo DNA slippage in a variety of DNA repair mutant backgrounds (Tran *et al.* 1997), and such events occur at an especially high frequency in MMR defective strains. For example, *msh2*Δ strains display a 10,000-fold increase in mutation rate compared to wild type in strains bearing the *lys2*A₁₄ reporter (Tran *et al.* 1997).

The mutation reporter plasmids contain *ARS*–*CEN* and *NatMX* markers and a *kanMX*::*insE*-A₁₄ construct whose expression is driven by *URA3* or *LEU2* promoters. These promoters replaced the strong *TEF* promoter that drives *KanMX* expression in pFA6–*KanMX*; we found that the *TEF* promoter prevented the detection of large differences in reversion frequency between in- and out-of-frame *KanMX*::*insE* constructs, presumably due to high rates of transcriptional slippage. In the *URA3* promoter-*kanMX*::*insE*-A₁₄ reporter in pEAA613, a 55-bp sequence containing a +1 frameshift in the 14-bp homopolymeric A run (*insE*-A₁₄; Tran *et al.* 1997) was inserted immediately after the *URA3* ATG. This sequence was immediately followed by codons 18–269 of the *KanMX* ORF. Derivatives were constructed that contained a 10-bp in-frame insertion (pEAA611), or utilized the *LEU2* promoter to drive expression (pEAA616; Table S3 in File S3).

We tested the sensitivity of our reporter assay by transforming pEAA613 and pEAA616 into compatible, incompatible, and MMR defective (*msh2*Δ) S288c strains (Table 5 and Table S6 in File S3). For strains containing pEAA613, we found that the *msh2*Δ strain displayed a 2000-fold higher rate of reversion to genecitin resistance compared to the compatible strain, and that the incompatible strain showed a 10-fold higher mutation rate compared to the compatible strain (Figure 5 and Table 5; *Materials and Methods*). Importantly, sequencing of the *kanMX*::*insE*-A₁₄ reporter from genecitin-resistant clones showed that a −1 frameshift had occurred in the homopolymeric A sequence in all of the clones (*n* = 14). While this range is less sensitive than was seen in the *lys2*-A₁₄ reversion assay (~75-fold in an assay with a

10,000-fold range between wild type and MMR defective; Table 4), this reporter construct provides the opportunity to estimate mutation rates in natural strains that lack genetic markers. We then transformed the *URA3* promoter-*kanMX*::*insE*-A₁₄ and *LEU2* promoter-*kanMX*::*insE*-A₁₄ plasmids into YJM555 to determine whether this strain showed a mutator phenotype consistent with a MMR incompatibility. As shown in Figure 5, Table 5, and Table S6 in File S3, the overall mutation rate in YJM555 was similar to that seen in the S288c strain, indicating that YJM555, a well-behaved strain derived from a YJM523 spore clone (Strope *et al.* 2015), compensated for the increased mutation rate conferred by the *MLH1*–*PMS1* incompatibility.

Suppression of incompatibility in YJM555 appears to involve more than one locus

To determine whether a single suppressor locus is present in YJM555 that suppressed *MLH1*–*PMS1* incompatibility, we mated spores of YJM555 to the incompatible strain EAY3235 (relevant genotype *lys2*-A₁₄, *MLH1*_{S288c}::*KanMX*, *PMS1*_{S288c}-R818K::*HIS3*) and sporulated the resulting diploids. We used the *lys2*-A₁₄ reversion assay to analyze spore clones because it is currently the most sensitive assay to measure DNA mismatch repair incompatibility (Heck *et al.* 2006). While it is possible that lysine auxotrophy could alter a complex phenotype, our initial goal was to determine whether suppression involved the contributions of more than one locus.

We picked 42 *lys2*-A₁₄ spore clones and tested them for reversion to Lys⁺ using a colony reversion patch assay (*Materials and Methods*). As shown in Figure 6 and Table S7 in File S3, the compatible control strain EAY3234 displayed a median reversion of 0 colonies (*n* = 10) and the incompatible control strain EAY3235 showed a median reversion of 91 (*n* = 10). These values fit with the approximately two orders of magnitude difference in mutation rate between the two strains (Table 4; Heck *et al.* 2006). It is important to note that YJM555 displayed a mutation rate that was slightly higher than observed in S288c strains (Table 5), and so it is likely that the median colony number would be slightly higher than seen in EAY3234 if YJM555 bearing a *lys2*-A₁₄ allele was tested for Lys⁺ reversion.

As shown in Figure 6, the mutator phenotype of the 42 spore clones distributed into roughly four groups, with one group showing Lys⁺ colony numbers close to EAY3234

Figure 5 Use of a *URA3* promoter-*kanMX::insE-A₁₄* plasmid to measure mutation rates in natural yeast isolates. (A) The *ARS-CEN* vector pEAA613 contains a *NatMX* selectable marker and a frameshift reporter in which the *insE-A₁₄* sequence from Tran *et al.* (1997) was inserted immediately after methionine 17 in the *KanMX* ORF. In this reporter, the *URA3* gene promoter drives expression of *KanMX*, using the methionine 17 in the *KanMX* ORF as the initiation codon. The resulting construct contains a +1 frameshift mutation that disrupts *KanMX* function. A reporter construct that contains the *insE-A₁₀* insertion and does not disrupt the *KanMX* ORF is shown as an in-frame control. Frameshift mutation events (e.g., a –1 deletion in the homopolymeric A run) are detected on YPD plates containing nourseothricin and geneticin. (B) Examples of reversion assays performed using the *URA3* promoter-*kanMX::insE-A₁₄* plasmid. EAY1369 (*cMLH1-cPMS1* compatible), EAY1370 (*cMLH1-kPMS1* incompatible) and EAY1372 (*msh2Δ*) were transformed with pEAA613 and plated in dilutions from 10 μ l of a 10 $\times$ concentrated overnight culture to 10 μ l of 10⁰, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ dilutions onto YPD nourseothricin (50 μ g/ml), geneticin (300 μ g/ml) plates. (C) Example of the reversion assay performed for YJM555 is shown.

(three spore clones, 1–6 median colony number). A second group displayed Lys⁺ colony numbers that fell between those found for the compatible and incompatible controls (18 spore clones, 18–48 median colony number). A third group displayed phenotypes similar to EAY3235 (eight spore clones, 68–107 median colony number), and a fourth displayed colony numbers that were higher than EAY3235 (13 spore clones, >120 median colony number). If a single locus was responsible for a suppressor phenotype, and suppression was specific to *PMS1*_{YJM555} (the *MLH1* ORF sequence is the same in *MLH1*_{S288c} and *MLH1*_{YJM555}; Figure 4), then 25% of the spore clones would have been expected to show a suppressor phenotype, with the remainder showing phenotypes similar to that seen in EAY3235 (showing a bimodal distribution). Fifty percent of spore clones would show a suppressed mutator phenotype if a single locus suppressor could interact with either *PMS1*_{YJM555} or *PMS1*_{S288c-R818K} (also showing a bimodal distribution). The fact that we see a range of mutator phenotypes, rather than a bimodal distribution, with only 3/42 spore clones showing phenotypes similar to compatible, and 13/42 showing phenotypes more severe than the incompatible, suggests that the suppression of mutator phenotype in YJM555 is unlikely to involve a single suppressor locus. A reasonable way to explain how the suppressor in YJM555 arose is that incompatible and compatible ancestral strains had mated, yielding spores that were incompatible and displayed an initial adaptive advantage. These spore clones then incurred a large number of mutations, some of which were beneficial, and in combination, suppressed the incompatible mutator phenotype, ultimately yielding YJM555. We cannot exclude the possibility that the spectrum of mutator phenotypes seen in the progeny results from suppressor loci interactions in combination with incompatibilities involving other genome stability loci (Demogines *et al.* 2008a).

Discussion

MMR incompatibility is effective only as a short-term strategy for adaptation to new environments

We performed competitions involving populations of yeast that contained different combinations of evolved, unevolved, MMR compatible, and MMR incompatible genotypes (Figure 2 and Figure 3). In these repetitive transfer experiments we observed long-term fitness costs for MMR incompatibility, suggesting that there is an elevated mutation rate associated with MMR incompatibility that could not be stably maintained in natural populations. We proposed that following a transient adaptive advantage provided by MMR incompatibility, incompatible strains can mate to compatible strains to maintain beneficial mutations, and thus return to MMR compatibility. Such a strategy would avoid the long-term fitness cost of being a mutator. This model is consistent with our survey of 1010 yeast isolates showing that while there are a significant number (18 isolates; Table 1) heterozygous for MMR incompatibility, which is recessive, only one (YJM523) is homozygous, and is not maintained as a mutator (Table 1 and Table 5; Argueso *et al.* 2003; Heck *et al.* 2006; Demogines *et al.* 2008b; Wielgoss *et al.* 2013; Bui *et al.* 2015).

As indicated above, we identified a single yeast isolate from 1010 sampled (YJM523) that is homozygous for the incompatible genotype. Importantly, our data suggest that there are unknown mutations elsewhere in the genome of YJM523 that contribute to suppressing the mutator phenotype associated with incompatibility (see below). The molecular evolution experiments presented in Figure 3, our genotyping analysis of 1010 yeast isolates, and the associated contingency test, suggest that once an adaptive mutation reached fixation a mutator state would no longer be favorable and would in fact become a liability. Consistent with this idea are observations made by Taddei *et al.* (1997), who proposed that a modestly

Table 5 Reversion assay using the *URA3 promoter-kanMX::insE-A₁₄* plasmid

Strain	Genotype	Reversion G418 ^r (10 ⁻⁷)	95% C.I. (10 ⁻⁷)	Relative rate	n
EAY1369	Wild type	6.1	3.1–7.0	1	17
EAY1370	Incompatible	59.8 ^a	25.7–239	9.9	17
EAY1372	<i>msh2Δ</i>	13,451 ^a	7230–46,090	2142	11
YJM555		9.8 ^b	6.5–10.7	1.6	14

The indicated strains (Table S1 in File S3) were transformed with *ARS-CEN URA3 promoter-kanMX::insE-A₁₄* plasmid pEAA613. Independent cultures (n) were examined for reversion to genecitin resistance as described in *Materials and Methods*. Median mutation rates are presented with 95% confidence intervals, and relative mutation rates compared to EAY1369 (S288c compatible) are shown.

^a Significantly different from EAY1369 ($P < 0.001$, Mann–Whitney test).

^b Significantly different from EAY1369 ($P < 0.01$, Mann–Whitney test).

elevated rate in an *Escherichia coli* population would facilitate adaptation, but once adaptive mutations were fixed, the mutation load present in individuals in the population, through the accumulation of deleterious mutations throughout their genomes, would lead to a decrease in their frequency. Genomic analyses (Figure 4B; Strobe *et al.* 2015) and direct mutation rate measurements indicate that YJM523, the parent of YJM555, is not a mutator (Table 5 and Table S6 in File S3; Heck *et al.* 2006; Demogines *et al.* 2008b). YJM555 displays an overall mutation rate similar to that seen for S288c compatible strains, and whole-genome neighbor joining analysis suggests that YJM555 is not an outlier compared to other genomes (Figure 4B, Table 5, and Table S6 in File S3; Strobe *et al.* 2015). As outlined below, we hypothesize that the *MLH1-PMS1* incompatibility seen in YJM523 was formed through a single mating between S288c and SK1 groups (Figure 1), and that these strains rapidly acquired extragenic suppressors that lowered their cellular mutation rates. In this scenario, incompatible strains containing a beneficial mutation for adaptation continue to rapidly acquire mutations; among these are suppressors of the mutator phenotype that reduce genetic load (Wielgoss *et al.* 2013). Such a scenario has been observed in single cell organisms that have relatively large population sizes (e.g., *E. coli*; Wielgoss *et al.* 2013).

There are likely to be other strategies to suppress the mutator phenotype associated with incompatibility. For example, among the 1010 isolates analyzed, the frequency of a known intragenic suppressor of the mutator phenotype (*MLH1-L271P*) (Table 3; Demogines *et al.* 2008b) is very high. In total, 812 of 937 isolates (73 could not be genotyped due to ambiguity at the 271 position, possibly due to hetero-

zygosity) contained this polymorphism, but YJM523 does not contain it. The fact that the *MLH1-P271* suppressor is so common supports the idea that there is an abundant availability of this apparently nondeleterious suppressor polymorphism that can allow strains to minimize mutation rates.

We recently learned that Helen Murphy and colleagues (Skelly *et al.* 2016) analyzed strains presented in Strobe *et al.* (2015) for incompatible *MLH1-PMS1* combinations. They also found that YJM555, which contains the incompatible *MLH1-PMS1* combination, displays a much lower mutation rate than predicted based on its incompatible genotype, and their genomic analysis does not suggest evidence for an elevated mutator phenotype in YJM555. They postulated, as we demonstrated in our work, that *MLH1-PMS1* incompatibility is suppressed by background genetic modifiers.

Finally, in this study we focused on a haploid model because we can uncover recessive phenotypes and monitor genotypes rapidly. However it is clear that most yeast in nature are diploid (1002 Yeast Genomes Project; <http://1002genomes.u-strasbg.fr/>). Our identification of isolates heterozygous for incompatibility indicate that in stress conditions that result in sporulation, haploid progeny will be produced, which display incompatible *MLH1-PMS1* genotypes. At present we cannot determine whether the incompatible haploids will diploidize before their contribution of adaptation, but we plan to further characterize the heterozygous diploids (Table 1), with the hope that future analyses will better address these concerns. For example, we are intrigued about the possibility that loss of heterozygosity, which can result in the rapid fixation of recessive mutations (Gerstein *et al.* 2014), provides a means to generate a homozygous incompatible genotype in the absence of sporulation.

How was the *MLH1-PMS1* incompatible in YJM523 generated?

YJM523 is a clinical isolate from Stanford University hospital; it appears to have an unmapped mutator suppressor(s) elsewhere in its genome because *MLH1-PMS1* combinations from this isolate conferred a higher mutation rate in the S288c background compared to its native backgrounds (compare data in Table 4 and Table 5). How it acquired extragenic suppressors remains unclear. The SNP polymorphism data presented in Figure 4 appear consistent with YJM523 and YJM320 being created through a single mating between S288c and SK1 group strains followed by sporulation and

Figure 6 Mutator phenotype of 42 spore clones derived from a YJM555 × EAY3235 cross. A total of 42 *lys2-A₁₄* spore clones were tested for reversion to Lys⁺ in a colony patch assay (*Materials and Methods*). The median number of colonies from each spore clone assay is shown, from lowest to highest. Assays performed on compatible (EAY3234) and incompatible (EAY3235) control strains are shown in red.

the segregation of markers. This model is consistent with the presence of *PMS1* SNP variants that are identical in YJM523 (incompatible) and YJM320 (compatible) (Figure 4). Furthermore, there are four *PMS1* SNPs (Figure 4B) and two *PMS1* amino acid polymorphisms (V392 and V564) (Table 3) in these two isolates that are not found in 1007 of the 1010 yeast isolates [they were found in the closely related strain YJM554 (Strope *et al.* 2015)], and YJM320 is on the same branch as YJM555 (Figure 4B; Strope *et al.* 2015). Taken together, this pattern suggests that SK1 and S288c group isolates mated to yield compatible and incompatible *MLH1*–*PMS1* genotypes. This suggests that YJM523 experienced an elevated mutation rate, presumably providing an adaptive advantage to a stress environment, which was subsequently suppressed by an extragenic suppressor(s) (Table 4 and Table 5). However, we cannot exclude the possibility that YJM523 never encountered situations where it had entered a mutator state in response to a changing environment and was instead created in a background where the initial mutation rate was low and could tolerate an incompatible genotype. Unfortunately, the data are not available to further test this idea. We do not favor such a hypothesis based on the complexity of the suppression phenotype seen in YJM555 (Figure 6), and previous work showing that genomic mutation rates tend to vary by less than an order of magnitude in organisms such as *E. coli*, *Neurospora crassa*, and *S. cerevisiae* (Drake *et al.* 1998; Zeyl and DeVisser 2001), suggesting that deviations from a typical rate would be selected against.

Development of a reporter construct to sensitively detect mutation rates in natural yeast isolates

The *ARS*–*CEN* *KanMX::insE-A₁₀₋₁₄* reporter constructs described in the paper provides a new approach to measure mutation rates in a large number of isolates that lack markers that are typically used for genetic manipulation and whose chromosome content can vary with respect to ploidy and chromosome copy number (Storchova 2014; Strope *et al.* 2015). In these constructs, the *NatMX* antibiotic resistance marker is used to select for the plasmid, and the *KanMX* marker is used to identify frameshift reversion events (Figure 5). The pEAA613 construct displayed a 2000-fold difference in mutation rate between wild type and MMR null. This difference is only fivefold lower than that seen using an integrated *lys2-A₁₄* reporter, which to our knowledge is the most sensitive assay developed to measure differences in frameshift reversion frequency between wild type and MMR mutants (Table 5; Tran *et al.* 1997). Importantly, our reporter plasmid assay was capable of distinguishing between mutation rates in wild-type and MMR incompatible strains. In the *lys2-A₁₄* assay, this difference was 75–100-fold in a 10,000-fold range; in the *KanMX::insE-A₁₀₋₁₄* assay it was 10-fold in a 2000-fold range (Table 4 and Table 5). We believe that this sensitive plasmid-based assay, in conjunction with whole-genome sequencing data and classical mutation accumulation analyses (Nishant *et al.* 2009, 2010; Lujan *et al.* 2014), will allow groups to accurately measure variation in mutation rate in natural isolates and determine whether this rate varies under dif-

ferent environmental conditions. Furthermore, one can modify the sequences present in the *insE* part of the reporter to examine reversion to genecitin resistance due to base substitution and other types of frameshift events. pEAA613 and derivatives can also be easily modified to integrate the *KanMX::insE-A₁₀₋₁₄* reporters at a specific chromosomal location.

Acknowledgments

We thank Claudia Caradec, members of the Alani lab, and Jae Young Choi for helpful comments and technical advice; John McCusker for advice and for providing us with the YJM521 and YJM523 natural isolates; and Helen Murphy for sharing unpublished data. J.S. is a member of the Institut Universitaire de France. D.T.B., N.A.-S., and E.A. were supported by National Institutes of Health (NIH) grant GM53085. C.F.A. was supported by NIH grant GM095793. The 1002 Yeast Genomes Project (J.S., G.L., and A.F.) was funded by France Génomique (ANR-10-INBS-09-08). D.T.B. was a fellow of the Vietnam Education Foundation. N.A.-S. is supported by a scholarship from the Saudi Arabian Cultural Mission. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Literature Cited

- Argueso, J. L., A. W. Kijas, S. Sarin, J. Heck, M. Waase *et al.*, 2003 Systematic mutagenesis of the *Saccharomyces cerevisiae* *MLH1* gene reveals distinct roles for Mlh1p in meiotic crossing over and in vegetative and meiotic mismatch repair. *Mol. Cell. Biol.* 23: 873–886.
- Boe, L., M. Danielsen, S. Knudsen, J. B. Petersen, J. Maymann *et al.*, 2000 The frequency of mutators in populations of *Escherichia coli*. *Mutat. Res.* 448: 47–55.
- Bui, D. T., E. Dine, J. B. Anderson, C. F. Aquadro, and E. E. Alani, 2015 A genetic incompatibility accelerates adaptation in yeast. *PLoS Genet.* 11: e1005407.
- Chao, L., and E. C. Cox, 1983 Competition between high and low mutating strains of *Escherichia coli*. *Evolution* 37: 125–134.
- Demogines, A., E. Smith, L. Kruglyak, and E. Alani, 2008a Identification and dissection of a complex DNA repair sensitivity phenotype in Baker's yeast. *PLoS Genet.* 4: e1000123.
- Demogines, A., A. Wong, C. Aquadro, and E. Alani, 2008b Incompatibilities involving yeast mismatch repair genes: a role for genetic modifiers and implications for disease penetrance and variation in genomic mutation rates. *PLoS Genet.* 4: e1000103.
- Denamur, E., G. Lecointre, P. Darlu, O. Tenaillon, C. Acquaviva *et al.*, 2000 Evolutionary implications of the frequent horizontal transfer of mismatch repair genes. *Cell* 103: 711–721.
- Dixon, W. J., and F. J. Massey, 1969 *Introduction to Statistical Analysis*. McGraw-Hill, New York.
- Dobzhansky, T., 1936 Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics* 21: 113–135.
- Drake, J. W., B. Charlesworth, D. Charlesworth, and J. F. Crow, 1998 Rates of spontaneous mutation. *Genetics* 148: 1667–1686.
- Eyre-Walker, A., and P. D. Keightley, 2007 The distribution of fitness effects of new mutations. *Nat. Rev. Genet.* 8: 610–618.
- Gerstein, A. C., A. Kuzmin, and S. P. Otto, 2014 Loss-of-heterozygosity facilitates passage through Haldanes sieve for *Saccharomyces cerevisiae* undergoing adaptation. *Nat. Commun.* 5: 3819.

- Gietz, R. D., and R. H. Schiestl, 2007 Large-scale high-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nat. Protoc.* 2: 38–41.
- Giraud, A., I. Matic, O. Tenaillon, A. Clara, M. Radman *et al.*, 2001 Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. *Science* 291: 2606–2608.
- Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15: 1541–1553.
- Hartl, D., and A. Clark, 2007 *Principles of Population Genetics*, Ed. 4. Sinauer Associates, Sunderland, MA.
- Heck, J. A., J. L. Argueso, Z. Gemici, R. G. Reeves, A. Bernard *et al.*, 2006 Negative epistasis between natural variants of the *Saccharomyces cerevisiae* *MLH1* and *PMS1* genes results in a defect in mismatch repair. *Proc. Natl. Acad. Sci. USA* 103: 3256–3261.
- Ho, S. N., H. D. Hunt, R. M. Horton, J. K. Pullen, and L. R. Pease, 1989 Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* 77: 51–59.
- Hoffman, C. S., and F. Winston, 1987 A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* 57: 267–272.
- Holm, C., D. W. Meeks-Wagner, W. L. Fangman, and D. Botstein, 1986 A rapid, efficient method for isolating DNA from yeast. *Gene* 42: 169–173.
- Kimura, M., 1967 On the evolutionary adjustment of spontaneous mutation rates. *Genet. Res.* 9: 23–34.
- LeClerc, J. W., B. Li, W. L. Payne, and T. A. Cebula, 1996 High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* 274: 1208–1211.
- Liti, G., and E. J. Louis, 2005 Yeast evolution and comparative genomics. *Annu. Rev. Microbiol.* 59: 135–153.
- Liti, G., D. M. Carter, A. M. Moses, J. Warringer, L. Parts *et al.*, 2009 Population genomics of domestic and wild yeasts. *Nature* 458: 337–341.
- Lujan, S. A., A. R. Clausen, A. B. Clark, H. K. MacAlpine, D. M. MacAlpine *et al.*, 2014 Heterogeneous polymerase fidelity and mismatch repair bias genome variation and composition. *Genome Res.* 24: 1751–1764.
- Magwene, P. M., Ö. Kayıkçı, J. A. Graneek, J. M. Reininga, Z. Scholl *et al.*, 2011 Outcrossing, mitotic recombination, and life-history trade-offs shape genome evolution in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 108: 1987–1992.
- Mann, H. B., and D. R. Whitney, 1947 On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* 18: 50–60.
- Marsit, S., and S. Dequin, 2015 Diversity and adaptive evolution of *Saccharomyces wine* yeast: a review. *FEMS Yeast Res.* 15: fov067.
- Muller, H. J., 1939 Reversibility in evolution considered from the standpoint of genetics. *Biol. Rev. Camb. Philos. Soc.* 14: 261–280.
- Muller, H. J., and G. Pontecorvo, 1940 Recombinants between *Drosophila* species the F1 hybrids of which are sterile. *Nature* 146: 199–200.
- Murphy, H. A., and C. W. Zeyl, 2010 Yeast sex: surprisingly high rates of outcrossing between asci. *PLoS One* 5: e10461.
- Nishant, K. T., N. D. Singh, and E. Alani, 2009 Genomic mutation rates: what high-throughput methods can tell us. *BioEssays* 31: 912–920.
- Nishant, K. T., W. Wei, E. Mancera, J. L. Argueso, A. Schlattl *et al.*, 2010 The baker's yeast diploid genome is remarkably stable in vegetative growth and meiosis. *PLoS Genet.* 6: e1001109.
- Orr, H. A., 1995 The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics* 139: 1805–1813.
- Raynes, Y., M. R. Gazzara, and P. D. Sniegowski, 2012 Contrasting dynamics of a mutator allele in asexual populations of differing size. *Evolution* 66: 2329–2334.
- Reuter, M., G. Bell, and D. Greig, 2007 Increased outbreeding in yeast in response to dispersal by an insect vector. *Curr. Biol.* 17: R81–R83.
- Rose, M. D., F. Winston, and P. Hieter, 1990 *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Ruderfer, D. M., S. C. Pratt, H. S. Seidel, and L. Kruglyak, 2006 Population genomic analysis of outcrossing and recombination in yeast. *Nat. Genet.* 38: 1077–1081.
- Schacherer, J., J. A. Shapiro, D. M. Ruderfer, and L. Kruglyak, 2009 Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. *Nature* 458: 342–345.
- Skelly, D. A., P. M. Magwene, B. Meeks, and H. A. Murphy, 2016 The effect of known mutator alleles in a cluster of clinical *Saccharomyces cerevisiae* strains. *bioRxiv* <https://doi.org/10.1101/090498>.
- Steinmetz, L. M., H. Sinha, D. R. Richards, J. I. Spiegelman, P. J. Oefner *et al.*, 2002 Dissecting the architecture of a quantitative trait locus in yeast. *Nature* 416: 326–330.
- Storchova, Z., 2014 Ploidy changes and genome stability in yeast. *Yeast* 31: 421–430.
- Strope, P. K., D. A. Skelly, S. G. Kozmin, G. Mahadevan, E. A. Stone *et al.*, 2015 The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. *Genome Res.* 25: 762–774.
- Taddei, F., M. Radman, J. Maynard-Smith, B. Toupance, P. H. Gouyon *et al.*, 1997 Role of mutator alleles in adaptive evolution. *Nature* 387: 700–702.
- Tanaka, M. M., C. T. Bergstrom, and B. R. Levin, 2003 The evolution of mutator genes in bacterial populations: the roles of environmental change and timing. *Genetics* 164: 843–854.
- Thompson, D. A., M. M. Desai, and A. W. Murray, 2006 Ploidy controls the success of mutators and nature of mutations during budding yeast evolution. *Curr. Biol.* 16: 1581–1590.
- Townsend, J. P., K. M. Nielsen, D. S. Fisher, and D. L. Hartl, 2003 Horizontal acquisition of divergent chromosomal DNA in bacteria: effects of mutator phenotypes. *Genetics* 164: 13–21.
- Tran, H. T., J. D. Keen, M. Krickler, M. A. Resnick, and D. A. Gordin, 1997 Hypermutability of homonucleotide runs in mismatch repair and DNA polymerase proofreading yeast mutants. *Mol. Cell. Biol.* 17: 2859–2865.
- Wielgoss, S., J. E. Barrick, O. Tenaillon, M. J. Wiser, W. J. Dittmar *et al.*, 2013 Mutation rate dynamics in a bacterial population reflect tension between adaptation and genetic load. *Proc. Natl. Acad. Sci. USA* 110: 222–227.
- Wilcoxon, F., 1945 Individual comparisons by ranking methods. *Biom. Bull.* 1: 80–83.
- Winston, F., C. Dollard, and S. L. Ricupero-Hovasse, 1995 Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* 11: 53–55.
- Zeyl, C., and J. A. DeVisser, 2001 Estimates of the rate and distribution of fitness effects of spontaneous mutation in *Saccharomyces cerevisiae*. *Genetics* 157: 53–61.

Communicating editor: J. A. Nickoloff

GENETICS

Supporting Information

www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.199513/-/DC1

Mismatch Repair Incompatibilities in Diverse Yeast Populations

Duyen T. Bui, Anne Friedrich, Najla Al-Sweel, Gianni Liti, Joseph Schacherer, Charles F. Aquadro and Eric Alani