Interspecific Hybrids Show a Reduced Adaptive Potential Under DNA Damaging Conditions

AUTHOR INFORMATION:

Carla Bautista[1,2,3,5], Souhir Marsit [1,2,3,5], Christian R Landry [1,2,3,4,5]

1. Regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines (PROTEO), Québec G1V 0A6, QC, Canada
2. Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada
3. Centre de Recherche en Données Massives de l'Université Laval, Université Laval, Québec, QC, Canada
4. Département de biochimie, de microbiologie et de bio-informatique, Université Laval, G1V 0A6 Québec, QC, Canada
5. Département de biologie, Université Laval, G1V 0A6 Québec, QC, Canada

Correspondence:

1. Corresponding Author: Carla Bautista (c.bautistarourjc@gmail.com) and Christian R Landry (Christian.landry@bio.ulaval.ca)
2. Other e-mail addresses: Souhir Marsit: (souhir.marsit@gmail.com)

SHORT RUNNING TITLE: Hybridization constraints adaptation under UV radiation

KEYWORDS: adaptation, experimental evolution, extreme conditions, selection, hybridization, polyploidy, yeast

AUTORSHIP: CB and CRL designed the research. CB performed the experiments, collected and analyzed the data with the assistance of SM and CRL. CB wrote the manuscript with the assistance of CRL. SM, and CRL edited the manuscript. CRL was responsible for funding acquisition. All authors read and approved the final manuscript.

ABSTRACT

Hybridization may increase the probability of adaptation to extreme ecological stresses. This advantage could be caused by an increased genome plasticity in hybrids, which could accelerate the search for adaptive mutations. Here we test if hybridization accelerates adaptive evolution in response to DNA damage using yeast as a model. We exposed 180 populations of hybrids between species (*Saccharomyces cerevisiae* and *Saccharomyces paradoxus*) and their parental strains to UV mimetic and control conditions for 200 generations. Although we found that adaptation occurs in both hybrids and parents, hybrids achieved a lower rate of adaptation. Adaptation to DNA damaging conditions comes with a large and similar cost for parents and hybrids, suggesting that this cost is not responsible for the lower adaptability of hybrids. We suggest that the lower adaptive potential of hybrids under UV mimetic conditions may result from the interaction between DNA damage and the inherent genetic instability of hybrids.

INTRODUCTION

Heterogeneous environments constantly challenge organisms by changing which phenotypes are optimal. Understanding which mechanisms accelerate or slow down adaptation to environmental heterogeneity is a central question in evolutionary biology (Bleuven & Landry 2016). If changes are rapid and drastic, unless they move to another environment, populations may collapse before adapting. These movements often impact geographical distributions, which can promote encounters between incipient species, enhancing the probability of hybridization and introgression (Gómez *et al.* 2015).

Although hybridization can have many negative impacts on the long-term genetic integrity of some taxa (Lowe *et al.* 2015; Todesco *et al.* 2016), it can also create favorable conditions for rapid adaptive evolution. The adaptive role of hybridization has been shown in the colonization of new niches (Lewontin & Birch 1966; Heil *et al.* 2017; Herbst *et al.* 2017), during speciation (Schumer *et al.* 2014), and during adaptive radiation (Ballerini 2012). Prime examples include Darwin finches, which benefited from hybridization during adaptation to the adverse climatic conditions (drought, flooding, etc.) caused by the exceptionally severe El Niño events (Grant & Grant 1996). In such cases, hybridization may facilitate adaptation through genomic admixture and its associated increase in phenotypic diversity, which could be particularly critical in extreme environments (Grant & Grant 1996; Lexer *et al.* 2003; Mallet 2005; Martin *et al.* 2006; Heil *et al.* 2017). In addition, hybrids often show phenotypes outside of the range observed in parental species because of transgressive segregation or heterosis and these phenotypes may be adaptive (Vega & Frey 1980; Rieseberg *et al.* 1999; Nolte & Sheets 2005; Landry *et al.* 2007).

Another important feature that may influence the evolution of hybrids is their inherent genomic instability. Certain models and experiments show that increase in mutation rates may accelerate adaptation (Taddei *et al.* 1997; Schaaff *et al.* 2002; Labat *et al.* 2005; Le Bars *et al.* 2014; Bui *et al.* 2015; Couce *et al.* 2017). Genomic instability includes higher rate of DNA damage (Wang *et al.* 2014; Herbst *et al.* 2017), chromosomal rearrangements (Baack & Rieseberg 2007; Mwathi *et al.* 2019), gene and chromosome copy number variation (Morales & Dujon 2012; Dion-Côté & Barbash 2017) and the multiplication of repetitive and transposable elements (Fontdevila 2005; Guerreiro 2014). These changes often lead to aneuploidy, which has also been shown to be a mechanism of adaptation in stressful conditions (Mulla *et al.* 2014; Sunshine *et al.* 2015) and hybrids may be particularly prone to producing aneuploid progeny (Gilchrist & Stelkens 2019). Another consequence of genomic instability is changes in ploidy (Mulla *et al.* 2014). Hybridization followed by whole genome duplication giving rise to polyploid hybrids has been observed in plants, animals and fungi (Alves *et al.* 2001; Soltis & Soltis 2009; Xiao *et al.* 2011; Marcet-Houben & Gabaldón 2015; Session *et al.* 2016; Charron *et al.* 2019). Because polyploidy itself could increase the rate of beneficial mutations (Selmecki *et al.* 2015), hybrids that polyploidize could have access to this feature as well. Indeed, hybridization followed by polyploidization has been directly related with adaptive diversification in plants (Matsuoka 2011; Alix *et al.* 2017) and in fish (Nolte *et al.* 2005; Saitoh *et al.* 2010).

However, there are likely limits to the adaptive potential of hybrids caused by their enhanced genomic instability. For instance, genomic changes of large effects, such as changes in ploidy, lead to hybrid inviability (Burton & Husband 2000). Similarly, high rates of genomic changes could lead to an increased acquisition rate of deleterious mutations (Bashir *et al.* 2014; Xie *et al.* 2016). Recently, Sprouffske et al. (2018) showed how *Escherichia coli* strains with a 100-fold increase in the mutation rate experienced a reduction in adaptation in many environments. In addition, the type of mutations that contribute to adaptation in hybrids may be particularly deleterious in other environments, leading to strong trade-offs. This is the case for aneuploid yeast strains, which typically show a large

condition-specific response due to pleiotropic effects (Sunshine *et al.* 2015). Finally, the accelerated rate of evolution could lead to the rapid accumulation of conditionally neutral mutations (Cooper 2014; Kawecki & Ebert 2018), leading to fitness trade-offs in other environments and thus limiting the long-term potential of hybrids.

The *Saccharomyces* yeast species have been widely used in experimental research due to their dual sexual and asexual mode of reproduction, their short doubling time and their deep genetic characterization (Landry *et al.* 2006; Replansky *et al.* 2008; Marsit *et al.* 2017). They have been used to study adaptation to high salinity (Posas *et al.* 2000; Dhar *et al.* 2011), to extreme temperatures (Salvadó *et al.* 2011), to dehydration (Khroustalyova *et al.* 2019), to heavy metals (Adamo *et al.* 2012; Herrera *et al.* 2014; Gorter *et al.* 2017) and to UV radiation (Lawrence and Christensen 1976). Despite the presence of interspecific reproductive barriers, introgression among yeast species has been observed in nature (Barbosa *et al.* 2016; Leducq *et al.* 2016) and in industrial conditions (Gibson & Liti 2015; Lopandic 2018). Evidence for the superiority of hybrids in response to extreme stress also comes from experimental evolution. For instance, Stelkens *et al*. (2014) showed that yeast hybrids are more likely to be evolutionarily rescued than parental species in stressful environments. Overall, these properties make yeast an optimal evolutionary model to examine the role of hybridization in the adaptability to stressful conditions.

A prominent environmental stressor is UV radiation (Björn 1996; Rozema *et al.* 1997), which has been intensified with human-induced environmental change due to, among other factors, the depletion of the ozone layer (Caldwell & Flint 1994; Van der Leun & de Gruijl 2002; Häder *et al.* 2011). Radiation represents a particular challenge in terms of adaptability by affecting DNA integrity and by increasing mutation rates (Hanway *et al.* 2002; Torres *et al.* 2013). Here we examine the rate of adaptation of two yeast species, *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*, and their hybrids, in the face of an environmental stressor, a DNA damaging agent mimicking the effects of UV radiation. These two species diverge by about 15% at the nucleotide level (Cliften *et al.* 2001) and naturally hybridize in the wild (Barbosa *et al.* 2016; Peter *et al.* 2018), although the F1 diploid hybrid is almost completely sterile (Murphy *et al.* 2006). We hypothesize that, because of the evolutionary potential of hybridization brought by genomic instability, hybrids will adapt faster than parental species. On the other hand, DNA damage may enhance genetic instability to a point where it prevents adaptation of the hybrid populations. To test these hypotheses, we experimentally evolved 180 populations of diploid hybrids and diploid parental strains through 200 generations in UV-mimicking conditions and matching control conditions.

MATERIALS AND METHODS

Yeast Strains and Media

The *S. cerevisiae* and *S. paradoxus* strains used in this study derive from natural strains LL13_054 and MSH-604 that were isolated in natural forests in North America. The *HO* locus was replaced by homologous recombination with different resistance cassettes to prevent mating type switching in haploids (Hygromycin B and Nourseothricin) as described in Güldener et al. (1996). The haploid yeast strains used (described in Table S1) were LL13_054 MATa HO::Hygromycin B and LL13_054 MATα HO::Nourseothricin for *S. cerevisiae* and MSH-604 MATα HO::Nourseothricin and MSH-604 MATa HO::Hygromycin B for *S. paradoxus* (Leducq *et al.* 2016; Charron *et al.* 2019). As a neutral growth condition, cells were grown in YPD (1% yeast extract, Fisher BioReagents™, USA; 2% tryptone, BioShop®, Canada; and 2% D-glucose, BioShop®, Canada). The change in the batch of the yeast

extract (Fisher BioReagents™, USA) used during experimental evolution may be responsible for the slight decrease in the growth rate we notified in control conditions after 200 generations.

Experimental Crosses

All incubation steps were performed at 25ºC. Haploid strains were grown overnight in 5 mL of YPD. Pre-cultures were diluted to OD_{595} of 1.0 in 500 µL aliquots. The aliquots from pairs of strains to be crossed were mixed in a tube and 5 µL were used to inoculate 200 µL of YPD medium in 30 replicates. Therefore, all starting diploid populations derive from independent mating events. Mixed haploid strains were incubated for 6 hours after which 5 µL of the mating cultures were spotted on a double selection YPD solid medium (100 µg/mL of Nourseothricin and 250 µg/mL of Hygromycin B). From each of the 30 spots per genotype, 1 colony was picked as a founder population for the evolution experiment, resulting in 30 independent lines for each of the 3 genotypes (parental species 1: *S. cerevisiae,* parental species 2: *S. paradoxus,* and their hybrids).

UV Mimetic Tolerance Assays

In order to reproduce UV radiation conditions, we used 4-Nitroquinoline 1-oxide (4-NQO) (Sigma–Aldrich, cat. no. N8141, batch #WXBC3635V) a UV mimetic molecule (Felkner & Kadlubar 1968; Jung *et al.* 2004). 4-NQO targets DNA by forming bulky adducts, which are formed by covalently attached bases, disrupting base-pairing and altering DNA structure (Malkin & Zahalsky 1966). 4-NQO was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 400 mM in individual aliquots that were stored at −20°C. To estimate cell tolerance before experimental evolution, 20 random lines of our three genotypes were inoculated in liquid cultures with gradually increasing 4-NQO concentrations (YPD + 4 µM, 8 µM, 16 µM, 24 µM, 32 µM and 40 µM of 4-NQO) and growth was monitored for 24 h.

Experimental Evolution

30 parallel lines for each genotype were pre-cultured in 1 mL of YPD liquid cultures in 96 deep-well plates (2 mL) and incubated for 24 h at 25°C. Subsequently, 20 µL of these 90 parallel lines (Fig. S1) were transferred in 96-well flat-bottomed culture plates with 180 µL of media (YPD or YPD + 4-NQO), resulting in an initial OD_{595} of approximately 0.1. The borders of the plates did not contain strains and were filled with sterile media to avoid border effects related to evaporation. Overall, a total of 180 cultures were maintained in parallel, 30 replicates for each of the 3 genotypes in YPD and 30 replicates for each of the 3 genotypes in YPD + 4-NQO (Fig. S1). Every 24 h, each culture was diluted 10-fold by transferring 20 µL of grown culture into 180 µL of fresh culture medium to initiate a new round of growth at an OD_{595} of approximately 0.1. A total of 21 such transfer cycles were carried out for a total of approximately 200 generations (each transfer cycle involved approximately ~ 10 generations). Incubation at 25ºC was performed directly in 3 temperature-controlled spectrophotometers (Infinite® 200 PRO, Tecan, Reading, UK) that read the OD_{595} at intervals of 15 minutes. Archives were maintained for every transfer cycle by mixing 80 µL of the evolved lines with 170 µL of 80% glycerol in 96 well plates and stored in a −80°C freezer.

Fitness Assays at the End of the Experimental Evolution

Ancestor strains (n = 90) as well as the lines evolved for 200 generations in YPD (n = 90) and in YPD+4-NQO (n = 90) were thawed from glycerol stocks on solid YPD omnitray plates (25ºC, 72 h). They were pre-cultured in 1 mL of YPD liquid cultures in 96 deep-well plates (2 ml) and incubated for 24 h at 25°C. Subsequently, 20 µL of these pre-cultures were grown in 96-well flat-bottomed culture plates in 180 µL of media (YPD or YPD + 4 µM, 8 µM, 16 µM, 24 µM, 32 µM and 40 µM of 4-NQO), resulting in an initial OD_{595} of approximately 0.1. Incubation at 25ºC was performed directly in 3 temperature-controlled spectrophotometers (Infinite® 200 PRO, Tecan, Reading, UK) that read the OD_{595} at intervals of 15 minutes.

Flow Cytometry Analysis of DNA Content

DNA content was measured by flow cytometry using the SYTOX™ green staining assay (Thermo Fisher, Waltham, USA) as in Charron *et al.* (2019). The strain *S. cerevisiae* (LL13_054) in both haploid and diploid states were used as controls. Cells were first thawed from glycerol stocks on solid YPD omnitray plates (25ºC, 72 h). They were inoculated in 1 mL of YPD liquid cultures in 96 deep-well plates (2 mL) and incubated for 24 h at 25°C. Cells were subsequently prepared as in Gerstein *et al.* (2006). They were fixed in 70% ethanol for at least 1 h at 25ºC. RNA was eliminated using 0.25 mg/ml of RNAse A overnight at 37 °C. Cells were washed twice with sodium citrate (50 mM, pH 7) and stained with a SYTOX™ green concentration of 0.6 µM for 1 h at 25ºC in the dark. The volume of cells was adjusted in sodium citrate (50 mM, pH 7) to be around a cell concentration of 500 cells/µL. Five thousand cells for each sample were analyzed on a Guava® easyCyte 8HT flow cytometer using 96-well microplates. Cells were excited with the blue laser at 488 nm and fluorescence was measured with a green fluorescence detection channel (peak at 512 nm). The distributions of the green fluorescence values were processed to find the two main density peaks, which correspond to the two cell populations, respectively in G1 and G2 phases.

Statistical Analysis

We used a series of linear mixed effect models (LMMs, lme package; (Bates *et al.* 2015)) to test for differences in growth rates of the growth curves (with normal distribution fit) between groups. The base model (lme (growth ~ genotype*time*treatment, random =1) contained genotype (Parent 1, Parent 2 and Hybrid), time (number of generations), treatment (UV or control), and their interaction as fixed effects. Paired t-tests were also used for paired data when comparing the same lines at different times (generation 0 and generation 200) or the same lines evolved in different media (control or 4-NQO). Kruskal-Wallis and Wilcoxon non-parametric tests were also used to test for the effect of ploidy. Multiple pairwise comparisons were used to test for differences between groups. For hypothesis testing, we always considered as significant a P-value <0.05. Growth of each experimental line was measured as the growth rate (r), the maximum slope of the growth curve fitted using the "Growthcurver" package (Sprouffske & Wagner 2016) in R. Similarly, we calculated the carrying capacity (K), which represents the maximum population size a particular environment can support, for further correlation analysis to test its relationship with growth rate (r). The data was analyzed using R version 3.4.1. The code is available at https://github.com/Landrylab/Hybridization_and_stress.

RESULTS

Experimental Evolution

We tested whether yeast hybrids would adapt faster to DNA stress conditions than parental species. We carried out an experiment with 90 independent populations of two parental species (*S. cerevisiae* and *S. paradoxus*) and their hybrid. These populations were grown for 200 generations in rich media (YPD) and in the presence of a UV radiation mimetic chemical, 4-nitroquinoline-1-oxide (4NQO) (Fig. 1A). In parallel, we evolved the same 90 starting strains for 200 generations in control conditions (YPD only) (Fig. 1A). A total of 180 populations were evolved. We will refer to the *S. cerevisiae*, *S. paradoxus* and hybrid crosses as the three genotypes.

In order to determine the proper UV mimetic chemical concentration to use, we first performed dose–response experiments across concentrations ranging from 0 to 40 µM of UV mimetic chemical in 20 randomly chosen populations. Growth was inhibited with increasing concentrations (Fig. S2A). We observed a steady reduction of growth rates from about 20% at 4 µM to nearly 80% at 40 µM across the three genotypes (Fig. S2B). We first tested 16 µM as concentration for experimental

evolution but populations failed to grow in the second round of serial dilution (Fig. S3B). We selected 4 µM as concentration, which led to approximately 25-30% growth rate diminution for the three genotypes (Fig. 1B). In general, *S. cerevisiae* was slightly less sensitive than the hybrid, which was less sensitive than *S. paradoxus* (Fig. S2B*)*.

By comparing the maximum growth rate (r) in UV mimetic condition through time, we found that all 3 genotypes experienced a significant increase in growth rate (Fig. 2A) and have thus adapted. The UV mimetic adapted lines reached on average 76%, 64% and 41% of the initial growth rate in control conditions for *S. cerevisiae*, *S. paradoxus* and the hybrids respectively (percentages calculated using T30 in Fig. 2B). We found a slight decrease in growth rate in control conditions when comparing data gathered during experimental evolution (Fig. S4A). However, we find that this was caused by a slight change in experimental conditions during the experiment (see methods) since these same strains grown in YPD once the experimental evolution ended show no significant differences (Fig. S4B). This change in media was previously remarked by Charron *et al.* (2019) in our laboratory. We measured the growth rate of the evolved strains from T200 across the gradient of UV mimetic concentrations tested above and found that fitness of evolved populations increased compared to the ancestral ones (Fig. S5). This result shows that there was a general decrease in sensitivity to the UV mimetic chemical and thus that adaptation is not specific to the concentration used for experimental evolution but extends to other concentrations as well.

We quantified the rate of adaptation as the increase of growth rate through time (Fig. 2B). We first examined whether the pattern of increase better fitted a quadratic model, as observed in other studies (de Visser & Lenski 2002). We found that the linear model, which explained 81% of the variability observed in growth rates (Table S2), was a better model than the quadratic one (Table S3). We observed a significant effect of the interaction between condition (UV mimetic versus control) and time (linear model, $p < 2e\text{-}16$) as revealed by a slope that is different from 0 in UV mimetic conditions (Fig. 2B). The slope in control conditions (Fig. 2B) was not different from 0 (m = -1.38e-04, m = -2.06e-04, m = -7.23e-05 respectively for *S. cerevisiae*, *S. paradoxus* and the hybrid) (Fig. S4). Growth rate did not systematically increase at the beginning of the experiment. This is possibly caused by strains having a strong sensitivity and a lack of physiological acclimatization in the first generations, which made the final OD before transfer more variable due to a delay in reaching stationary phase. We also observed more variability among replicates of the hybrid and *S. paradoxus* genotypes in UV mimetic conditions at T200 (Fig. 2A).

The comparison of fitness gains between the final and initial time points (Fig. 2C) shows that the extent of adaptation in the hybrid was lower than in the two parental genotypes (pairwise comparisons, $p = 6.3e\text{-}06$ and $p = 1.8e\text{-}11$, respectively for *S. cerevisiae* and *S. paradoxus*), which rejects our hypothesis that hybrids would show a greater rate of adaptation. We noticed the same result when observing the slopes that reflect the rate of adaptation through time. The hybrid showed a slope (m = 0.0007) in UV mimetic conditions that was significantly shallower (Fig. 2B, Table S2) than the parental species *S. cerevisiae* (m = 0.0016) and the parental species *S. paradoxus* (m = 0.0013) (linear model, $p < 2.59e\text{-}15$ and $p < 5.53e\text{-}09$ respectively). This result is also seen when we exposed the 3 genotypes to a gradient of concentration of the UV mimetic chemical in which the increased tolerance is less pronounced in the hybrid at high doses (Fig. S5).

Finally, we analyzed the correlation between the two parameters that characterize density-dependent population growth: the maximum growth rate per individual (r), as used above, and the carrying capacity (K), which is the optical density in saturated cultures. It was recently shown that the *r–K* correlation is negative in optimal conditions and positive in stressful conditions (Wei & Zhang 2019). Accordingly, we hypothesized that the r-K correlation would be positive in UV mimetic conditions, and

that its value would decrease as the strains adapted because the extent of stress would decrease. We found that the values of the *r–K* correlations are negative in control conditions and positive in UV mimetic conditions (Fig. 2D). The correlation in UV mimetic conditions increase slightly in the hybrid while it remained stable (*S. cerevisiae*) or decreased (*S. paradoxus*) in parental species (Fig. 2D). This supports that adaptation was less pronounced in the hybrid compared to the parents, although the differences are subtle.

Cost of Adaptation

We tested whether adaptation to UV mimetic conditions would lead to a fitness trade-off in other conditions, and if this trade-off was stronger for hybrids compared to parents. Such a trade-off could come from the cost of adapting to UV mimetic conditions, or to the accumulation of mutations that are neutral in the stress conditions but would be deleterious in the control conditions, both of which would be visible in control conditions. To test this, we measured the growth rate of all the strains in control conditions (YPD). As shown above, we observed no growth rate improvement in control conditions for the strains evolved in control conditions compared to the ancestors (Fig. 3A) (Paired t-tests, $p = 0.66$, $p = 0.68$, $p = 0.089$, respectively for *S. cerevisiae*, *S. paradoxus* and hybrid). However, strains evolved in UV mimetic conditions grew slower than both ancestors and strains evolved in control conditions (Fig. 3A) when grown in control conditions. Therefore, all 3 genotypes showed this fitness trade-off once adapted to UV mimetic conditions. Hybrid genotypes did not show stronger trade-off than the parental genotypes (Fig. 3B). Overall, we observed a correlation between the extent of the trade-off and the extent of adaptation (Fig. 3C : Spearman's rank correlation coefficient $r = 0.33$, $p = 0.0016$). There were no significant differences among genotypes in the trade-off/adaptation ratio, supporting the observation that adaptation in hybrids is not more costly than in the parents. Some strains showed negative trade-off/adaptation ratios (Fig. 3D) and those come either from negative trade-off or null or slight loss of fitness (no adaptation, values below 0). This is caused by the fact that 12.22% of the strains evolved in the UV mimetic conditions have lower growth rates at the end of the experiment than their ancestors. Almost all of these (10/11) values belong to hybrids, consistent with their lower rate of adaptation.

Effect of Ploidy Variation on Adaptation

Recent studies showed that hybrids between *S. cerevisiae* and *S. paradoxus* (Charron *et al.* 2019) and other yeast hybrids (Morard *et al.* 2020) can change ploidy. Because ploidy can affect the rate of adaptation (Selmecki *et al.* 2015), especially in stressful environments (Schoenfelder & Fox 2015), we examined whether such changes could explain some of the differences we detected among genotypes. We found that the three genotypes contained a few founding triploid populations (Fig. S6). However, hybrids did not show more changes in ploidy than parental species (Fig. S6). We nevertheless reexamined the results observed above and found that hybrids still adapted slower if we considered only ancestrally diploid strains. Mulla et al. (2014) reported that some aneuploid *S. cerevisiae* showed improved fitness compared to parental wild-type strains under 4-NQO conditions. Therefore, we also examined if exposure to UV mimetic conditions could itself favor the increase in frequency of polyploid strains during the experiment. We found few changes in ploidy (from diploids to triploids) after 200 generations in UV mimetic conditions (Fig. S6) but they were not more frequent than among strains evolved in control conditions, suggesting that changes in ploidy are not particularly favored in UV mimetic conditions. Finally, we examined if changes in ploidy impacted fitness in UV mimetic conditions. We compared the growth rate of the different ploidies. We found no significant differences among groups (Fig. 4). Overall, these analyses indicate that ploidy does not have a significant effect on adaptation to UV mimetic conditions nor do they explain the lower evolutionary potential of the hybrid under these conditions. However, it is important to consider that due to the low number of polyploidization events, the statistical power of the inference is reduced.

DISCUSSION

Identifying which factors favor adaptation to environmental stressors is an important goal in ecology and evolution. Here we tested if hybrids would adapt faster than parental species when exposed to intense stress, using yeast and UV radiation mimetic conditions as models. Our hypothesis was based on previous observations suggesting that hybrids may have an adaptive advantage when faced with stressful conditions (Whitney *et al.* 2006; Stelkens *et al.* 2014; Gibson & Liti 2015; Peris *et al.* 2017). Previous studies reported rapid adaptation in yeast (50-300 generations) in stresses such as salt, ethanol and copper (Dhar *et al.* 2011; Chang *et al.* 2013; Voordeckers *et al.* 2015; Peris *et al.* 2017) and also to UV radiation both in natural strains (Lidzbarsky *et al.* 2009; Pulschen *et al.* 2015) and in laboratory conditions (Lawrence & Christensen 1976). We found that all three genotypes adapted to UV mimetic conditions but the rate of adaptation and the extent achieved in 200 generations were lower for hybrids.

Why do hybrids show a slower rate than parental strains? One possibility would be that hybrids are more sensitive to the chemical so it makes them incapable of adapting to this media. This is unlikely since the hybrid genotype is less sensitive than *S. paradoxus* at the concentration used. We rather hypothesize that in the specific case of stress caused by DNA damage, hybrids may be at a disadvantage because they are genetically unstable (Fontdevila 2005; Marfil *et al.* 2006; Baack & Rieseberg 2007; Morales & Dujon 2012; Guerreiro 2014; Xie *et al.* 2016; Dion-Côté & Barbash 2017; Mwathi *et al.* 2019) and DNA damaging agents may further enhance this instability, preventing the occurrence or fixation of adaptive mutations. Consistent with the instability of hybrids, recent study of mutation accumulation in yeast reported that hybrids from more divergent parents lines were lost at a greater rate than the less divergent ones (Charron *et al.* 2019). As the loss occurred in the first 250 generations, the authors suggested that it was mostly due to the genomic instability that arises rapidly after hybridization rather than spontaneous mutations. Thus, we suggest that the lower adaptive potential of our hybrids in UV mimetic conditions may result from the interaction between DNA damage and the inherent genetic instability of hybrids. To support this hypothesis, it would be necessary to compare the rate of adaptation of hybrids in other DNA damaging conditions and other stresses non-related to DNA damage, and also to confirm that genome instability is enhanced in hybrids. It would also be useful to identify the mechanistic basis of adaptation to UV mimetic chemicals in hybrids and parental species, as it is possible that hybrids do not have access to the same adaptive mutations as parental species. Some mechanisms of resistance have been characterized in *S. cerevisiae* and could vary with respect to hybrids, for instance through the perturbation of the proteasome (Karpov *et al.* 2019) and pathways involved in multi-drug resistance transporters (Rong-Mullins *et al.* 2018).

Adaptation to one environment can decrease fitness in another, revealing evolutionary trade-offs (Cooper & Lenski 2000). Such trade-off can be the consequence of mutations that are beneficial in stressful environments but detrimental in non-selective environments, also called fitness cost. For instance, Arctic *Daphnia* naturally exposed to UV evolved a melanic morph that is more resistant to UV and that is competitively inferior to the non-melanic ones in laboratory conditions (Hessen 1996). Such trade-off can limit the long-term success of populations that adapt to acute stress because they lead to specialized genotypes (Anderson et al. 2013) that would lose their advantage when normal conditions return. The accumulation of neutral mutations in strong selective environments, but that are deleterious in non-selective environments, can also cause trade-offs (Mee & Yeaman 2019). The latter mechanism may be accelerated in the presence of UV mimetic chemicals and lead to genomic decomposition, as seen in mutator strains (Couce et al., 2017).

All strains showed a significant fitness reduction in non-selective control conditions once adapted to UV mimetic conditions. We also found that across strains, the extent of trade-off is correlated with the extent of adaptation, an observation that is more consistent with an actual cost of adaptation than with the accumulation of conditionally deleterious mutations, which, in principle, should be independent from the extent of adaptation. We did not find that the hybrids suffer from a higher cost than the parental species. It therefore remains unclear whether the higher cost to adaptation and more accumulation of mutations in the hybrids contribute to limiting their rate of adaptation.

Experimental evolution comes with limitations (McDonald 2019). For instance, serial transfers produce populations that are usually subject to bottlenecks (Wahl *et al.* 2002). In our study, every serial transfer produced an approximately 10-fold decrease in population sizes with varying severity. As a result, we observed some bottlenecks, especially among the first generations when strains are less acclimated and adapted. Consequently, alternative evolutionary pathways could be lost, limiting the number of different adaptive peaks visited (Poelwijk *et al.* 2007; Zheng *et al.* 2019). However, this did not prevent adaptation from occurring. Another limit is that we are studying first generation, F1 hybrids, which have not undergone sexual reproduction and cannot exploit the power of recombination to remove deleterious combination of alleles and favor the maintenance of advantageous ones. These experimental hybrids rely only on the loss of heterozygosity (Charron *et al.* 2019) for the removal of alleles, which meiosis could achieve by recombination in hybrids (Schumer *et al.* 2018; Zhang *et al.* 2020). Sexually recombining hybrids may therefore not suffer from the same limits as F1 hybrids in the conditions we used. This will need to be examined in the future.

The study of hybridization has been a prominent and challenging research theme in biology (Anderson 1953; Dobzhansky & Pavlovsky 1958; Grant & Grant 1996; Seehausen 2004; Mitchell *et al.* 2019). Understanding why hybridization may lead to advantages or disadvantages is crucial for the conservation of ecosystems (Becker *et al.* 2013; Hamilton & Miller 2016; Chan *et al.* 2018), for crop improvement (Waara & Glimelius 1995; Liu *et al.* 2005) and in industry (Bellon *et al.* 2015; Gibson & Liti 2015; Lopandic 2018). Our results show that studying hybrids in conditions that promote DNA damage may be a powerful context in which study the limits of hybridization as a mechanism that may promote adaptation in stress conditions.

Acknowledgments
We thank Nadia Aubin-Horth, Angel Cisneros, Guillaume Charron and Marika Drouin for comments on the manuscript, Guillaume Charron for help with the strain construction, Angel Cisneros for help in data analysis and members of the Landry Lab for helpful discussions.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding
CB was supported by “la Caixa” Foundation (ID 100010434), under agreement LCF/BQ/AA16/11580051) and by the Fonds de recherche du Québec - Nature et technologies (FRQNT) (#274987). SM is supported by Fonds de recherche du Québec – Santé (FRQS) (#270686) post-doctoral fellowship. CRL’s work on hybridization is supported by a Natural Sciences and Engineering Research Council (NSERC) Discovery grant. CRL holds the Canada Research Chair in Evolutionary Cell and Systems Biology.

REFERENCES

Adamo, G.M., Brocca, S., Passolunghi, S., Salvato, B. & Lotti, M. (2012). Laboratory evolution of copper tolerant yeast strains. *Microb. Cell Fact.*, 11, 1.

Alix, K., Gérard, P.R., Schwarzacher, T. & Heslop-Harrison, J.S.P. (2017). Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. *Ann. Bot.*, 120, 183–194.

Alves, M.J., Coelho, M.M. & Collares-Pereira, M.J. (2001). Evolution in action through hybridisation and polyploidy in an Iberian freshwater fish: a genetic review. *Genetica*, 111, 375–385.

Anderson, E. (1953). Introgressive Hybridization. *Biol. Rev. Camb. Philos. Soc.*, 28, 280–307.

Baack, E.J. & Rieseberg, L.H. (2007). A genomic view of introgression and hybrid speciation. *Curr. Opin. Genet. Dev.*, 17, 513–518.

Ballerini, E.S. (2012). Natural hybridization as a catalyst of rapid evolutionary change. *Rapidly Evolving Genes and Genetic Systems*, 256–265.

Barbosa, R., Almeida, P., Safar, S.V.B., Santos, R.O., Morais, P.B., Nielly-Thibault, L., *et al.* (2016). Evidence of Natural Hybridization in Brazilian Wild Lineages of Saccharomyces cerevisiae. *Genome Biol. Evol.*, 8, 317–329.

Bashir, T., Sailer, C., Gerber, F., Loganathan, N., Bhoopalan, H., Eichenberger, C., *et al.* (2014). Hybridization alters spontaneous mutation rates in a parent-of-origin-dependent fashion in Arabidopsis. *Plant Physiol.*, 165, 424–437.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.*, 67.

Becker, M., Gruenheit, N., Steel, M., Voelckel, C., Deusch, O., Heenan, P.B., *et al.* (2013). Hybridization may facilitate in situ survival of endemic species through periods of climate change. *Nat. Clim. Chang.*, 3, 1039–1043.

Bellon, J.R., Yang, F., Day, M.P., Inglis, D.L. & Chambers, P.J. (2015). Designing and creating Saccharomyces interspecific hybrids for improved, industry relevant, phenotypes. *Appl. Microbiol. Biotechnol.*, 99, 8597–8609.

Björn, L.O. (1996). Effects of ozone depletion and increased UV-B on terrestrial ecosystems. *Int. J. Environ. Stud.*, 51, 217–243.

Bleuven, C. & Landry, C.R. (2016). Molecular and cellular bases of adaptation to a changing environment in microorganisms. *Proc. Biol. Sci.*, 283.

Bui, D.T., Dine, E., Anderson, J.B., Aquadro, C.F. & Alani, E.E. (2015). A Genetic Incompatibility Accelerates Adaptation in Yeast. *PLoS Genet.*, 11, e1005407.

Burton, T.L. & Husband, B.C. (2000). Fitness differences among diploids, tetraploids, and their triploid progeny in Chamerion angustifolium: mechanisms of inviability and implications for polyploid evolution. *Evolution*, 54, 1182–1191.

Caldwell, M.M. & Flint, S.D. (1994). Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Clim. Change*, 28, 375–394.

Chang, S.-L., Lai, H.-Y., Tung, S.-Y. & Leu, J.-Y. (2013). Dynamic large-scale chromosomal rearrangements fuel rapid adaptation in yeast populations. *PLoS Genet.*, 9, e1003232.

Chan, W.Y., Peplow, L.M., Menéndez, P., Hoffmann, A.A. & van Oppen, M.J.H. (2018). Interspecific Hybridization May Provide Novel Opportunities for Coral Reef Restoration. *Frontiers in Marine Science*, 5, 160.

Charron, G., Marsit, S., Hénault, M., Martin, H. & Landry, C.R. (2019). Spontaneous whole-genome duplication restores fertility in interspecific hybrids. *Nat. Commun.*, 10, 4126.

Cliften, P.F., Hillier, L.W., Fulton, L., Graves, T., Miner, T., Gish, W.R., *et al.* (2001). Surveying Saccharomyces genomes to identify functional elements by comparative DNA sequence analysis. *Genome Res.*, 11, 1175–1186.

Cooper, V.S. (2014). The origins of specialization: insights from bacteria held 25 years in captivity. *PLoS Biol.*, 12, e1001790.

Cooper, V.S. & Lenski, R.E. (2000). The population genetics of ecological specialization in evolving Escherichia coli populations. *Nature*, 407, 736–739.

Couce, A., Caudwell, L.V., Feinauer, C., Hindré, T., Feugeas, J.-P., Weigt, M., *et al.* (2017). Mutator genomes decay, despite sustained fitness gains, in a long-term experiment with bacteria. *Proc. Natl. Acad. Sci. U. S. A.*, 114, E9026–E9035.

Dhar, R., Sägesser, R., Weikert, C., Yuan, J. & Wagner, A. (2011). Adaptation of Saccharomyces cerevisiae to saline stress through laboratory evolution. *J. Evol. Biol.*, 24, 1135–1153.

Dion-Côté, A.-M. & Barbash, D.A. (2017). Beyond speciation genes: an overview of genome stability in evolution and speciation. *Curr. Opin. Genet. Dev.*, 47, 17–23.

Dobzhansky, T. & Pavlovsky, O. (1958). Interracial hybridization and breakdown of coadapted gene complexes in Drosophila paulistorum and Drosophila willistoni. *Proceedings of the National Academy of Sciences*, 44, 622–629.

Felkner, I.C. & Kadlubar, F. (1968). Parallel between ultraviolet light and 4-nitroquinoline-1-oxide sensitivity in Bacillus subtilis. *J. Bacteriol.*, 96, 1448–1449.

Fontdevila, A. (2005). Hybrid genome evolution by transposition. *Cytogenetic and Genome Research*.

Gerstein, A.C., Chun, H.-J.E., Grant, A. & Otto, S.P. (2006). Genomic convergence toward diploidy in Saccharomyces cerevisiae. *PLoS Genet.*, 2, e145.

Gibson, B. & Liti, G. (2015). Saccharomyces pastorianus: genomic insights inspiring innovation for industry. *Yeast*, 32, 17–27.

Gilchrist, C. & Stelkens, R. (2019). Aneuploidy in yeast: Segregation error or adaptation mechanism? *Yeast*, 36, 525–539.

Gómez, J.M., González-Megías, A., Lorite, J., Abdelaziz, M. & Perfectti, F. (2015). The silent extinction: climate change and the potential hybridization-mediated extinction of endemic high-mountain plants. *Biodivers. Conserv.*, 24, 1843–1857.

Gorter, F.A., Derks, M.F.L., van den Heuvel, J., Aarts, M.G.M., Zwaan, B.J., de Ridder, D., *et al.* (2017). Genomics of Adaptation Depends on the Rate of Environmental Change in Experimental Yeast Populations. *Mol. Biol. Evol.*, 34, 2613–2626.

Grant, B.R. & Grant, P.R. (1996). High Survival of Darwin's Finch Hybrids: Effects of Beak Morphology and Diets. *Ecology*, 77, 500–509.

Guerreiro, M.P.G. (2014). Interspecific hybridization as a genomic stressor inducing mobilization of transposable elements in Drosophila. *Mob. Genet. Elements*, 4, e34394.

Güldener, U., Heck, S., Fielder, T., Beinhauer, J. & Hegemann, J.H. (1996). A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res.*, 24, 2519–2524.

Häder, D.-P., Helbling, E.W., Williamson, C.E. & Worrest, R.C. (2011). Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochem. Photobiol. Sci.*, 10, 242–260.

Hamilton, J.A. & Miller, J.M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conserv. Biol.*, 30, 33–41.

Hanway, D., Chin, J.K., Xia, G., Oshiro, G., Winzeler, E.A. & Romesberg, F.E. (2002). Previously uncharacterized genes in the UV- and MMS-induced DNA damage response in yeast. *Proc. Natl. Acad. Sci. U. S. A.*, 99, 10605–10610.

Heil, C.S.S., Smukowski Heil, C.S., DeSevo, C.G., Pai, D.A., Tucker, C.M., Hoang, M.L., *et al.* (2017). Loss of Heterozygosity Drives Adaptation in Hybrid Yeast. *Mol. Biol. Evol.*, 34, 1596–1612.

Herbst, R.H., Bar-Zvi, D., Reikhav, S., Soifer, I., Breker, M., Jona, G., *et al.* (2017). Heterosis as a consequence of regulatory incompatibility. *BMC Biol.*, 15, 38.

Herrera, R., Alvarez, M.C., Gelis, S., Kodedová, M., Sychrová, H., Kschischo, M., *et al.* (2014). Role of Saccharomyces cerevisiae Trk1 in stabilization of intracellular potassium content upon changes in external potassium levels. *Biochim. Biophys. Acta*, 1838, 127–133.

Hessen, D.O. (1996). Competitive trade-off strategies in Arctic Daphnia linked to melanism and UV-B stress. *Polar Biol.*, 16, 573–579.

Jung, H.J., Hwang, J.N. & Seo, Y.R. (2004). The protective effect of mild hyperthermia against UV-mimetic agent 4-nitroquinoline-1-oxide (4NQO) in human colon cells. *Res. Commun. Mol. Pathol. Pharmacol.*, 115-116, 123–133.

Karpov, D.S., Spasskaya, D.S., Nadolinskaia, N.I., Tutyaeva, V.V., Lysov, Y.P. & Karpov, V.L. (2019). Deregulation of the 19S proteasome complex increases yeast resistance to 4-NQO and oxidative stress via upregulation of Rpn4- and proteasome-dependent stress responsive genes. *FEMS Yeast Res.*, 19, foz002.

Kawecki, T.J. & Ebert, D. (2018). Conceptual issues in local adaptation. *Ecol. Lett.*, 1225–1241.

Khroustalyova, G., Giovannitti, G., Severini, D., Scherbaka, R., Turchetti, B., Buzzini, P., *et al.* (2019). Anhydrobiosis in yeasts: Psychrotolerant yeasts are highly resistant to dehydration. *Yeast*, 36, 375–379.

Labat, F., Pradillon, O., Garry, L., Peuchmaur, M., Fantin, B. & Denamur, E. (2005). Mutator phenotype confers advantage in Escherichia coli chronic urinary tract infection pathogenesis. *FEMS Immunol. Med. Microbiol.*, 44, 317–321.

Landry, Hartl & Ranz. (2007). Genome clashes in hybrids: insights from gene expression. *Heredity*, 99, 483–493.

Landry, Townsend, J.P., Hartl, D.L. & Cavalieri, D. (2006). Ecological and evolutionary genomics of Saccharomyces cerevisiae. *Mol. Ecol.*, 15, 575–591.

Lawrence, C.W. & Christensen, R. (1976). UV mutagenesis in radiation-sensitive strains of yeast. *Genetics*, 82, 207–232.

Le Bars, H., Bonnaure-Mallet, M., Barloy-Hubler, F., Jolivet-Gougeon, A. & Bousarghin, L. (2014). Strong mutator phenotype drives faster adaptation from growth on glucose to growth on acetate in Salmonella. *Microbiology*, 160, 2264–2271.

Leducq, J.-B., Nielly-Thibault, L., Charron, G., Eberlein, C., Verta, J.-P., Samani, P., *et al.* (2016). Speciation driven by hybridization and chromosomal plasticity in a wild yeast. *Nat Microbiol*, 1, 15003.

Lewontin, R.C. & Birch, L.C. (1966). Hybridization as a source of variation for adaptation to new environments. *Evolution*, 20, 315–336.

Lexer, C., Welch, M.E., Durphy, J.L. & Rieseberg, L.H. (2003). Natural selection for salt tolerance quantitative trait loci (QTLs) in wild sunflower hybrids: implications for the origin of Helianthus paradoxus, a diploid hybrid species. *Mol. Ecol.*, 12, 1225–1235.

Lidzbarsky, G.A., Shkolnik, T. & Nevo, E. (2009). Adaptive Response to DNA-Damaging Agents in Natural Saccharomyces cerevisiae Populations from “Evolution Canyon”, Mt. Carmel, Israel. *PLoS ONE*, 4, e5914.

Liu, J., Xu, X. & Deng, X. (2005). Intergeneric somatic hybridization and its application to crop genetic improvement. *Plant Cell Tissue Organ Cult.*, 82, 19–44.

Lopandic, K. (2018). Saccharomyces interspecies hybrids as model organisms for studying yeast adaptation to stressful environments. *Yeast*, 35, 21–38.

Lowe, W.H., Muhlfeld, C.C. & Allendorf, F.W. (2015). Spatial sorting promotes the spread of maladaptive hybridization. *Trends Ecol. Evol.*, 30, 456–462.

Malkin, M.F. & Zahalsky, A.C. (1966). Interaction of the water-soluble carcinogen 4-nitroquinoline N-oxide with DNA. *Science*, 154, 1665–1667.

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends Ecol. Evol.*, 20, 229–237.

Marcet-Houben, M. & Gabaldón, T. (2015). Beyond the Whole-Genome Duplication: Phylogenetic Evidence for an Ancient Interspecies Hybridization in the Baker’s Yeast Lineage. *PLoS Biol.*, 13, e1002220.

Marfil, C.F., Masuelli, R.W., Davison, J. & Comai, L. (2006). Genomic instability in Solanum tuberosum x Solanum kurtzianum interspecific hybrids. *Genome*, 49, 104–113.

Marsit, S., Leducq, J.-B., Durand, É., Marchant, A., Filteau, M. & Landry, C.R. (2017). Evolutionary biology through the lens of budding yeast comparative genomics. *Nat. Rev. Genet.*, 18, 581–598.

Martin, N.H., Bouck, A.C. & Arnold, M.L. (2006). Detecting adaptive trait introgression between Iris fulva and I. brevicaulis in highly selective field conditions. *Genetics*, 172, 2481–2489.

Matsuoka, Y. (2011). Evolution of Polyploid Triticum Wheats under Cultivation: The Role of Domestication, Natural Hybridization and Allopolyploid Speciation in their Diversification. *Plant Cell Physiol.*, 52, 750–764.

McDonald, M.J. (2019). Microbial Experimental Evolution – a proving ground for evolutionary theory and a tool for discovery. *EMBO Rep.*, 20.

Mee, J.A. & Yeaman, S. (2019). Unpacking Conditional Neutrality: Genomic Signatures of Selection on Conditionally Beneficial and Conditionally Deleterious Mutations. *Am. Nat.*, 194, 529–540.

Mitchell, N., Owens, G.L., Hovick, S.M., Rieseberg, L.H. & Whitney, K.D. (2019). Hybridization speeds adaptive evolution in an eight-year field experiment. *Sci. Rep.*, 9, 6746.

Morales, L. & Dujon, B. (2012). Evolutionary role of interspecies hybridization and genetic exchanges in yeasts. *Microbiol. Mol. Biol. Rev.*, 76, 721–739.

Morard, M., Benavent-Gil, Y., Ortiz-Tovar, G., Pérez-Través, L., Querol, A., Toft, C., *et al.* (2020). Genome structure reveals the diversity of mating mechanisms in Saccharomyces cerevisiae x Saccharomyces kudriavzevii hybrids, and the genomic instability that promotes phenotypic diversity. *Microbial Genomics*, mgen000333.

Mulla, W., Zhu, J. & Li, R. (2014). Yeast: a simple model system to study complex phenomena of aneuploidy. *FEMS Microbiol. Rev.*, 38, 201–212.

Murphy, H.A., Kuehne, H.A., Francis, C.A. & Sniegowski, P.D. (2006). Mate choice assays and mating propensity differences in natural yeast populations. *Biol. Lett.*, 2, 553–556.

Mwathi, M.W., Schiessl, S.V., Batley, J. & Mason, A.S. (2019). “Doubled-haploid” allohexaploid Brassica lines lose fertility and viability and accumulate genetic variation due to genomic instability. *Chromosoma*, 128, 521–532.

Nolte, A.W., Freyhof, J., Stemshorn, K.C. & Tautz, D. (2005). An invasive lineage of sculpins, Cottus sp. (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. *Proceedings of the Royal Society B: Biological Sciences*, 272, 2379–2387.

Nolte, A.W. & Sheets, H.D. (2005). Shape based assignment tests suggest transgressive phenotypes in natural sculpin hybrids (Teleostei, Scorpaeniformes, Cottidae). *Front. Zool.*, 2, 11.

Peris, D., Moriarty, R.V., Alexander, W.G., Baker, E., Sylvester, K., Sardi, M., *et al.* (2017). Hybridization and adaptive evolution of diverse Saccharomyces species for cellulosic biofuel production. *Biotechnol. Biofuels*, 10, 78.

Peter, J., De Chiara, M., Friedrich, A., Yue, J.-X., Pflieger, D., Bergström, A., *et al.* (2018). Genome evolution across 1,011 Saccharomyces cerevisiae isolates. *Nature*, 556, 339–344.

Poelwijk, F.J., Kiviet, D.J., Weinreich, D.M. & Tans, S.J. (2007). Empirical fitness landscapes reveal accessible evolutionary paths. *Nature*, 445, 383–386.

Posas, F., Chambers, J.R., Heyman, J.A., Hoeffler, J.P., de Nadal, E. & Ariño, J. (2000). The transcriptional response of yeast to saline stress. *J. Biol. Chem.*, 275, 17249–17255.

Pulschen, A.A., Rodrigues, F., Duarte, R.T.D., Araujo, G.G., Santiago, I.F., Paulino-Lima, I.G., *et al.* (2015). UV-resistant yeasts isolated from a high-altitude volcanic area on the Atacama Desert as eukaryotic models for astrobiology. *Microbiologyopen*, 4, 574–588.

Replansky, T., Koufopanou, V., Greig, D. & Bell, G. (2008). Saccharomyces sensu stricto as a model system for evolution and ecology. *Trends Ecol. Evol.*, 23, 494–501.

Rieseberg, L.H., Archer, M.A. & Wayne, R.K. (1999). Transgressive segregation, adaptation and speciation. *Heredity* , 83 (Pt 4), 363–372.

Rong-Mullins, X., Ayers, M.C., Summers, M. & Gallagher, J.E.G. (2018). Transcriptional Profiling of Saccharomyces cerevisiae Reveals the Impact of Variation of a Single Transcription Factor on Differential Gene Expression in 4NQO, Fermentable, and Nonfermentable Carbon Sources. *G3* , 8, 607–619.

Rozema, J., van de Staaij, J., Björn, L.O. & Caldwell, M. (1997). UV-B as an environmental factor in plant life: stress and regulation. *Trends Ecol. Evol.*, 12, 22–28.

Saitoh, K., Chen, W.-J. & Mayden, R.L. (2010). Extensive hybridization and tetrapolyploidy in spined loach fish. *Mol. Phylogenet. Evol.*, 56, 1001–1010.

Salvadó, Z., Arroyo-López, F.N., Guillamón, J.M., Salazar, G., Querol, A. & Barrio, E. (2011). Temperature adaptation markedly determines evolution within the genus Saccharomyces. *Appl. Environ. Microbiol.*, 77, 2292–2302.

Schaaff, F., Reipert, A. & Bierbaum, G. (2002). An elevated mutation frequency favors development of vancomycin resistance in Staphylococcus aureus. *Antimicrob. Agents Chemother.*, 46, 3540–3548.

Schoenfelder, K.P. & Fox, D.T. (2015). The expanding implications of polyploidy. *The Journal of Cell Biology*, 209, 485–491.

Schumer, M., Rosenthal, G.G. & Andolfatto, P. (2014). How common is homoploid hybrid speciation? *Evolution*, 68, 1553–1560.

Schumer, M., Xu, C., Powell, D.L., Durvasula, A., Skov, L., Holland, C., *et al.* (2018). Natural selection interacts with recombination to shape the evolution of hybrid genomes. *Science*, 360, 656–660.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends Ecol. Evol.*, 19, 198–207.

Selmecki, A.M., Maruvka, Y.E., Richmond, P.A., Guillet, M., Shoresh, N., Sorenson, A.L., *et al.* (2015). Polyploidy can drive rapid adaptation in yeast. *Nature*, 519, 349–352.

Session, A.M., Uno, Y., Kwon, T., Chapman, J.A., Toyoda, A., Takahashi, S., *et al.* (2016). Genome evolution in the allotetraploid frog Xenopus laevis. *Nature*, 538, 336–343.

Soltis, P.S. & Soltis, D.E. (2009). The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.*, 60, 561–588.

Sprouffske, K., Aguilar-Rodríguez, J., Sniegowski, P. & Wagner, A. (2018). High mutation rates limit evolutionary adaptation in Escherichia coli. *PLoS Genet.*, 14, e1007324.

Sprouffske, K. & Wagner, A. (2016). Growthcurver: an R package for obtaining interpretable metrics from microbial growth curves. *BMC Bioinformatics*, 17, 172.

Stelkens, R.B., Brockhurst, M.A., Hurst, G.D.D. & Greig, D. (2014). Hybridization facilitates evolutionary rescue. *Evol. Appl.*, 7, 1209–1217.

Sunshine, A.B., Payen, C., Ong, G.T., Liachko, I., Tan, K.M. & Dunham, M.J. (2015). The fitness consequences of aneuploidy are driven by condition-dependent gene effects. *PLoS Biol.*, 13, e1002155.

Taddei, F., Radman, M., Maynard-Smith, J., Toupance, B., Gouyon, P.H. & Godelle, B. (1997). Role of mutator alleles in adaptive evolution. *Nature*, 387, 700–702.

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., *et al.* (2016). Hybridization and extinction. *Evol. Appl.*, 9, 892–908.

Torres, N.P., Lee, A.Y., Giaever, G., Nislow, C. & Brown, G.W. (2013). A high-throughput yeast assay identifies synergistic drug combinations. *Assay Drug Dev. Technol.*, 11, 299–307.

Van der Leun, J.C. & de Gruijl, F.R. (2002). Climate change and skin cancer. *Photochem. Photobiol. Sci.*, 1, 324–326.

Vega, U. & Frey, K.J. (1980). Transgressive segregation in inter and intraspecific crosses of barley. *Euphytica*, 29, 585–594.

de Visser, J.A.G.M. & Lenski, R.E. (2002). Long-term experimental evolution in Escherichia coli. XI. Rejection of non-transitive interactions as cause of declining rate of adaptation. *BMC Evol. Biol.*, 2, 19.

Voordeckers, K., Kominek, J., Das, A., Espinosa-Cantú, A., De Maeyer, D., Arslan, A., *et al.* (2015). Adaptation to High Ethanol Reveals Complex Evolutionary Pathways. *PLoS Genet.*, 11, e1005635.

Waara, S. & Glimelius, K. (1995). The potential of somatic hybridization in crop breeding. *Euphytica*, 85, 217–233.

Wahl, L.M., Gerrish, P.J. & Saika-Voivod, I. (2002). Evaluating the impact of population bottlenecks in experimental evolution. *Genetics*, 162, 961–971.

Wang, Z., Yin, H., Lv, L., Feng, Y., Chen, S., Liang, J., *et al.* (2014). Unrepaired DNA damage facilitates elimination of uniparental chromosomes in interspecific hybrid cells. *Cell Cycle*, 13, 1345–1356.

Wei, X. & Zhang, J. (2019). Environment-dependent pleiotropic effects of mutations on the maximum growth rate r and carrying capacity K of population growth. *PLoS Biol.*, 17, e3000121.

Whitney, K.D., Randell, R.A. & Rieseberg, L.H. (2006). Adaptive introgression of herbivore resistance traits in the weedy sunflower Helianthus annuus. *Am. Nat.*, 167, 794–807.

Xiao, J., Zou, T., Chen, Y., Chen, L., Liu, S., Tao, M., *et al.* (2011). Coexistence of diploid, triploid and tetraploid crucian carp (Carassius auratus) in natural waters. *BMC Genetics*, 12, 20.

Xie, Z., Wang, L., Wang, L., Wang, Z., Lu, Z., Tian, D., *et al.* (2016). Mutation rate analysis via parent–progeny sequencing of the perennial peach. I. A low rate in woody perennials and a higher mutagenicity in hybrids. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20161016.

Zhang, Z., Bendixsen, D.P., Janzen, T., Nolte, A.W., Greig, D. & Stelkens, R. (2020). Recombining your way out of trouble: The genetic architecture of hybrid fitness under environmental stress. *Mol. Biol. Evol.*, 37, 167–182.

Zheng, J., Payne, J.L. & Wagner, A. (2019). Cryptic genetic variation accelerates evolution by opening access to diverse adaptive peaks. *Science*, 365, 347–353.

FIGURES AND LEGENDS

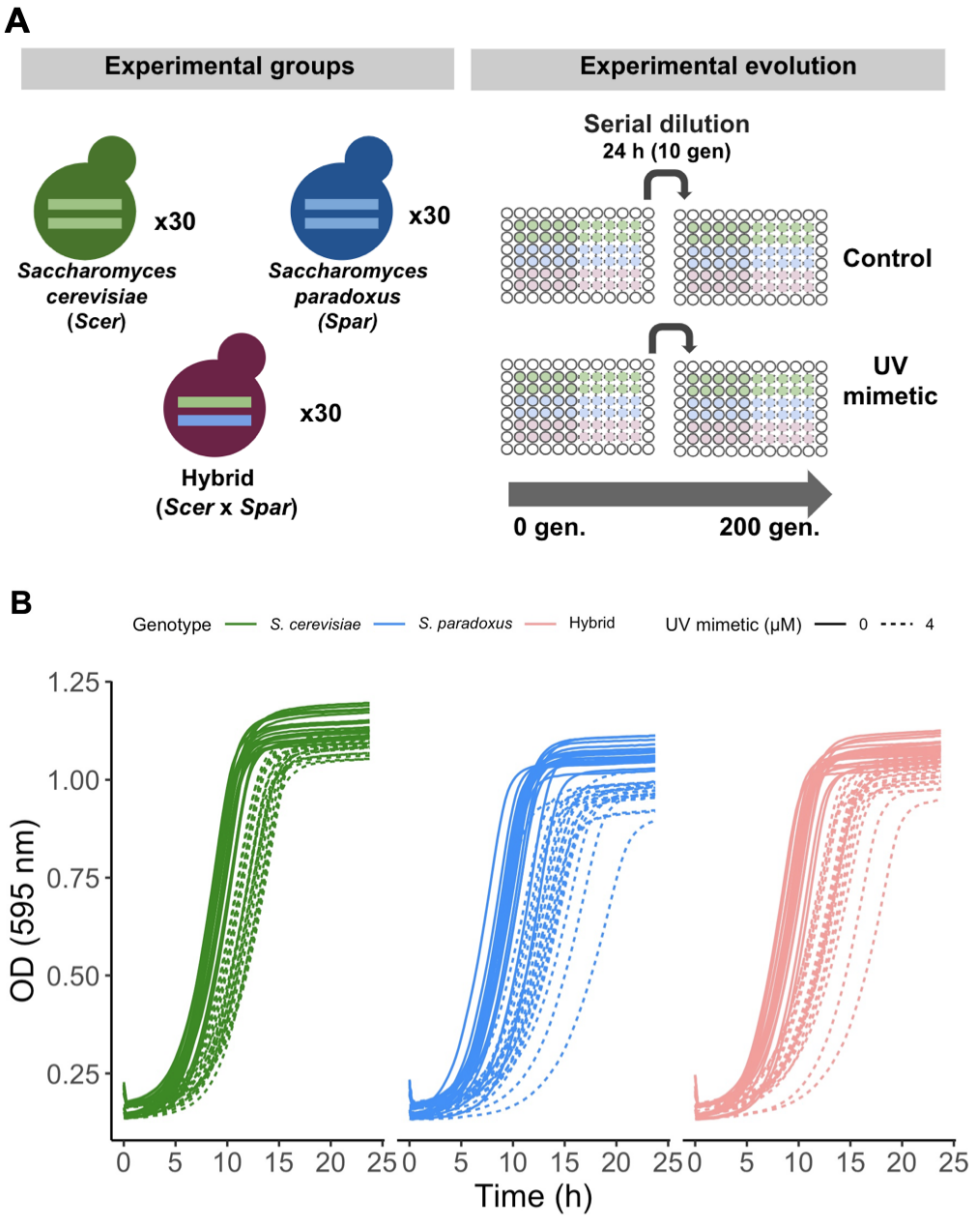

Figure 1. Experimental design. A) 30 independent populations of each genotype (*S. cerevisiae*, *S. paradoxus* and their hybrid) were evolved for 200 generations in control YPD and in YPD + UV mimetic chemical (4 μM of 4-NQO). Each 24 hour, a new population was founded by transferring about 10% of the previous population to fresh media. B) Optical density as a function of time for 20 of the initial populations of each genotype in control conditions and in UV mimetic conditions (4 μM of 4-NQO).

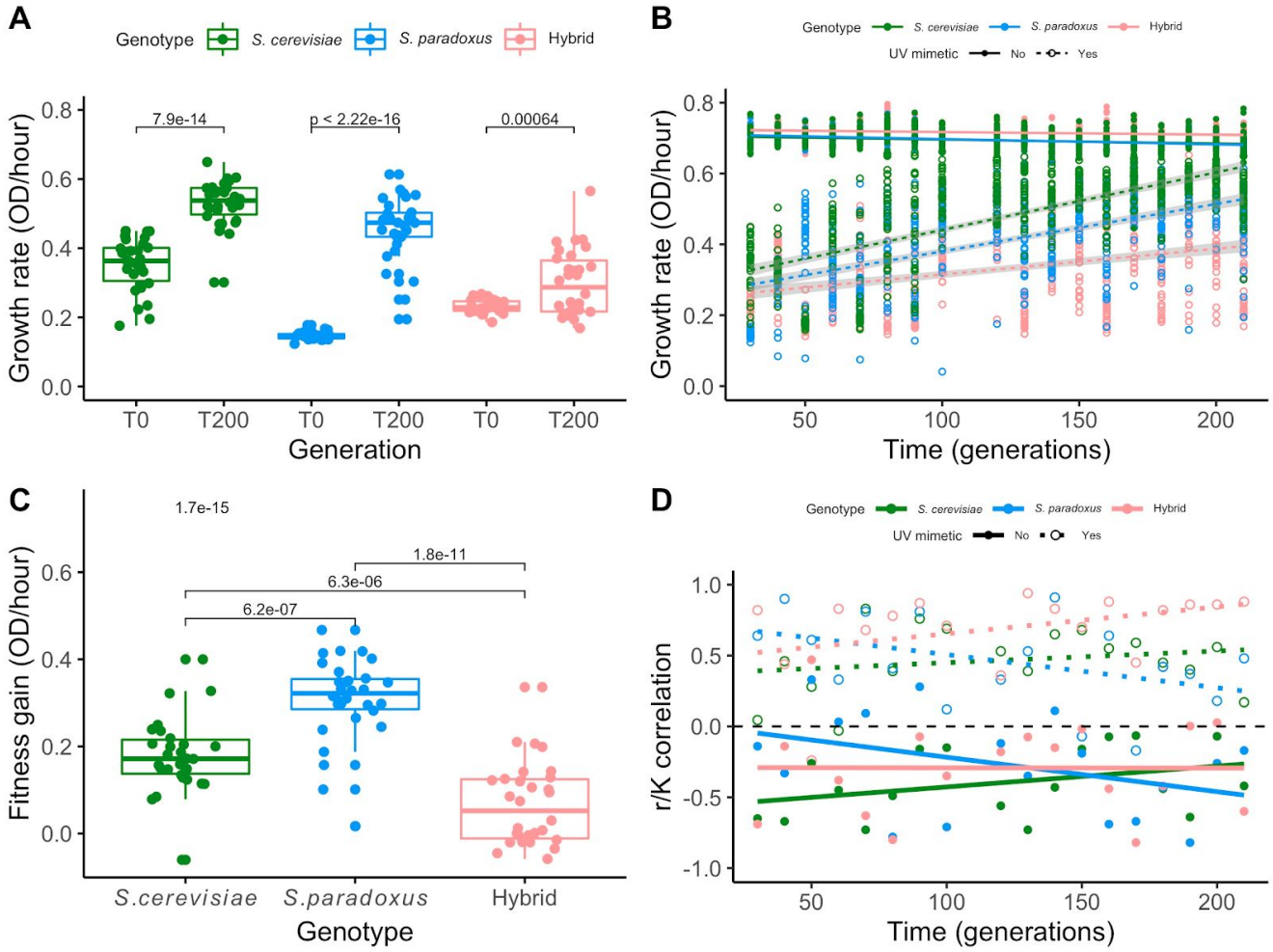

Figure 2. Adaptation of parents and hybrids in UV mimetic conditions. A) Growth in UV mimetic conditions for the 30 lines of the three genotypes at the initial (T30) and final (T200) time points. Paired t-tests were performed between growth rate (r) at T30 and T200 for each genotype. P-values are shown above. For these analyses, we considered the initial growth as the growth estimated from the third cycle (day 3) because we did not monitor growth continuously in the first and second cycles due to technical problems. In any case, not including the first two cycles may be required because cells sensitivity to the UV mimetic chemical increased during that period (Fig. S3), most likely because it takes time before cells are sensitized to the new condition. B) The growth rate (r) of the different genotypes is shown as a function of the number of generations. Each data point is a growth rate estimated per population per time cycle. A 95% confidence interval is shown for the regression lines (n = 30 populations for each genotype). C) Fitness gain: change in the growth rate between initial and final time points calculated by subtracting growth rate in UV mimetic conditions at T30 from the growth rate at T200. ANOVA and pairwise comparisons were performed among genotypes (n = 30 populations for each genotype). Global p-value for ANOVA test (above) and pairwise p-values are shown. D) Spearman's rank correlations between growth rate (r) and carrying capacity (K) through generations for populations grown in control and in UV mimetic condition. Each point represents the value of the Spearman's rank correlation between r and K among the n = 30 populations for each genotype for a given day and a given condition.

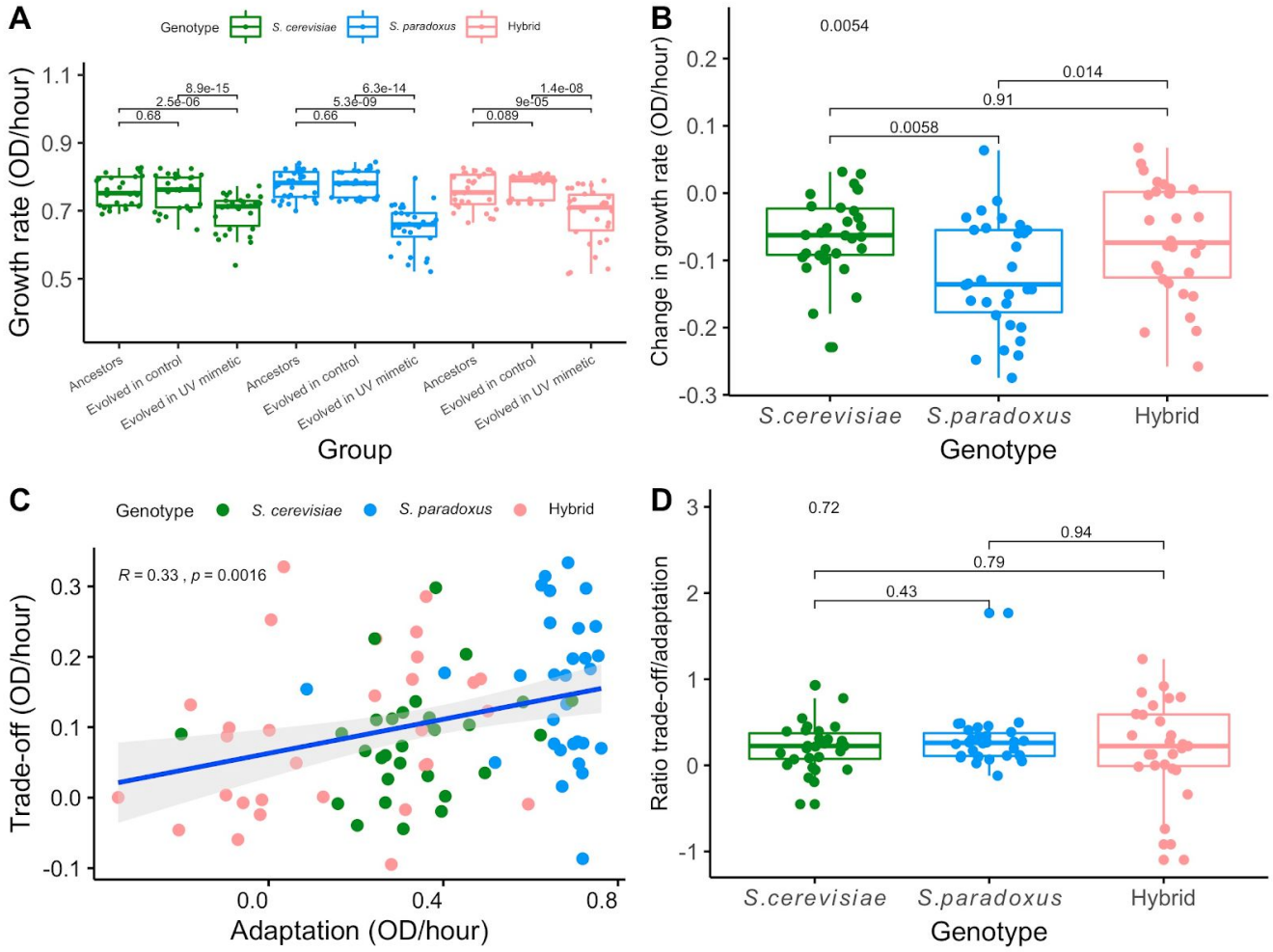

Figure 3. Strains adapted to a UV mimetic chemical show a trade-off under control conditions. A) Growth rate (r) in control conditions of ancestral strains, of strains evolved in controlled conditions (200 generations) and of strains adapted to UV mimetic conditions (200 generations). Paired t-tests were performed by matching derived and ancestral strains (n = 30 populations for each genotype). P-values are shown above. B) Change in growth rate between strains evolved in UV mimetic conditions and ancestors calculated by subtracting growth rate in control of ancestors (T0) from the growth rate in control of the strains evolved in UV mimetic conditions (T200). ANOVA and pairwise comparisons were performed among genotypes (n = 30 populations for each genotype). Global p-value for ANOVA test (above) and pairwise p-values are shown. C) Trade-off depends on the extent of adaptation to UV mimetic conditions. Adaptation values, expressed as fitness increase, were obtained by subtracting the growth in UV mimetic conditions at T0 from the growth in UV mimetic conditions at T200. Trade-off values were obtained by subtracting the growth rate of the strains evolved in UV mimetic conditions (T200) grown in control conditions from the growth of the ancestors (T0) grown in control conditions. Spearman's rank correlation was performed (n = 30 populations for each genotype). Spearman's rank coefficient and associated p-value are shown. D) Cost of adaptation as the trade-off/adaptation ratio calculated by dividing the values of trade-off by the values of adaptation for each genotype. ANOVA and pairwise comparisons were performed among genotypes (n = 30 populations for each genotype). Global p-value for ANOVA test (above) and pairwise p-values are shown.

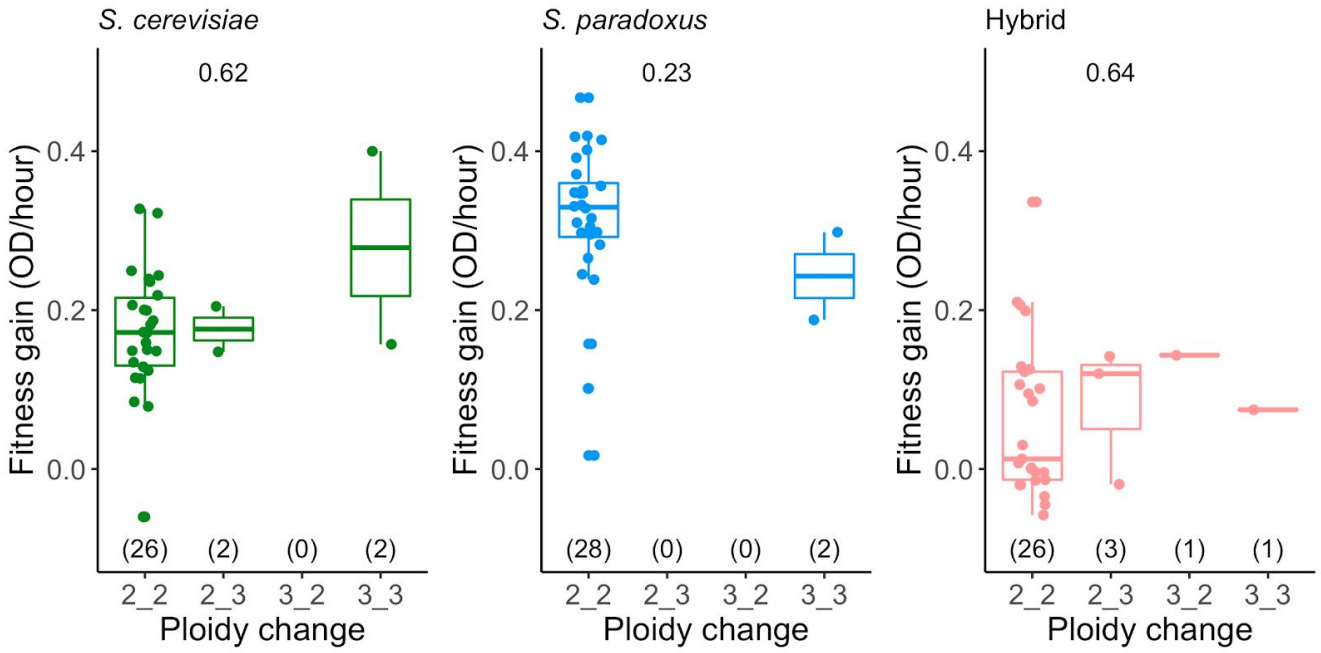

Figure 4. Changes in ploidy do not significantly impact fitness. Fitness gains across experimental evolution in UV mimetic conditions as a function of ploidy change. Numbers before the underscore (X_) represent the starting ploidy and numbers after the underscore (_X) represent the final ploidy. Each point represents the change in growth rate between T200 and T0 in UV mimetic conditions for each experimental line. The number of lines is shown in parentheses below the boxes. A non-parametric Kruskal-Wallis H test was used (n = 30 populations for each genotype). P-values are shown above.

Supporting Information for:

Interspecific Hybrids Show a Reduced Adaptive Potential Under DNA Damaging Conditions

AUTHOR INFORMATION:

Carla Bautista[1,2,3,5], Souhir Marsit [1,2,3,5], Christian R Landry [1,2,3,4,5]

1. Regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines (PROTEO), Québec G1V 0A6, QC, Canada
2. Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada
3. Centre de Recherche en Données Massives de l'Université Laval, Université Laval, Québec, QC, Canada
4. Département de biochimie, de microbiologie et de bio-informatique, Université Laval, G1V 0A6 Québec, QC, Canada
5. Département de biologie, Université Laval, G1V 0A6 Québec, QC, Canada

Correspondence:

1. Corresponding Author: Carla Bautista (c.bautistarourjc@gmail.com) and Christian R Landry (Christian.landry@bio.ulaval.ca)
2. Other e-mail addresses: Souhir Marsit: (souhir.marsit@gmail.com)

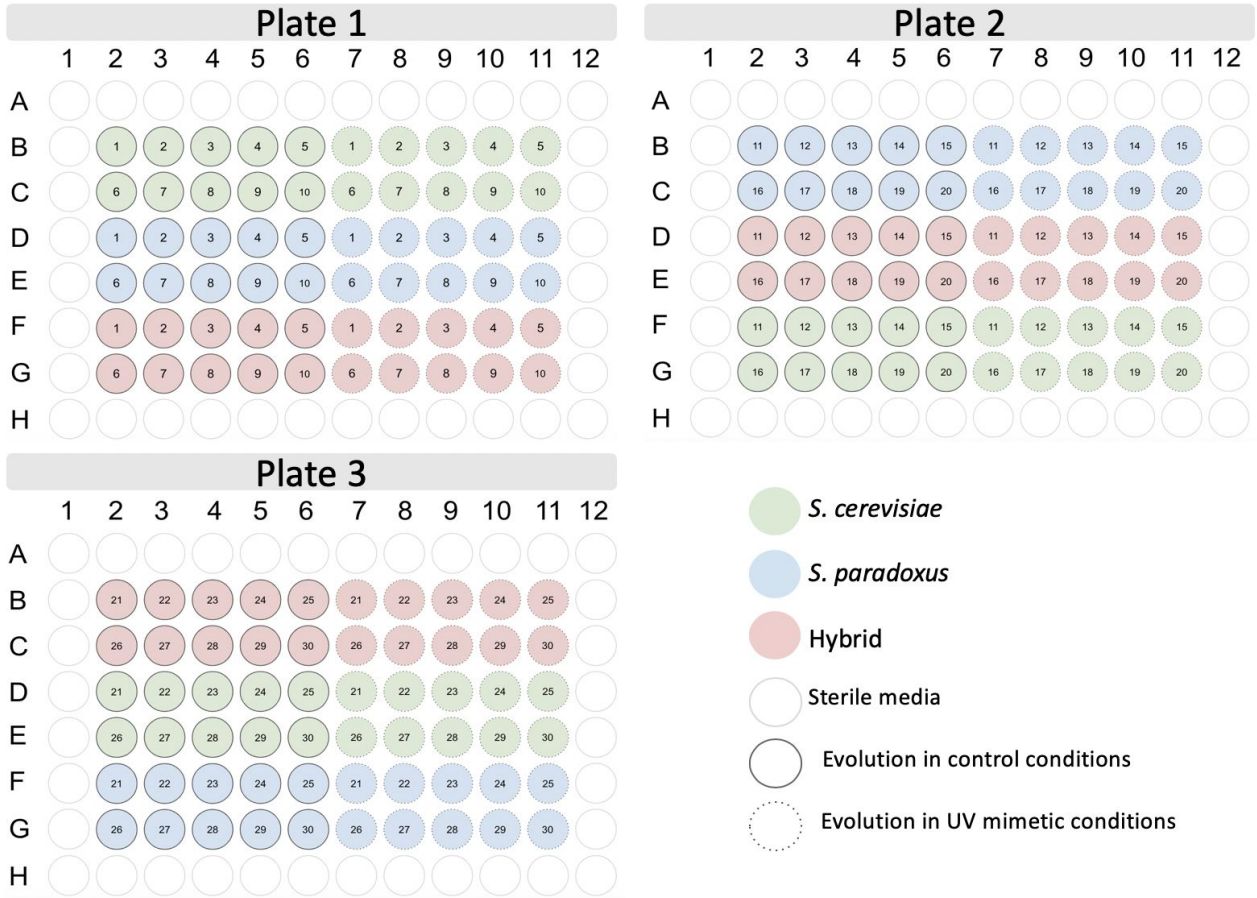

Figure S1. Design of the experimental evolution plates. 30 parallel lines of each genotype were evolved under control and UV mimetic conditions in three different plates. The arrangement of the plates, with the genotypes changed position on each plate, allowed to limit the effect of position on the plate. The borders of the plates did not contain strains and were filled with sterile media to avoid border effects caused by evaporation.

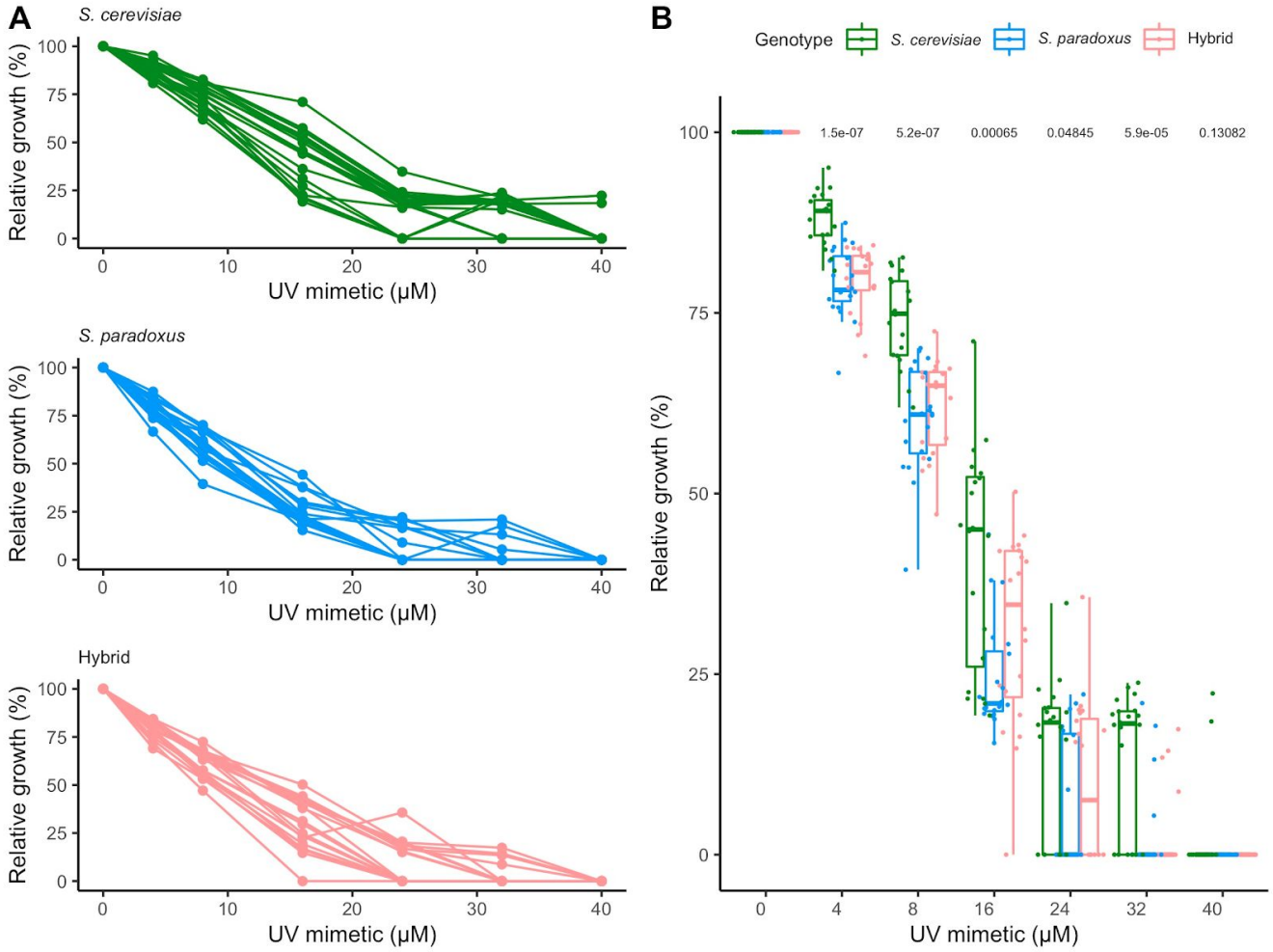

Figure S2. Effect of UV mimetic on growth. Relative growth is calculated by normalizing the growth rate (r) in each concentration with respect to the growth rate (r) of each strain in 0 μM of UV mimetic chemical (n = 20 populations for each genotype). A) Growth of each population of each genotype in control (YPD) and in increasing UV mimetic concentrations (0 to 40 μM) B) Growth of each genotype in control (YPD) cultures and in increasing UV mimetic concentrations (0 to 40 μM). ANOVA among genotypes in each concentration was performed (n = 20 populations for each genotype). P-values are shown above each concentration.

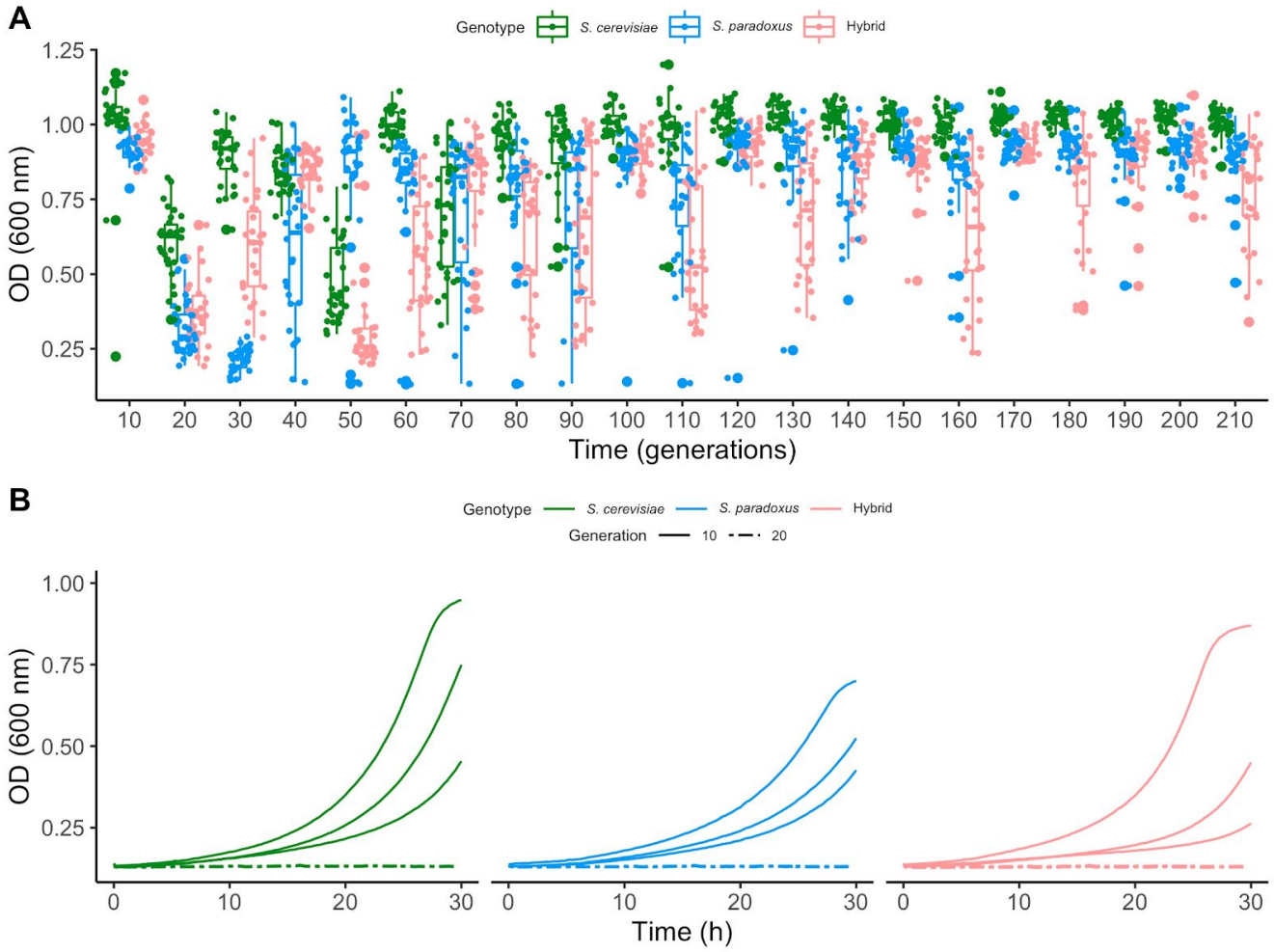

Figure S3. Cell sensitivity to the UV mimetic chemical increased during the two first cycles. A) Final optical density as a function of time (measured in generations) of each genotype in conditions UV mimetic conditions (4 µM of 4-NQO) (n = 30 populations for each genotype). B) Optical density as a function of time (over 30 h) of each genotype in conditions UV mimetic conditions (16 µM of 4-NQO) (n = 3 populations for each genotype)

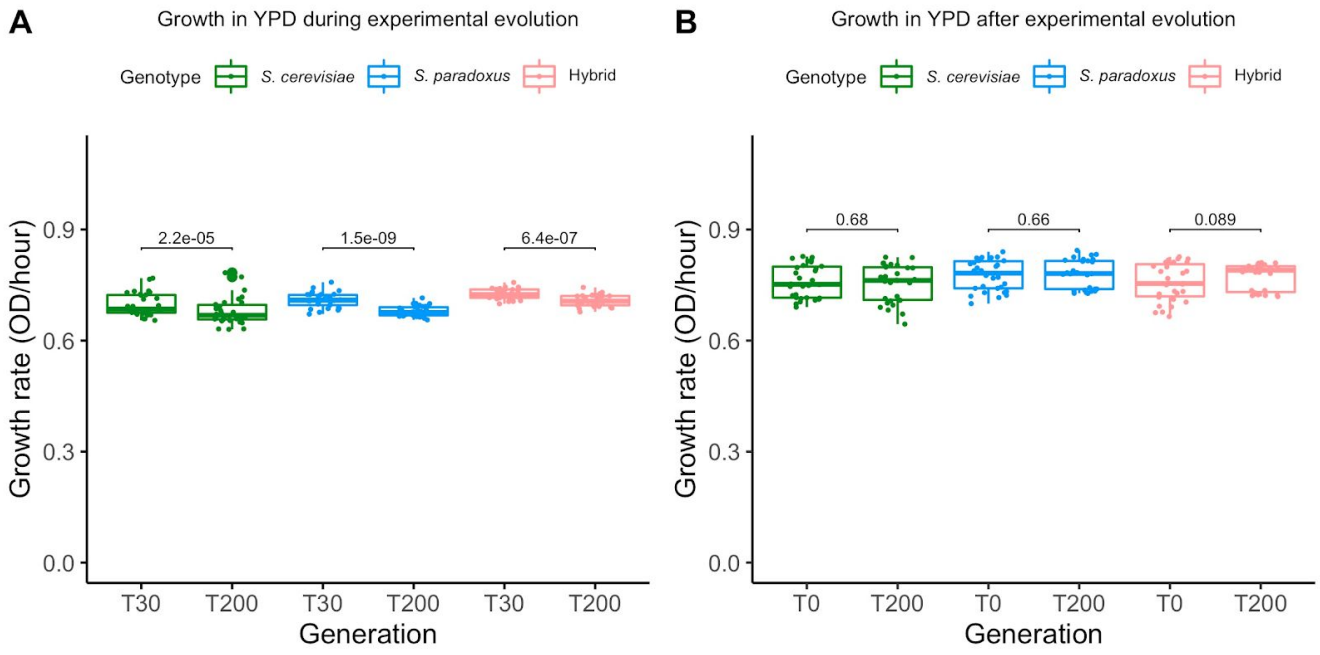

Figure S4. Growth of parents and hybrids in control conditions. A) Growth rates in control conditions for the three genotypes at T30 and T200 during experimental evolution (n = 30 populations for each genotype). B) Growth rates in control conditions for the three genotypes at T0 (ancestors from glycerol) and T200 (evolved at control from glycerol) after experimental evolution. Paired t-tests were performed between growth rate (r) at 0 generations and at 200 generations for each genotype. P-values are shown (n = 30 populations for each genotype).

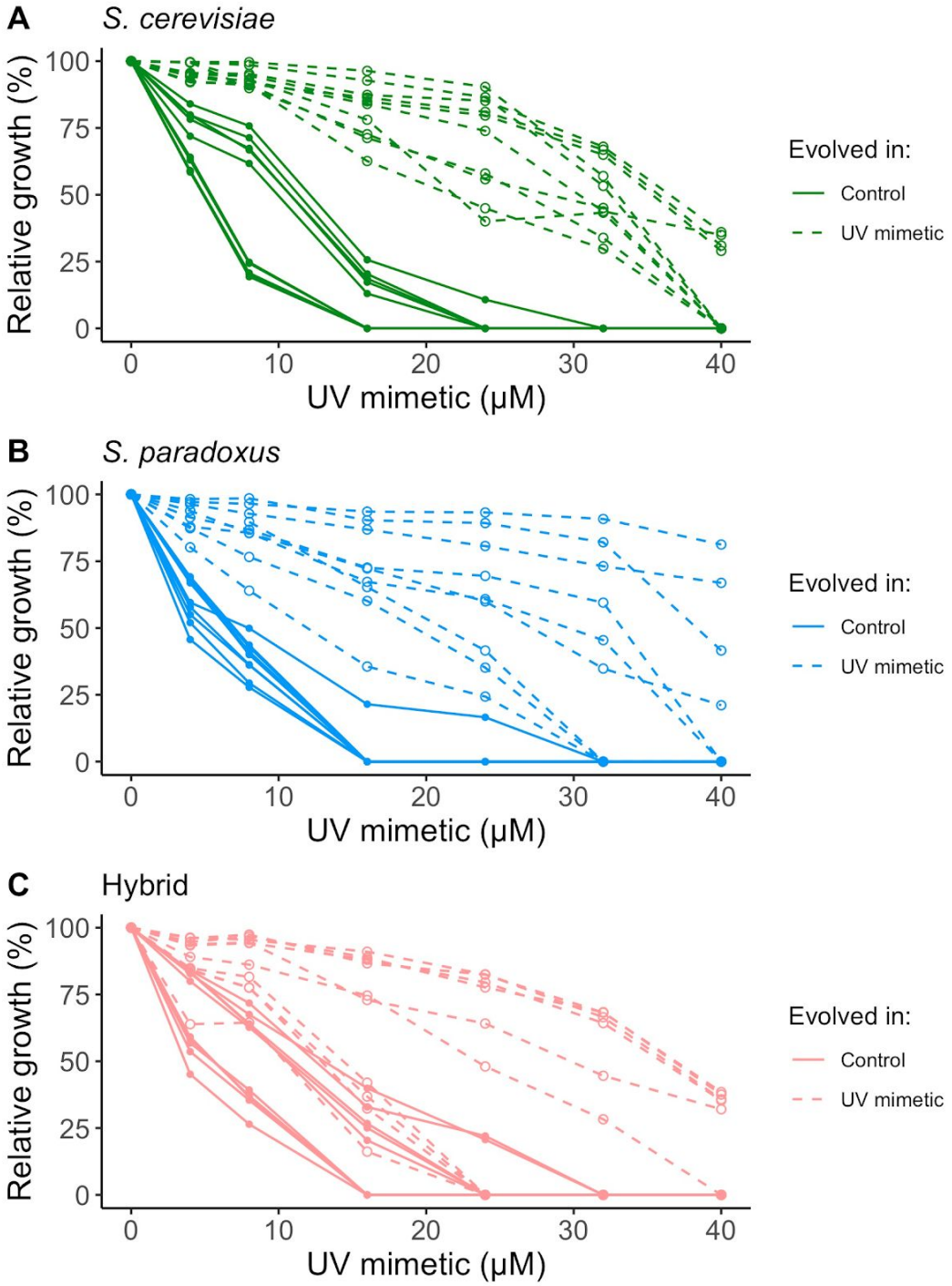

Figure S5. Adaptation to low concentration of UV mimetic leads to adaptation to higher concentrations. A) Growth of each population of each genotype evolved in UV mimetic or control conditions in increasing UV mimetic concentrations (0 to 40 μM) (n = 10 populations for each genotype).

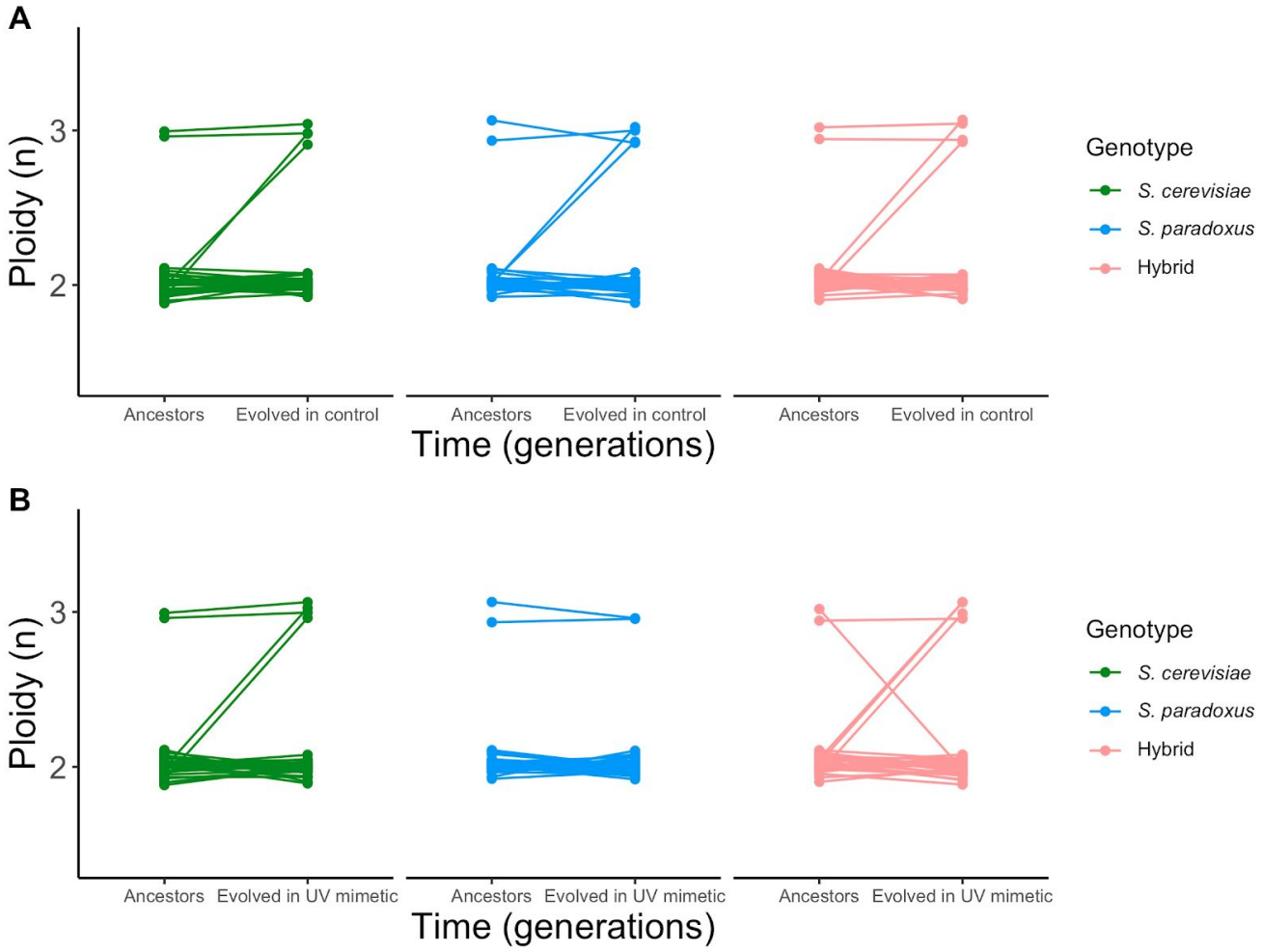

Figure S6. Variation in ploidy across the experiment. A,B) The trajectories of the ploidy of each replicate from the ancestors to the generation 200 are shown both in control (A) and in UV mimetic (B) evolution (n = 30 populations for each genotype).

Tables S1, S2 and S3.
https://docs.google.com/spreadsheets/d/1g1pAOih7rq4US6y498StJ5pWAGdmzmJ6gb5edv45www/edit?usp=sharing