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The Genomic Landscape and Evolutionary Resolution of Antagonistic Pleiotropy in Yeast

Wenfeng Qian,[1] Di Ma,[2,3] Che Xiao,[1,4,5] Zhi Wang,[1,6] and Jianzhi Zhang[1,*]
[1]Department of Ecology and Evolutionary Biology
[2]Department of Cell and Developmental Biology
[3]Life Sciences Institute
University of Michigan, Ann Arbor, MI 48109, USA
[4]School of Life Sciences, Peking University, Beijing 100871, China
[5]Present address: Graduate Program in Genetics, Stony Brook University, Stony Brook, NY 11794, USA
[6]Present address: The Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA
*Correspondence: jianzhi@umich.edu
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SUMMARY

Antagonistic pleiotropy (AP), or genetic tradeoff, is an important concept that is frequently invoked in theories of aging, cancer, genetic disease, and other common phenomena. However, the prevalence of AP, which genes are subject to AP, and to what extent and how AP may be resolved remain unclear. By measuring the fitness difference between the wild-type and null alleles of ~5,000 nonessential genes in yeast, we found that in any given environment, yeast expresses hundreds of genes that harm rather than benefit the organism, demonstrating widespread AP. Nonetheless, under sufficient selection, AP is often resolvable through regulatory evolution, primarily by *trans*-acting changes, although in one case we also detected a *cis*-acting change and localized its causal mutation. However, AP is resolved more slowly in smaller populations, predicting more unresolved AP in multicellular organisms than in yeast. These findings provide an empirical foundation for AP-dependent theories and have broad biomedical and evolutionary implications.

INTRODUCTION

Antagonistic pleiotropy (AP) is a form of pleiotropy (Wagner and Zhang, 2011) in which the relative advantage of two alleles of a gene is reversed in different components of fitness, such as different sexes, developmental stages, and external environments. AP is commonly invoked in explanations and models of senescence (Williams, 1957), cancer (Rodier et al., 2007), genetic disease (Carter and Nguyen, 2011), sexual conflict (Rice, 1992; Innocenti and Morrow, 2010), cooperation (Foster et al., 2004), evolutionary constraint (Carroll, 2005; He and Zhang, 2006), adaptation (Fisher, 1930; Orr, 2000; Wang et al., 2010; Pavlicev and Wagner, 2012), neofunctionalization (Hughes, 1994), and speciation (Berlocher and Feder, 2002). For instance, a prevailing theory of aging asserts that mutations that accumulated during evolution due to their benefits for development and reproduction in early stages of life tend to be deleterious later in life and cause senescence (Williams, 1957). AP dictates that a mutation is unlikely to be advantageous to multiple traits or in multiple environments, leading to compromises among adaptations of different traits or in different environments (Fisher, 1930). This fundamental property limits the extent and rate of adaptation (Orr, 2000), and guarantees that no species will outperform all others in all environments (Levins, 1968).

In contrast to the prominent roles of AP in many theories, our empirical knowledge of AP is limited. Early artificial-selection experiments showed that improving one trait often worsens another, suggesting that AP is not uncommon (Mather and Harrison, 1949; Rice, 1992; Cooper and Lenski, 2000; Ostrowski et al., 2005). In a study in *Drosophila*, Innocenti and Morrow (2010) proposed >1,000 candidate genes that are subject to sexual antagonism, based on correlations between gene expression levels and organismal fitness across 15 genotypes and two sexes. However, because correlation does not imply causation, the actual AP genes remain elusive. As such, neither the prevalence of AP nor the identity of AP genes is known at the whole-genome scale, although individual cases of AP genes have been reported in recent years (Lang et al., 2009; Magwire et al., 2010; Wenger et al., 2011). The extent to which AP may be resolved evolutionarily, which genetic mechanisms are mainly responsible for AP resolution, and which population genetic parameters are conducive to AP resolution also remain unclear. Here we address these fundamental questions by using a combination of genomics, genetics, and modeling, based on the principle that AP of a gene between two environments is proven when deletion of the gene lowers the organismal fitness in one of the environments but improves it in the other.

RESULTS

Identification of AP Genes

To quantify AP at the genomic scale, we took advantage of a collection of yeast gene deletions that Giaever et al. (2002) constructed by individually knocking out 4,642 nonessential genes and 11 pseudogenes from a laboratory strain of *Saccharomyces*

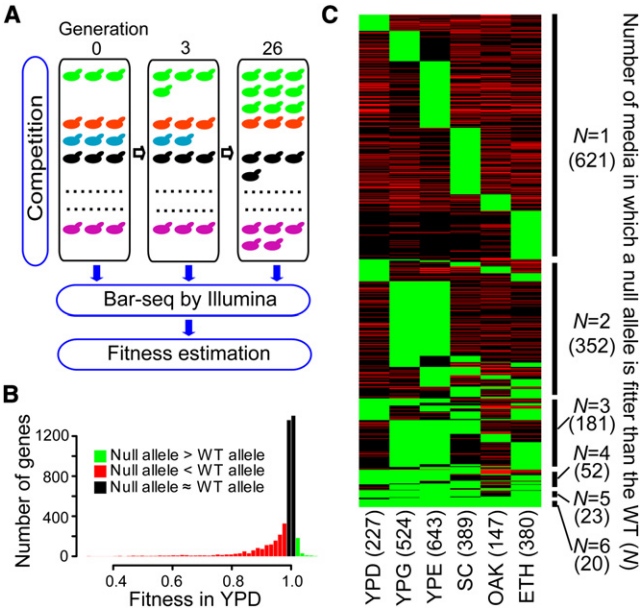

Figure 1. Genome-wide Identification of Yeast Genes Subject to AP in Six Environments

(A) High-throughput fitness estimation. All ~5,000 nonessential gene deletion strains were grown together in one of six different media. Fitness was estimated from strain frequencies quantified by Bar-seq at the beginning (0th generation) and end (3rd or 26th generation) of each competition. Each color depicts one yeast genotype.

(B) The fitness spectrum of gene deletion strains, relative to the WT, in YPD. >, significantly fitter; <, significantly less fit; ≈, no significant difference in fitness.

(C) Genes with null alleles fitter than WT in at least one medium. Each row represents a gene and each column represents a medium. The color scheme is the same as in (B). The number of genes whose null alleles are significantly fitter than WT in each of the six media is shown in parentheses following the medium. The number of genes whose null alleles are significantly fitter than WT in N = 1, 2, …, and 6 media is indicated in parentheses below the N values. See also Figure S1, and Tables S1 and S2.

cerevisiae, and placing in each deletion strain a unique 20-nucleotide barcode that can be amplified by universal primers. We grew all of the homozygous deletion strains together and quantified their relative frequencies at multiple time points by amplifying and then sequencing the barcodes using the Illumina-based Bar-seq method (Smith et al., 2009), which provides a large dynamic range and low background noise (Smith et al., 2009; Figure 1A; Table S1; see Experimental Procedures). Bar-seq digitally counts every strain, whereas the previous microarray-based method (Giaever et al., 2002) does not provide a signal that is linear with the frequency of a strain. Although amplification biases from PCR may exist in library preparation for Illumina sequencing, the biases should not affect our fitness measurement because fitness is estimated by comparing the frequency of a strain between two samples obtained at different time points, and the PCR biases are canceled out from the between-sample comparisons. Similarly, Illumina sequencing biases (Dohm et al., 2008) do not affect our fitness measurement because the effects are canceled out when two samples obtained from two time points are compared. It has been reported that Illumina sequencing has a relative high sequencing error (1%). However, such errors do not affect our results because any two barcodes differ from each other by at least five nucleotides (Shoemaker et al., 1996), beyond what sequencing errors can do. We discarded all sequencing reads that differed from the known barcodes by more than one nucleotide.

Fitness was measured in six distinct media representing a subset of the diverse environments that wild, domesticated, and laboratory yeast strains have experienced, termed the rich medium (YPD), glycerol medium (YPG), ethanol medium (YPE), synthetic complete medium (SC), synthetic oak exudate medium (OAK), and rich medium with 6% ethanol (ETH). We estimated the fitness of each deletion strain relative to the wild-type (WT) by using the 11 pseudogene deletion strains as 11 biological replicates of the WT. By contrast, previous high-throughput fitness quantifications lacked WT references and effectively used the weighted average strain in the whole population as the reference (Giaever et al., 2002; Steinmetz et al., 2002; Deutschbauer et al., 2005; Dudley et al., 2005; Hillenmeyer et al., 2008), which would be problematic for identifying beneficial null alleles, for two reasons. First, because the frequencies of low-fitness strains decrease in competition, the fitness of the weighted average strain increases during competition, which makes fitness estimation unreliable. Second, because there are many low-fitness strains in the population, the average fitness of the population is lower than the fitness of the WT. Thus, a strain that was found to be fitter than the population average in earlier studies may not be fitter than the WT. In our study, we used the 11 pseudogene deletion strains to estimate the SD of our fitness measurement, which allowed us to estimate the probability (p value) that the fitness of a deletion strain equals the WT and the corresponding Q value after considering multiple testing (see Experimental Procedures).

Under YPD, 62.2% of the nonessential gene deletion strains are not significantly different from the WT in fitness ($Q > 0.01$), while 32.6% are significantly less fit ($Q < 0.01$) and 5.1% are significantly fitter ($Q < 0.01$; Figure 1B; Table S2). Qualitatively similar observations were made in the other five media (Figure S1; Table S2). The number of deletion strains that are significantly fitter than the WT varies from 147 to 643 in the six media (Figure 1C), with decreasing numbers of strains that are fitter than the WT in more media (Figure 1C).

The reliability of our fitness estimation is reflected by the high Pearson's correlation coefficient between two biological replicates ($r = 0.94$; Figure 2A), low false-negative rate (only one of 11 previously identified beneficial null alleles (Sliwa and Korona, 2005) was not rediscovered here; Table S3), and small fitness variation among the 11 pseudogene deletion strains in most media (Figure 2B; Table S4; see Extended Discussion). It is also important to estimate the false-positive rate, because the fitness of a strain in a pool of thousands of strains could be different from that in a pairwise competition with the WT, due to potential interactions among strains. To gauge the false-positive rate, we randomly chose 24 gene deletion strains that are fitter than the WT in Bar-seq, and remeasured their fitness by a more accurate low-throughput method involving pairwise competition with the WT (He et al., 2010). We found the fitness

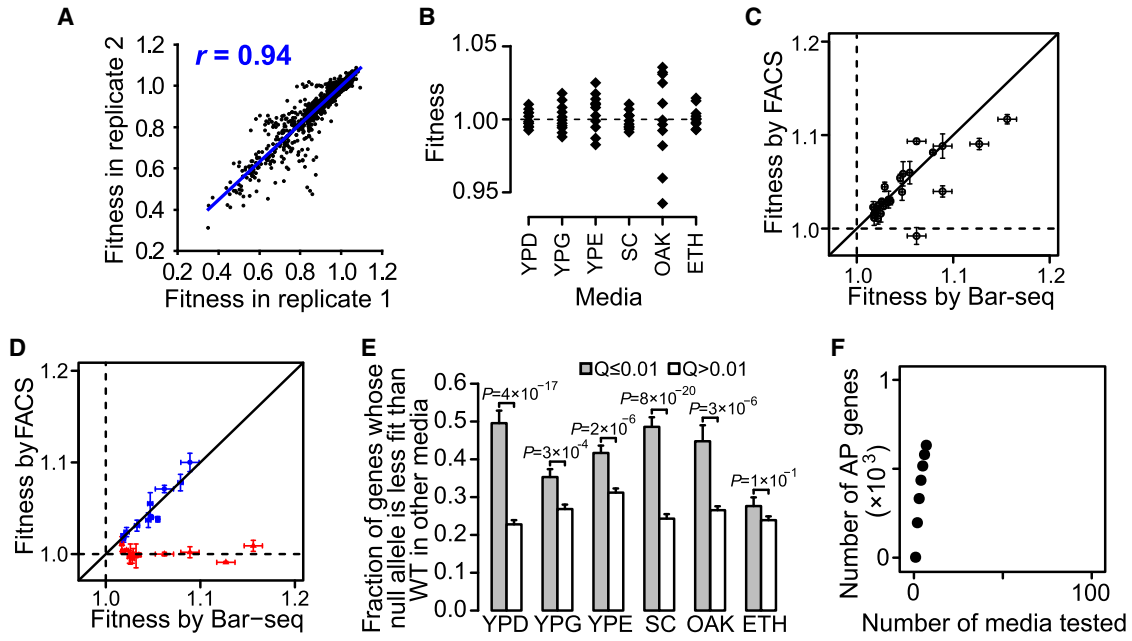

Figure 2. Validations of Bar-Seq Results

(A) Fitness estimates in YPD are highly correlated between two biological repeats ($r = 0.94$; $p < 10^{-4899}$).

(B) Fitness of 11 pseudogene deletion strains in six media. The unusually high variation under OAK is caused by the low number of sequencing reads obtained (see Extended Discussion and Table S1).

(C) Fitness values of 24 randomly chosen AP gene deletion strains estimated by Bar-seq and by a more accurate low-throughput method (fluorescence-activated cell sorting [FACS]). Error bars represent 1 SE.

(D) Fitness values of 24 randomly chosen AP gene deletion strains estimated by Bar-seq and those of their corresponding independently generated deletion strains measured by FACS. Those confirmed by FACS to be subject to AP (blue dots) show highly correlated fitness estimates by two methods ($y = 1.0819\,x - 0.0863$, $r = 0.95$, $p = 6.6 \times 10^{-6}$), whereas the unconfirmed values (red triangles) show no correlation ($y = 0.0003\,x + 1.0004$, $r = 0.002$, $p = 0.97$). Error bars represent 1 SE.

(E) Compared with gene deletion strains equally fit as the WT in a medium (open bar), those that are significantly fitter (gray bar) are more likely to be less fit than the WT in other media. Error bars represent 1 SE. Statistical significance (p value) is determined by chi-square test.

(F) The number of observed AP genes ($Q < 0.01$) increases with the number of media tested. The numbers have been corrected for false-positive and false-negative errors.

See also Tables S3, S4, and S5.

estimates from the two methods to be largely consistent ($r = 0.80$; $p = 3 \times 10^{-6}$), and 22 of the 24 strains were again significantly fitter than the WT (Figure 2C; Table S5). All of the above analyses are based on the assumption that the genotypes of the deletion strains are correct; however, a number of secondary mutations in the yeast gene deletion collection have been reported (Hughes et al., 2000). Because beneficial secondary mutations are more likely than deleterious ones to be included in the collection, secondary mutations tend to cause false positives. To estimate the impact of such mutations, we independently deleted the above 24 genes and measured the fitness values of these new deletion strains by the low-throughput method (He et al., 2010). We found that 46% of these genes could be confirmed (Figure 2D; Table S5). These results suggest that most false positives arose from secondary mutations accumulated in the gene deletion collection rather than from Bar-seq errors or strain-strain interactions.

Strictly speaking, AP is inferred when the null allele of a gene is (1) fitter than the WT in at least one condition and (2) less fit than the WT in at least one condition. We dropped the second criterion here because it should have been met by all of the genes examined; otherwise, the null allele would have been fixed in the species (Extended Discussion). Indeed, compared with null alleles having fitness similar to that of the WT ($Q > 0.01$) in a medium, those significantly fitter than the WT ($Q < 0.01$) in the medium tend to be less fit than the WT in other media (Figure 2E). Under the first criterion, we identified 1,249 AP genes, 566 of which also satisfy the second criterion. After considering the false-negative and false-positive rates, we estimated that $1249 \times (11/24) \times (11/10) = 630$ genes, or 13.6% of all nonessential genes examined, are subject to AP. This is likely a conservative estimate, for three reasons: first, although AP can potentially occur between any two alleles at a locus, only two specific alleles per locus are compared here. Second, because the number of identified AP genes increases with the number of media examined (Figure 2F), and because yeast experiences more than six environments in nature, the actual number of genes subject to AP should be much greater than estimated here. Third, although our fitness measure is more sensitive than all other high-throughput methods, its sensitivity (~0.01) is still lower than

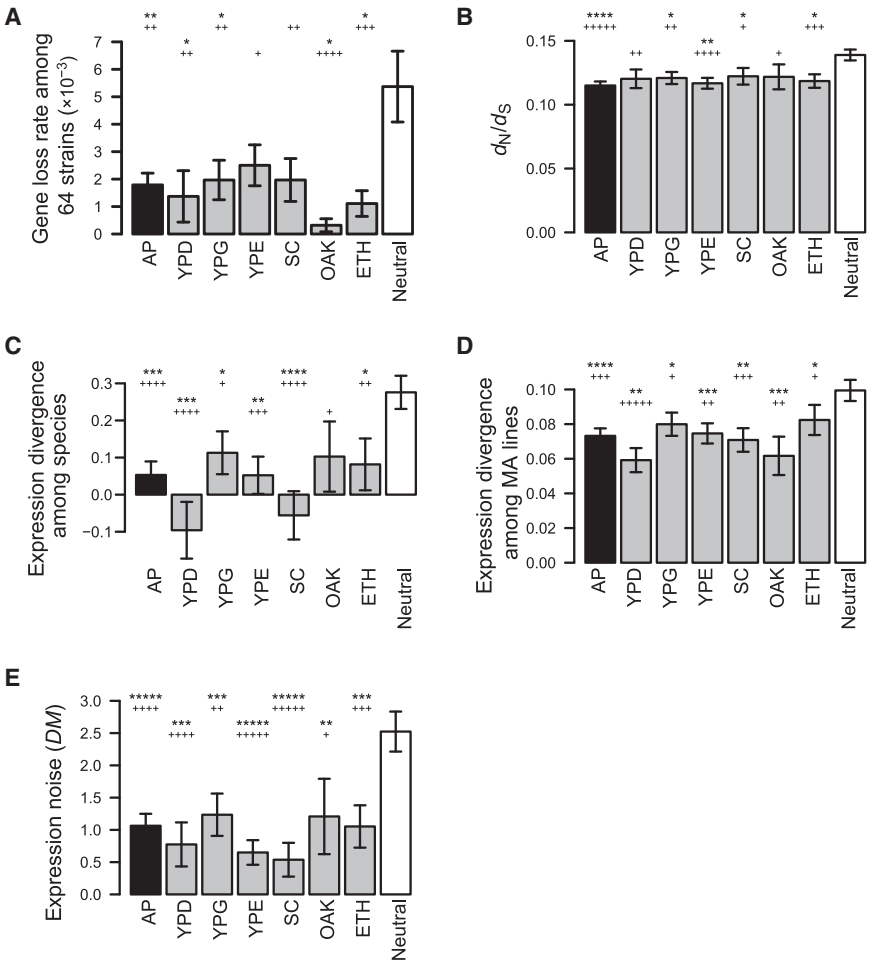

Figure 3. Properties of AP Genes Compared with Neutral Genes

AP genes are those whose null alleles are significantly fitter than the WT in at least one of the six media. Neutral genes are those whose null alleles do not significantly differ in fitness from the WT in any of the six media. In all panels, p values were obtained by Mann-Whitney U test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; *****$p < 0.00001$) or t test ($^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{++++}p < 0.0001$; $^{+++++}p < 0.00001$). Error bars indicate 1 SE.

(A) Gene loss rates (per gene per strain) in 64 strains of diverse origins are lower among the entire set of 1,249 AP genes (black bar) or AP genes identified from individual media (gray bars) than among 1,344 neutral genes (open bar).

(B) Ratios of the number of nonsynonymous substitutions per nonsynonymous site (d_N) and the number of synonymous substitutions per synonymous site (d_S) between *S. cerevisiae* and *S. paradoxus* are lower for AP genes than for neutral genes.

(C) Gene expression divergences among four *Saccharomyces* species are lower in AP genes than in neutral genes.

(D) Gene expression divergences among yeast MA lines are lower in AP genes than in neutral genes.

(E) Gene expression noise is lower for AP genes than for neutral genes.

that of natural selection, which can detect a fitness differential as small as the inverse of the effective population size (N_e), which is $\sim 10^7$ in yeast (Wagner, 2005). Thus, there are likely many more genes that are subject to milder AP than were detected here.

Properties of AP Genes

The identified AP genes differ in several aspects from "neutral genes," which showed similar fitness between null and WT alleles ($Q > 0.01$) in all six media examined. First, when we sampled a diverse panel of 64 strains from different environments, we found that AP genes were less likely to be lost than neutral genes (Figure 3A; see Experimental Procedures), suggesting that, overall, AP genes are more important and less dispensable than neutral genes. Second, natural selection acting on the coding sequence of a gene can be quantified by the ratio of the number of nonsynonymous substitutions per nonsynonymous site (d_N) to the number of synonymous substitutions per synonymous site (d_S). We found d_N/d_S to be lower for AP genes than for neutral genes when *S. cerevisiae* was compared with its sister species *S. paradoxus* (Figure 3B), suggesting a stronger purifying selection on the coding sequences of AP genes compared with neutral genes. Third, AP genes showed lower expression divergences than neutral genes when the microarray gene expression data from several yeast species (*S. cerevisiae*, *S. paradoxus*, *S. mikatae*, and *S. kudriavzevii*; Tirosh et al., 2006) were compared (Figure 3C). This difference could be due to smaller mutational target sizes and/or a stronger purifying selection on the expression of AP genes compared with neutral genes. We found that AP genes had lower expression divergences than neutral genes in a set of mutation accumulation (MA) lines of yeast (Landry et al., 2007; Figure 3D). Because MA lines are subject to virtually no natural selection, the above finding indicates that AP genes have smaller mutational targets for expression changes than neutral genes. Stochastic gene expression variation among isogenic cells, or expression noise (Newman et al., 2006), reflects the strength of purifying selection acting on gene expression variation (Batada and Hurst, 2007; Lehner, 2008; Wang and Zhang, 2011) and is not influenced by mutational target size. We found that AP genes have smaller expression noise than neutral genes (Figure 3E), suggesting a stronger purifying selection on expression level in AP genes compared with neutral genes. Therefore, both smaller mutational target sizes and stronger purifying selection contribute to the lower expression divergences of AP genes compared with neutral genes. Taken together, all of these analyses consistently show a stronger purifying selection acting on AP genes than on neutral genes. Note that the above observations are valid not only for all AP genes as a whole (black bars in Figure 3) but also for AP genes identified from each environment (gray bars in Figure 3).

By definition, the expression of an AP gene reduces fitness in some environments. What are the underlying molecular mechanisms of these adverse effects? We found that AP genes are enriched or deprived in a number of Gene Ontology (GO) categories (Table S6; see Experimental Procedures). For instance, compared with all of the genes in the genome, genes with a null allele fitter than the WT allele under ETH are enriched in six GO categories, after controlling for multiple-hypothesis testing (Table S6). These six GO categories can be further divided into three groups: phospholipid transport, endoplasmic-reticulum-associated protein catabolic process, and heterochromatin (Figure 4), which appear to be related to the known cellular effects of ethanol. For example, ethanol influences cell membrane integrity (Ingram and Buttke, 1984), and ethanol tolerance relies on the phospholipid composition of the cell membrane (Mishra and Prasad, 1988). Phospholipid transporters enable directed movements of phospholipids and thus may be harmful under high ethanol concentrations. In addition, ethanol induces the production of endogenous DNA-damaging molecules (Brooks, 1997) and interferes with chromatin condensation (Talebi et al., 2011). Thus, the expression of genes related to heterochromatin could be deleterious in the presence of ethanol. Because ethanol metabolism disrupts protein catabolism (Donohue, 2009), the expression of genes involved in protein catabolism could be harmful in ETH. Although the exact molecular mechanisms of specific AP remain to be determined in future detailed studies, the enriched and deprived GO categories offer insights for such studies. Complementing most previous studies that provided lists of genes that are vital to specific traits or biological processes, our study provides lists of genes that are detrimental to these traits or processes. Such information is important for a complete understanding of the mechanisms underlying these traits or processes. Because false-positive detections of AP genes would have blurred the true differences between AP genes and neutral genes, our statements about the detected differences are conservative.

Evolutionary Resolution of AP

In theory, AP between a functional allele and a null allele of a gene can be resolved by lowering the expression of the functional allele in the environment where it is harmful. Two hypotheses may explain the unresolved AP in the laboratory yeast we studied: (1) there is a paucity of regulatory mutations that can resolve AP, or (2) there is a paucity of selection for the fixation of such mutations if the environment concerned is rarely encountered. To distinguish between these hypotheses, we examined four yeast strains that have adapted to their respective ecological niches. The second hypothesis is supported if AP involving the native environment of a strain has been largely resolved; otherwise, the first hypothesis is supported.

We began by confirming our prior knowledge (Warringer et al., 2011) about the adaptations of the four strains to their respective environments (Figure 5A) by measuring their relative fitness in four media that approximate the four environments (Figure 5B). For instance, the sake strain K12 is expected to (and indeed does) have the highest fitness in the rich medium with 6% ethanol (ETH) among the four media tested. If AP is resolvable by sufficient natural selection, we can make three predictions about a gene whose expression is beneficial in environment A but harmful in environment B. First, the expression level of the gene in B should be lower for a strain more adapted to B than for a strain less adapted to B. Second, for a strain adapted to both environments, the expression of the gene should be lower in B than in A. Third, a strain that has adapted to both A and B should have a greater expression difference between these two environments than a strain that has adapted to only one of the environments.

We tested these predictions by quantifying the expression levels of the validated AP genes in Figure 2C (see Experimental Procedures). For example, *PDR17* encodes a phosphatidylinositol transfer protein that participates in phospholipid synthesis and transport, and is involved in resistance to multiple drugs. Its null allele is fitter than the functional allele in YPG, but the opposite is true in ETH (Figure 5C). We measured the messenger RNA (mRNA) concentrations of *PDR17* from two strains (M22 and K12) in two media (YPG and ETH). We observed that (1) in YPG, *PDR17* expression is lower for the strain better adapted to YPG (M22) than for the strain less adapted to YPG (K12; Figure 5D); (2) for M22, *PDR17* expression is lower in YPG than in ETH (Figure 5E); and (3) the expression difference between the two media is greater for the strain adapted to both environments (M22) than for the strain adapted to only one environment (K12; Figure 5F). In total, the three predictions are respectively supported by 31 of 35 (Figure 5G; Table S7), 22 of 25 (Figure 5H; Table S8), and four of five (Figure 5I; Figure S2) cases examined.

In addition to transcriptional regulation, we observed protein subcellular relocalization (Komeili and O'Shea, 2000) in AP resolution. *MIG1* encodes a transcription factor that functions exclusively inside the nucleus in glucose repression (Schüller, 2003). Its functional allele is fitter than the null allele in YPD, but the opposite is true in OAK (Figure 5J). In the wild strain YPS1000, which is adapted to an environment mimicked by the OAK medium, MIG1 is localized to the nucleus under YPD. However, under OAK, where MIG1 would be deleterious, MIG1 is localized to the cytoplasm (Figure 5K) and hence imposes no harm. Together, the findings of many AP-mitigating regulations at the transcriptional or posttranscriptional levels strongly suggest that the unresolved AP in the laboratory strain is largely attributable to a paucity of selection rather than a paucity of mutation, consistent with a recent report that the mutational target size for expression alterations of a gene is substantial (Gruber et al., 2012). Also consistent with this conclusion is the observation that, in the laboratory strain that is adapted to YPD (Figure 5A), relatively few null alleles are fitter than the WT allele under YPD compared with other media (Figure 1C; Extended Discussion).

Genetic Mechanisms of AP Resolution

To understand the genetic basis of the environment-specific transcriptional regulation that mitigates AP, we investigated whether this regulation arose from *cis*-acting changes, which act through the same DNA molecule that encodes the focal gene, or *trans*-acting changes, which operate via diffusible molecules. We crossed two parental diploid strains (M22 and K12) to make a hybrid strain (M22 × K12) and used pyrosequencing to


Ubiquitin-dependent protein catabolic process
Proteasomal ubiquitin-dependent protein catabolic process
ER-associated protein catabolic process
Proteasomal protein catabolic process
Modification-dependent macromolecule catabolic process
Modification-dependent protein catabolic process
Proteolysis involved in cellular protein catabolic process
Proteolysis
Phospholipid transport
Macromolecule localization
Phospholipid transporter activity
Lipid transport
Localization
Metabolic process
Macromolecule metabolic process
Primary metabolic process
Protein metabolic process
Lipid transporter activity
Transport
Establishment of localization
Biological process
Catabolic process
Cellular protein catabolic process
Cellular macromolecule catabolic process
Protein catabolic process
Substrate-specific transporter activity
Transporter activity
Molecular function
Gene ontology
Cellular process
Cellular catabolic process
Macromolecule catabolic process
Cellular protein metabolic process
Cell
Cellular component
Cellular metabolic process
Cellular macromolecule metabolic process
Membrane-enclosed lumen
Cell part
Intracellular
Organelle
Non-membrane-bounded organelle
Organelle part
Intracellular non-membrane-bounded organelle
Intracellular part
Organelle lumen
Membrane-bounded organelle
Nuclear heterochromatin
Nuclear chromatin
Heterochromatin
Chromosome
Chromatin
Intracellular organelle part
Intracellular organelle
Chromosomal part
Nuclear chromosome, telomeric region
Nuclear telomeric heterochromatin
Intracellular organelle lumen
Nuclear lumen
Nuclear chromosome
Intracellular membrane-bounded organelle
Nucleus
Nuclear part
Nuclear chromosome part
Chromosome, telomeric region
Telomeric heterochromatin
P values
3×10-5
0.05
0
# of genes in the GO category
3
1000
4450
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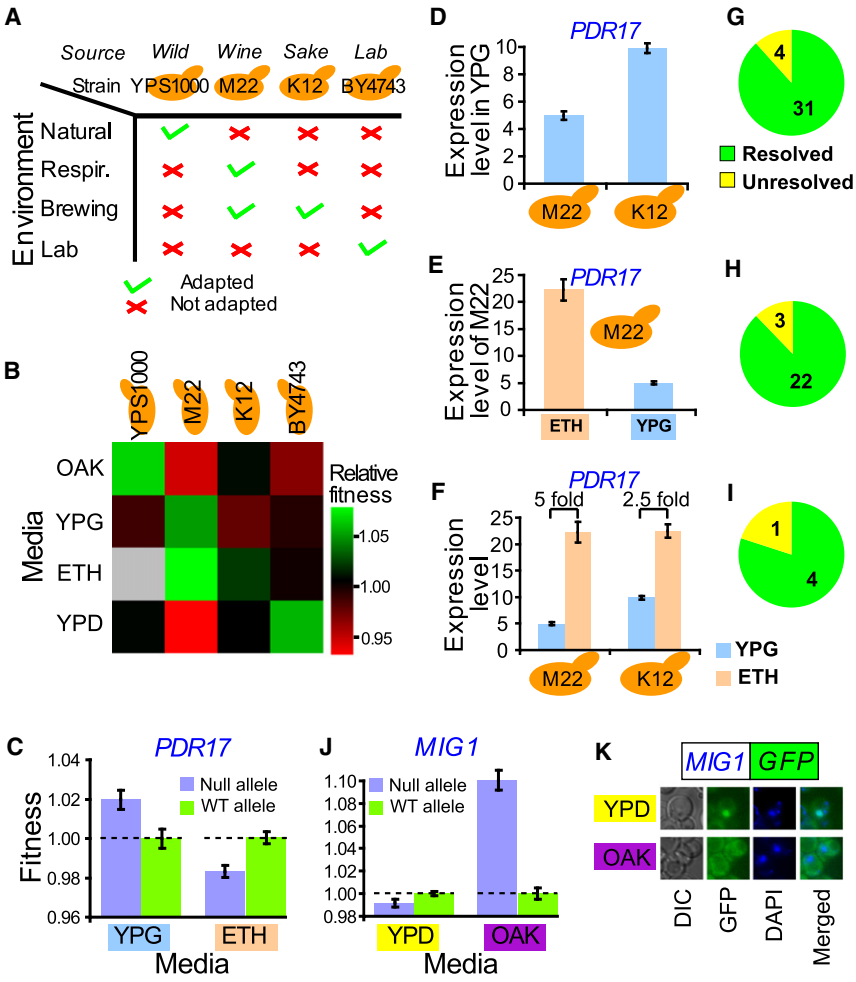

Figure 5. AP Is at Least Partially Resolved by Gene Regulation in the Presence of Sufficient Selection

(A) Prior knowledge about the native environments of various yeast strains. Respir., respiration.

(B) Relative fitness of four yeast strains in four media. The values are directly comparable across rows and across columns (see Experimental Procedures). Due to severe flocculation of YPS1000 in ETH, its fitness could not be measured.

(C) The null allele of *PDR17* is fitter than the WT in YPG ($p = 2 \times 10^{-5}$) but less fit than the WT in ETH ($p = 6 \times 10^{-8}$). In (C)–(F) and (J), error bars show 1 SE.

(D) *PDR17* expression under YPG is lower in M22 than in K12 ($p = 2 \times 10^{-4}$).

(E) *PDR17* expression of M22 is lower under YPG than under ETH ($p = 3 \times 10^{-3}$).

(F) The expression-level difference between YPG and ETH is greater for M22 than for K12 ($p = 0.004$).

(G–I) Numbers of examined genes in which AP is at least partially resolved (green) or unresolved (yellow) by transcriptional regulation, based on the same three tests shown for *PDR17* in (D)–(F), respectively.

(J) The null allele of *MIG1* is less fit than the WT in YPD ($p = 0.05$) but fitter than the WT in OAK ($p = 3 \times 10^{-48}$).

(K) In the wild strain YPS1000, MIG1 is localized in the nucleus under YPD, but in the cytoplasm under OAK. MIG1-green fluorescent protein (GFP) fusion protein allows the visualization of MIG1's subcellular localization. DAPI stains the nucleus in blue. DIC, differential interference contrast microscopy.

See also Figure S2, Table S7, and Table S8.

measure allele-specific expressions in the hybrid as well as in mixed parents. The expression difference between the two alleles in the hybrid is caused by *cis*-acting changes, whereas the difference in allele-specific expression ratio (M22/K12) between the hybrid and mixed parents is caused by *trans*-acting changes (Wittkopp et al., 2004).

We examined three AP genes with large environment-specific expression regulation. For *PDR17*, the M22/K12 expression ratio in the hybrid is not significantly different from one, under either YPG or ETH (Figure 6A; $p = 0.50$ and $p = 0.70$, respectively; two-tailed t test), suggesting a lack of *cis*-acting differences between the two strains. Consistent with the results in Figure 5F, the M22/K12 expression ratio is significantly <1 in mixed parents under YPG ($p = 0.01$) but not under ETH ($p = 0.44$; Figure 6A). Thus, the YPG-specific *PDR17* expression divergence between M22 and K12 is primarily caused by *trans*-acting changes. A similar conclusion can be made for the second examined gene, *APQ12* (Figure 6B; Figure S3).

The third gene studied, *STP4*, showed a different mechanism. *STP4* encodes a transcription factor that is involved in multiple cellular processes and drug resistance. The null allele is fitter than the functional allele in YPG, but this relation is reversed in ETH (Figure 6C). We found the M22/K12 expression ratio of *STP4* in YPG to be <1 by a similar amount in mixed parents and the hybrid ($p = 0.97$, two-tailed t test; Figure 6D), indicating that the *STP4* expression divergence between M22 and K12 in YPG is primarily caused by *cis*-acting changes. We suspected that a 250-nucleotide promoter region of *STP4* that harbors four single-nucleotide differences between the two strains is responsible for the expression divergence between them in YPD. To test this hypothesis, we swapped this region between the two strains in haploid cells. Indeed, *STP4* expression in

Figure 4. Significantly Overrepresented GO Categories for Genes whose Null Alleles are Fitter than the WT in ETH

GO categories and their "parents" in the GO hierarchical architecture are connected by arrows. Blue arrows represent the "is a" relation and green arrows represent the "part of" relation. Node colors represent the p values of overrepresentation, and the cyan circle around a node indicates a significantly over-represented GO category (FDR < 0.05). Node size reflects the number of genes in the GO category.

See also Table S6.

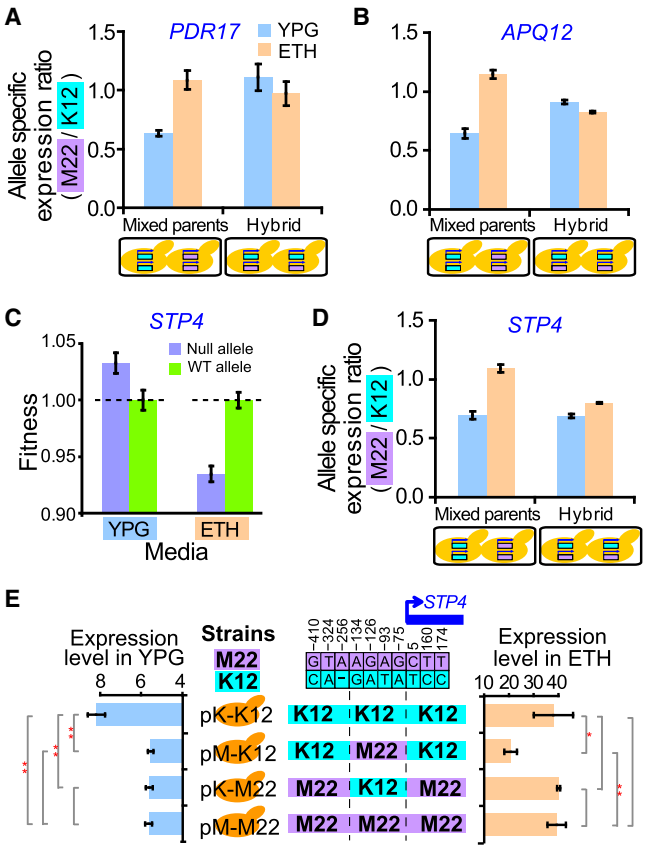

Figure 6. Genetic Mechanisms of AP-Alleviating, Environment-Specific Expression Regulations

(A) *PDR17* expression ratios between M22 (purple) and K12 (blue) alleles in mixed diploid parents and in hybrids.

(B) *APQ12* expression ratios between M22 (purple) and K12 (blue) alleles in mixed diploid parents and in hybrids.

(C) The null allele of *STP4* is fitter than the WT under YPG ($p = 5 \times 10^{-4}$) but less fit than the WT under ETH ($p = 5 \times 10^{-21}$).

(D) *STP4* expression ratios between M22 and K12 alleles in mixed diploid parents and in hybrids.

(E) Localization of causal mutation(s) responsible for the lowered *STP4* expression of M22, compared with K12, under YPG. Nucleotide differences between the two strains in the region between 442 nucleotides upstream and 238 nucleotides downstream of the translation starting site are presented together with their positions relative to the translation starting site. We swapped between haploid strains of M22 (pM-M22) and K12 (pK-K12), a 250-nucleotide proximate promoter region that contains four single-nucleotide differences, to create two mosaic strains (pM-K12 and pK-M22). The expression levels of *STP4* in the four strains under YPG and ETH are depicted. In all panels, error bars show 1 SE. The single red asterisk indicates significantly different expression levels at $p < 0.05$ between two genotypes connected by a gray line, and double red asterisks indicate $p < 0.01$.

See also Figure S3.

K12 was reduced to the M22 level when its promoter was replaced with that of M22 (Figure 6E), suggesting that one or more of the four-nucleotide mutations caused the expression difference between M22 and K12. Interestingly, *STP4* expression in M22 was not enhanced by use of the K12 promoter ($p = 0.84$; Figure 6E), demonstrating a genetic-background-specific effect of these regulatory changes.

The above regulatory mutations that are beneficial to M22 under YPG may be harmful under ETH, because *cis*-acting changes tend to affect gene expression in multiple conditions (Smith and Kruglyak, 2008). Indeed, the M22 allele had a lower expression compared with the K12 allele in the hybrid under ETH ($p = 0.001$; Figure 6D), and replacing the native promoter with the M22 promoter in K12 lowered *STP4* expression in ETH as in YPG ($p = 0.05$; Figure 6E). Nevertheless, this deleterious *cis* effect in ETH is compensated for by *trans*-acting changes, as is evident from the comparison of the M22/K12 expression ratio in mixed parents and the hybrid ($p = 0.0003$; Figure 6D). In summary, *trans*-acting changes were found in all three cases of AP resolution examined, whereas only one case involved an additional *cis*-acting change.

Population Genetics of AP Resolution

Our observation that although most AP is at least partially resolvable, AP was still present in many genes in the laboratory strain prompted us to determine which population genetic parameters are conducive to AP resolution. Specifically, we formulated the expected waiting time for an AP-alleviating mutation destined for fixation to appear in a population (i.e., time to mutation T_m) and the expected time from the appearance to the fixation of this mutation (i.e., time to fixation T_f; see Extended Discussion). The expected total waiting time for the appearance and fixation of the first AP-alleviating mutation is $T = T_m + T_f$. We assume that, relative to the WT, the mutant has a selective advantage of s in environment B but zero in environment A, and that the population spends a fraction (f) of its time in B and the rest of its time in A. We show in the Extended Discussion that the equivalent selection coefficient s_e equals sf. We considered two additional parameters: N_e and the equivalent number of nucleotide sites at which all point mutations alleviate AP (i.e., mutational target size L). The mutation rate per site per generation (u) is relatively constant among cellular organisms, and the estimate from yeast (3×10^{-10}) is used here (Lynch et al., 2008). A larger u has the same effect as a larger L, because uL is what matters. For yeast, $T_m/T_f < 1$ when $L > 2.5$ (Figure 7A), indicating that when the mutational target size is not extremely small, the time to AP resolution is primarily determined by the time to fixation rather than the time to mutation. However, in species with smaller N_e, the situation is easily reversed (Figure 7A). Although T_m/T_f is independent of s_e, T decreases with rising s_e (Figure 7B). For yeast, depending on its generation time (g) in nature, the time to AP resolution (gT) varies from 1 to 10,000 years (Figure 7C). For example, when $sf = 0.001$, $L = 4$ nucleotides, and $g = 16$ hr, gT is ~100 years. It is possible that nonrepetitive environmental changes occur so frequently that a yeast population cannot fix an AP-resolving allele before the specific environment vanishes. AP would be hard to resolve under this scenario.

DISCUSSION

By measuring the fitness effects of null mutations in almost all yeast nonessential genes under six different environments, we achieved a genome-scale quantification of AP. Although our AP quantification was performed in a laboratory strain of yeast, we believe that our conclusions extend to wild strains because,

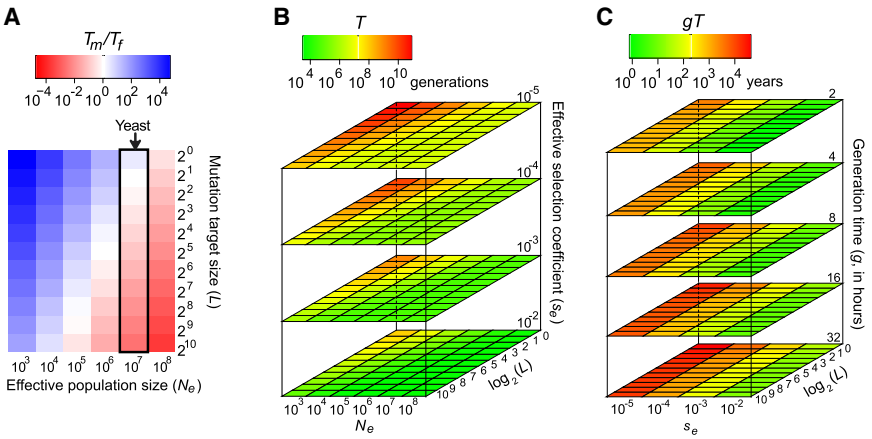

Figure 7. Expected Fixation Times of AP-Alleviating Alleles

(A) The ratio between the expected waiting time for the appearance of the first AP-alleviating allele that is destined for fixation (T_m) and the expected time required for this allele to become fixed from its first appearance (T_f) decreases with rising effective population size (N_e) and mutational target size (L). Yeast has an effective population size of $\sim 10^7$, as indicated.

(B) Expected total number of generations ($T = T_m + T_f$) required for the appearance and fixation of the first AP-alleviating allele decreases with rising L and effective selection coefficient (s_e). In almost all cases, T also decreases with rising N_e.

(C) Expected number of years (gT) required for the appearance and fixation of the first AP-alleviating allele in yeast under different L, s_e, and generation times (g).

similarly to many wild strains, the laboratory strain experiences multiple different environments, and because most of the six media under which AP was surveyed are routine laboratory media. This view is supported by the observation that even in YPD, the most frequently used medium for culturing the laboratory strain, there are >200 AP genes (114 after correcting for false negatives and false positives), and this number is likely a gross underestimate, as mentioned earlier. Although the specific genes subject to AP may vary among strains due to the different environments encountered by different strains, AP is probably more frequent in wild strains than in laboratory strains, because the number of environmental variables in the wild is likely greater than that commonly applied in the laboratory. Our finding that AP is often resolvable in strains that are well adapted to certain environmental factors (e.g., a high ethanol concentration) means that only AP related to this factor is resolved in these strains. However, they can and will have unresolved AP related to other environmental factors to which the strains are not well adapted (e.g., ambient temperature that varies both deterministically and stochastically). As long as the environment is not constant, AP is expected to exist. Our finding that under any condition yeast expresses hundreds of genes that are harmful rather than advantageous to the organism demonstrates the prevalence of AP and the importance of considering AP in understanding yeast biology.

AP is expected to be even more abundant in multicellular organisms than in yeast, for two reasons. First, although our yeast study focuses exclusively on AP in different external environments, multicellular organisms are subject to additional types of AP. For example, some alleles that are advantageous to one sex are known to be harmful to the other in *Drosophila* (Innocenti and Morrow, 2010). In humans, mutations that cause Huntington disease, a neurodegenerative disorder in which symptoms typically manifest after the reproductive age, are known to increase fecundity (Carter and Nguyen, 2011). The existence of sexes, tissues, and life stages in complex multicellular organisms creates a greater potential for AP. Second, our population genetic analysis showed that it takes longer to resolve AP when the effective population size is smaller or when the generation time is longer. Because multicellular organisms have much smaller effective population sizes and much longer generation times than yeast (Lynch, 2007), the fraction of AP that is unresolved is expected to be much greater in the former than in the latter. Thus, taking AP into consideration is likely to be important for understanding the biology of complex multicellular organisms.

Because AP is invoked in current explanations and models of many biomedical and evolutionary phenomena, as mentioned above, our finding of prevalent AP provides an empirical foundation for these theories and has profound implications for many areas of biology. In particular, if many disease-causing mutations are kept in the population because of their unexpected benefits in other aspects of life (e.g., development, fecundity, and host defense), as has been suggested for the mutations that cause Huntington disease, cystic fibrosis, sickle-cell anemia, glucose-6-phosphate dehydrogenase deficiency, cancer, and many other diseases (Carter and Nguyen, 2011), special precautions would be needed in treating these diseases, because a treatment could lead to adverse effects in other aspects of life. On the other hand, discerning the underlying mechanisms of AP in such diseases could lead to a better understanding and even improvement of antagonistic traits, such as host defense in relation to sickle-cell anemia. This so-called "positive biology" (Farrelly, 2012) could complement the common practice of focusing exclusively on diseases in biomedical research. The identified natural solutions to AP may also guide designs of synthetic genomes and organisms (Gibson et al., 2010) that need to perform well in multiple environments. When introducing a gene into a host genome, one should examine the effect of that introduction in multiple environments, sexes, tissues, and life stages, because a gene that is beneficial in one condition can be deleterious in another. To optimize the function of the synthetic organism, a well-designed expression regulation network is required to suppress the expression when it is harmful and to activate the expression when it is advantageous. AP among environments is also a special, strong type of genotype by environment (G × E) interaction in which a mutation has opposite fitness effects in two environments. Our study demonstrates the abundance of G × E interactions and offers a list of such interactions in yeast that will be useful for understanding the underlying

molecular mechanisms of G × E interactions. We hope that our genome-scale quantification of AP will stimulate additional studies in this area of universally recognized importance that to date has been largely untouched by systematic empirical analysis.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Media for Bar-Seq

The yeast single gene deletion collection (Giaever et al., 2002) was purchased from Invitrogen (Cat. No. 95401.H1Pool). The strains are diploid, with a homozygous deletion of a nonessential gene per strain. The yeast strains were competed in six media (see Extended Experimental Procedures for details of the media).

Fitness Measurement by Bar-Seq

The frequency of each strain was measured at generations 0, 3, and 26 by Bar-seq (Smith et al., 2009). We extracted the genomic DNAs from each yeast population, amplified the barcodes by PCR, and PCR-added sequences recognizable by Illumina sequencing primers. We used only the upstream barcode (Giaever et al., 2002) because the downstream barcode is known to be missing in some strains (Deutschbauer et al., 2005). We sequenced 40 nucleotides from one end of each PCR amplicon using one lane on an Illumina Genome Analyzer IIx at the University of Michigan DNA Sequencing Core. The Illumina Pipeline software version 1.5 was used for base calling from the image data. To guarantee high accuracy of fitness measurement, strains with <100 reads in generation 0 were not considered. See Extended Experimental Procedures for details of the fitness estimation.

Identification of AP Genes

We first used the *HO* deletion strain as the WT reference (Meiron et al., 1995; Ekino et al., 1999) to estimate the relative fitness of the ten additional pseudogene deletion strains. We then calculated the mean fitness of the 11 strains. As expected, the mean fitness was not significantly different from one in any medium (Table S4). We thus merged the reads of all 11 strains and considered them collectively as the WT reference. Using this reference, we calculated the fitness of every deletion strain, including the 11 pseudogene deletion strains. To determine whether the fitness of a deletion strain differed significantly from one, we conducted a *Z* test using the fitness values of the 11 pseudogene deletion strains as the null distribution. The p values from the *Z* test were further converted to *Q* values after the consideration of multiple testing (Storey, 2002).

Analysis of the Properties of AP Genes

See Extended Experimental Procedures for details regarding the bioinformatic analyses.

Relative Fitness of Four Yeast Strains in Four Media

By competition with a yellow fluorescent protein (YFP)-marked reference strain followed by cell counting using flow cytometry (He et al., 2010), we measured the relative fitness ($f_{i,j}$) of each of four strains (i = 1–4) in each of four media (j = 1–4). We then calculated the mean fitness of each strain in the four media (g_i) and the mean fitness of the four strains in each medium (h_j). The relative fitness of each strain in each medium was estimated by $(f_{i,j}/g_i)/h_j$.

Strain Construction

Strain construction was done according to standard methods in yeast genetics. See Extended Experimental Procedures for details. The nonlaboratory strains used in our study were described previously (Liti et al., 2009; Schacherer et al., 2009).

Microscopy

Yeast cells were grown in YPD or OAK overnight at 30°C to the stationary phase. The optical density (OD) of the yeast culture was measured at 660 nm with a spectrophotometer (GENESYS 5; Thermo Scientific). The yeast culture was diluted to OD_{660} = 0.1 by fresh medium supplemented with 1 μg/ml DAPI (Sigma) and was harvested when OD_{660} reached 0.5 (mid-log phase). The yeast cells were washed, condensed, and examined under a fluorescence microscope (DeltaVision Spectris; Applied Precision).

Expression Measurement by Quantitative PCR

We followed the standard molecular biology method for quantitative PCR (qPCR; see Extended Experimental Proceduresfor details). *ACT1* was used as an internal control.

Determination of Allele-Specific Gene Expression by Pyrosequencing

Pyrosequencing was performed according to published protocols (Wittkopp et al., 2004). See Extended Experimental Proceduresfor details.

ACCESSION NUMBERS

The Bar-seq data reported in this paper are available from the Zhang lab at http://www.umich.edu/~zhanglab/download.htm.

SUPPLEMENTAL INFORMATION

Supplemental Information includes an Extended Discussion, Extended Experimental Procedures, three figures, and eight tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.09.017.

LICENSING INFORMATION

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WEB RESOURCES

The URLs for data presented herein are as follows:

Bioconductor, http://www.bioconductor.org/
Broad Institute, http://www.broadinstitute.org/annotation/fungi/comp_yeasts/downloads.html
Illumina, http://www.illumina.com
Princeton, ftp://gen-ftp.princeton.edu/yeast_snps/schacherer2008/all_del.gff
Saccharomyces Genome Database, http://www.yeastgenome.org/

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