Starvation Induces Genomic Rearrangements and Starvation-Resilient Phenotypes in Yeast

Scott Coyle and Evgueny Kroll†*

*Department of Molecular and Cellular Biology, University of California, Berkeley; and †Department of Biological Sciences, University of Montana

Evolution has shaped a wide variety of genomes across eukaryotic taxa. However, the forces that shape the genomes are generally unknown. Because organisms in nature commonly experience prolonged periods of nutrient depletion, we posit that diverse demographic, physiological, and genomic responses to starvation can occur. To test for these possibilities, we subjected replicate yeast populations to prolonged starvation. We observed that clones repeatedly gave rise to descendants that were karyotypically diverse. After a 1-month starvation period, approximately 70% of randomly isolated members of starved populations harbored one or more genomic rearrangements. Further, we found that 5 of 16 karyotypically differentiated groups of isolates from starved populations were more resilient to starvation than nonstarved clones and their common ancestor. Phylogenetic analysis of these isolates suggests that genomic rearrangements that arose during starvation can be adaptive in the context of a nutrient-depleted environment. Altogether our data illustrate the profound influence of environmental conditions on adaptive genome evolution in eukaryotes.

Introduction

Phenotypic variation within a population depends, among other factors, on the rate of supply of new mutations. As most mutations are highly deleterious, mutation rate is kept low (Cairns 1998) and generally assumed to be constant under optimal conditions (Drake et al. 1998). However, during prolonged exposure to a stressful condition (defined here as an environmental change that lowers mean population fitness [Zhivotovsky 1997]), the potential benefit of generating a few adaptive variants may outweigh the cost of many deleterious mutations (Muller 1928; Sturtevant 1937; Radman 1999). So-called "mutator strains" that are defective in mismatch repair are commonly observed in natural isolates of *Escherichia coli* and other bacteria (LeClerc et al. 1996; Harris et al. 1999; Vulic et al. 1999; Oliver et al. 2002; Li et al. 2003). Theoretical models have shown that under strong selection in clonal populations, mutators enjoy a selective advantage that enables them to persist over many generations (Arjan et al. 1999; Tenaillon et al. 1999). These theoretical predictions have been verified experimentally (Sniegowski et al. 1997).

Suboptimal environmental conditions can influence the small- and large-scale genomic changes that occur in evolving populations. For example, exposure of *E. coli* cells to starvation, crowding, or nonoptimal temperature activates the SOS response (Dri and Moreau 1993; Lenski et al. 1994; Finkel and Kolter 1999; Bjedov et al. 2003; Hastings et al. 2004). SOS elevates rates of homologous and nonhomologous recombination, interferes with DNA polymerase proofreading, and induces error-prone replication (Abbott 1985; Woodgate et al. 1987; Napolitano et al. 2000). These alterations increase the rates of point mutation and genome rearrangement, making it likely that SOS plays a role in evolutionary change (Echols 1981; Radman et al. 1999).

Key words: genome rearrangements, starvation, *Saccharomyces cerevisiae*, genome evolution.

E-mail: evgueny.kroll@mso.umt.edu.

Mol. Biol. Evol. 25(2):310–318. 2008
doi:10.1093/molbev/msm256
Advance Access publication November 20, 2007

Although it is obvious that eukaryotic genomes are dynamic over evolutionary time, little is known about molecular mechanisms by which eukaryotes might respond to stress in a manner reminiscent of SOS. We do know that the genomes of *Saccharomyces* and other *Hemiascomycetes* genera have been reshuffled by numerous chromosomal rearrangements, apparently with rates that differ by several orders of magnitude among lineages (Dujon et al. 2004). Also, a number of experimental studies using chemostats have demonstrated that yeast growing under nutrient limitation frequently undergo genome rearrangements; in some cases, specific rearrangements appear to be adaptive (Adams et al. 1992; Bidenne et al. 1992; Brown et al. 1998; Dunham et al. 2002).

Because the chemostat is a simple, constant environment, it is unsuited for investigating the genomic consequences of drastic environmental changes. A more relevant experimental model would be the one in which populations undergo prolonged starvation after exhausting all available nutrients. In fact, such starvation can arise from a variety of environmental events, including changes in temperature and soil properties, niche invasion, and destruction of habitat. Owing to the general nature of starvation, it would not be unreasonable to expect that certain population responses to starvation would be of a general, blind character—similar to SOS—and that they would result in an overall increase in the number of genetic variants, some of which might be better adapted to the changed environmental conditions.

To test for this possibility, we exposed replicate populations of *Saccharomyces cerevisiae* to starvation for 1 month. We isolated clones from these populations, determined their karyotypes by pulsed-field gel electrophoresis, and then subjected them to a second round of starvation. Relative to nonstarved yeast, we observed that starved populations accumulated an exceptionally high number of genomic rearrangements. In contrast, fine-scale mutation frequency increased only severalfold. We also observed that a subset of karyotypically differentiated clones from starved populations displayed a heritable increase in starvation resilience. Starvation-resilient cells were frequently enriched with novel chromosomes, indicating a potential evolutionary role for changes in genome architecture that arise in response to starvation.

Materials and Methods

Starvation Method

DB146 (gift from B. Blondin and F. Vezinhet) is a mitochondrially marked derivative of diploid strain 8101 from a champagne vineyard (Bidenne et al. 1992). A single clone of DB146 was incubated in 5 ml rich medium at 30 °C overnight with aeration. We call this population "ancestral." Three 5-ml aliquots (of 2×10^5 cells/ml) were further incubated at 30 °C without shaking for 1 month in the spent medium, approximating the natural state of starvation. At the end of the incubation, we called each of the 3 cultures a "starved culture" (cultures 1, 2, and 3) and subclones from these cultures "starved isolates." For comparison, we grew a similar aliquot of the ancestral culture for approximately 20 generations in 100 ml of rich medium. We call subclones from the batch-grown culture "nonstarved isolates."

Karyotyping

Intact chromosomes were prepared from yeast in agarose plugs as described (Carle and Olson 1985) with minor modifications (we used only 2-ml cultures, Zymolyase 20T, and formed agarose plugs in the 2.2-ml centrifuge tubes). The chromosomes suspended in agarose plugs were then subjected to field-inversion gel electrophoresis (FIGE) (Heller and Pohl 1990) using a SwitchBack Pulse controller (Hoeffer, Scientific Instruments, San Francisco, CA) in 0.5× Tris-borate EDTA cooled to 12 °C, at 175 V, 2–70 s ramp for 24 h, with 3.6:1 forward–backward ratio. Several other settings (1–50 s and 40–90 s ramp and 3.2:1 ratio) were used to expand particular regions of the gel. This method provided sufficient resolution to consistently distinguish the 11 lowermost bands (chromosomes) on the gel. We ignored the uppermost bands where FIGE typically yields inconsistent results (Heller and Pohl 1990). The lowermost bands correspond (from small to large) to chromosomes Ia, Ib, VIa, VIb, III, IX, VIIIa, V, and VIIIb in the ancestral clone (Longo and Vezinhet 1993), where "a" and "b" denote length polymorphisms in homologues. We also followed 3 chromosomes (unid-1 to -3) that have yet to be identified in this strain. Gels were stained with SYBR Gold and scanned with Molecular Dynamics Storm 860 scanner.

Assays for Mutagenesis

An aliquot of 10^6 cells was collected from each starved and nonstarved culture, mixed into 5-ml yeast extract peptone dextrose (YPD), and grown overnight. The following day, the cultures were diluted and plated onto both rich media plates (to assess the cell viability) and onto selective media plates (to assess the proportion of cells bearing particular mutations). We independently measured the frequencies of mutant alleles that confer resistance to 2 drugs. For dominant cycloheximide resistance (mostly due to mutations in the *CYH4* gene), 10^8 cells from 5 ancestral and 3 starved populations of DB146 were plated on minimal plates containing 6.8 μM cycloheximide. For the *LYS2* forward mutagenesis (most of our mutants should be *lys2*/*lys2* double mutants), 10^8 cells were plated on minimal plates containing 0.2% L-α-aminoadipic acid. After 1 week (in the case of cycloheximide) and 3 days (in the case of L-α-aminoadipic acid), the number of colonies on each plate was scored and the mutation frequency was calculated as the square root of the frequency of the resistant colonies. As the frequency of the loss-of-function mutations in the *LYS2* locus is quite high, we were able to record the frequency of the double *lys2*/*lys2* mutants in our diploid strain.

Phylogenetic Analysis

Each distinct band observed on pulsed-field gels was assigned a number; a binary matrix was built for isolates based on the presence (1) or absence (0) of each band. Phylogenies were constructed with PHYLIP (Felsenstein 1989) using a Wagner maximum parsimony method (Eck and Dayhoff 1966) that forces the root of the tree to correspond to the karyotype of the ancestral clone. Genetic distance was calculated as the total number of differences (both appearances of new bands and disappearances of others) in karyotype between any pair of isolates (see supplementary table S2, Supplementary Material online).

Reproductive Capacity After Starvation

A total of 57 isolates from 1-month starved cultures (and 19 nonstarved isolates) were grown overnight in 200-μL YPD, diluted to 600 μL, and 200 μL deposited in triplicate into a well of three 96–deep-well plates. Plates were incubated at 30 °C with no shaking. At 2 weeks, a 50× dilution of each culture was spotted onto a YPD plate and incubated at 30 °C for 4 h. Reproductive capacity of each culture was defined as the fraction of cells that are capable of initiating a round of cell division and was measured microscopically as the fraction of cells that began budding after the 4-h incubation on a fresh YPD plate. This measurement approximates well the colony-forming ability of yeast cells (data not shown). Reproductive capacity was measured again for a select number of isolates at 4 weeks of restarvation using the same methods. We call reproductive capacity after starvation "resilience to starvation."

Statistical Methods

Nonparametric statistical methods were used in all cases as the assumptions of normality or sample size associated with parametric tests were not satisfied in our data sets. The Mann–Whitney *U* test and the Kruskal–Wallis test were used to test for differences in the median values between populations, whereas the Fisher's exact test was used to test for a relationship between categorical values. Statistical tests were performed using the R statistical software package (R Development Core Team [2007]. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

Results

Gross Genomic Rearrangements during Starvation

To examine how rates of genomic change in yeast populations might be affected by starvation, we starved 3 parallel cultures derived from one ancestor clone of

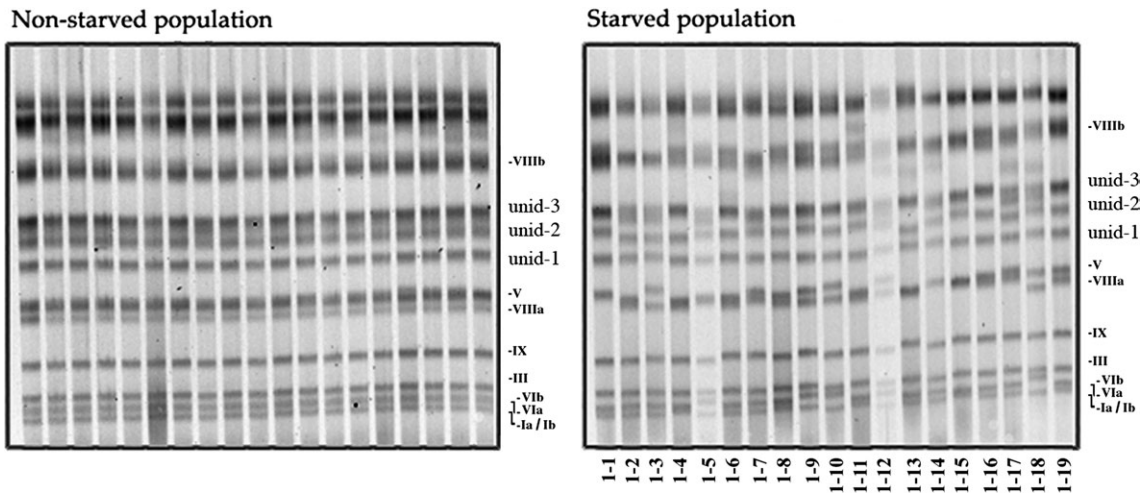

FIG. 1.—Electrokaryotypes of 19 isolates from the nonstarved control and the starved culture #1. Starved isolates are numbered underneath. Chromosomes are marked on the right as in Longo and Vezinhet (1993), also see Materials and Methods. Unidentified (but used in scoring) chromosomes are numbered unid-1, -2, and -3. A graphic editor was used to adjust the contrast.

DB146, a derivative of a *S. cerevisiae* champagne strain (Longo and Vezinhet 1993) at 30 °C for 1 month without agitation (see Materials and Methods). At the end of the 1-month incubation, cell titer increased from 10^5 to approximately 10^8 cells/ml. This means that cells underwent approximately 10 generations on average per cell, not accounting for autolysis of a subset of cells. The reproductive capacity of starving cells was measured each week and was never lower than 40%. Cultures were assessed weekly for sporulation by microscopic examination; at no point did we observe ascospore formation in starving cultures.

We then isolated 19 subclones (starved isolates) from each of the 3 starved populations by streaking starved populations onto solid rich medium and picking colonies after 2 days. For the nonstarved control, we serially propagated a culture of DB146 in rich medium at 30 °C for approximately 20 generations Then, as above, we isolated 19 subclones (nonstarved isolates) from the nonstarved culture by streaking cells onto rich solid medium.

We analyzed isolate karyotypes by pulsed-field gel electrophoresis. Figure 1 shows the electrokaryotypes for 19 nonstarved isolates and 19 starved isolates from culture #1. (for the other gels, see Supplementary Material online) We found, as expected, that the karyotypes of the nonstarved isolates did not deviate from the ancestral karyotype at least after 20 generations of growth in liquid rich medium (fig. 1). In contrast, we found that isolates from starved populations contained a dramatically increased number of chromosomal rearrangements (fig. 1 and supplementary fig. S4, Supplementary Material online). Overall, we scored 16 separate changes in the karyotypes of starved isolates, of which 9 were novel bands and 7 were a disappearance of a wild-type band. In all, 41 of 57 starved isolates harbored those changes, and of those 41 isolates, 35 showed more than one rearrangement. We found that the bands in the ancestor that changed most frequently in starved isolates corresponded to chromosomes III, V, VI, VIII, and IX. Our frequency estimates are conservative as isolates that appear to retain the ancestral banding pattern may still contain rearrangements not resolved by FIGE, for example, small-scale rearrangements, inversions of any size, and rearrangements of larger chromosomes.

Previous karyotypic analyses have shown that when DB146 is serially propagated in rich medium, approximately 10% of the resulting clones contain chromosomal rearrangements after 1 month or 145 generations (Longo and Vezinhet 1993). In contrast, 71% of our starved isolates acquired at least one rearrangement during month-long incubation, during which an average of only 10 cell generations occurred.

Estimating Rate versus Frequency of Genomic Change in Starved Cultures

It is not possible to accurately estimate rearrangement "rates" for these cultures because we cannot assume that all new rearrangements accumulated in a neutral fashion (Lea and Coulson 1949; Rosche and Foster 2000). However, even if some starved cells were the product of 10 cell generations, although other starving cells autolyzed, the rate per cell generation at which chromosomal rearrangements accumulate in starved populations is likely to be an order of magnitude (or more) greater than that of populations in serially propagated, nonstarved culture.

Some Novel Karyotypes Became Prevalent during Starvation

Within each starved culture, we often identified multiple isolates with the same apparent karyotype. These karyotypes always differed markedly from the common ancestor. We analyzed these similar isolates on FIGE gels under 3 different settings to expand the regions of the gel containing novel bands in these isolates; we found that mobility was the same in each FIGE condition set (see Supplementary Material online). Thus, either these isolates were clonally related or most rearrangements occurred at a small

number of "hot spots." However, were the latter true, we would expect to find the same rearrangements in parallel starved cultures: This was not observed (supplementary fig. S5, Supplementary Material online). We, therefore, conclude that certain novel karyotypes that arose during starvation became more prevalent during that treatment.

Most of the Genomic Rearrangements Occurred during Starvation

We observed 16 independent genomic changes distributed across 41 isolates in 57 subclones from starved cultures. No karyotypic changes were observed among 20 isolates from nonstarved cultures. These differences were highly significant ($P = 0.007$, Fisher exact test, 2-tail P), indicating that it is extremely unlikely that most rearrangements existed prior to starvation (otherwise, we should have seen some of the rearrangements among the nonstarved isolates.) Moreover, for preexisting rare rearrangements to rise to such a high frequency, they would have had to have been strongly selected during starvation. However, this is not true as only 5 of the 16 observed karyotypic changes awarded any significant advantage in restarvation experiments (see below). Lastly, we would expect that some preexisting rearrangements would rise to high frequency in multiple experimental populations as our parallel cultures were prepared as aliquots of a single master culture. Because each starved culture had a unique set of chromosomal rearrangements and because the nonstarved cultures had none, we conclude that most of the genomic changes we observed did not exist prior to starvation but developed during the starvation process itself.

Frequencies of Fine-Scale Mutations during Starvation

Fine-scale mutations are another form of genetic change that could accumulate in starving populations. We measured the frequency of mutation in starved and nonstarved populations using 2 independent assays. In the first assay, the ability to grow on plates with low levels of cycloheximide was used to compare the frequency of dominant mutation (in the *CYH4* gene) between starved and ancestral populations. We found that the mutation frequency was marginally higher in starvation-selected populations of DB146 (ancestor median frequency $= 1.0 \times 10^{-7}$, starved median frequency $= 3.3 \times 10^{-7}$). We confirmed these results in a second assay using resistance to L-α-aminoadipic acid, which is conferred by double mutation in the *LYS2* gene (ancestor median frequency $= 1.5 \times 10^{-7}$, starved median frequency $= 5.7 \times 10^{-7}$). Thus, we observed only a slight increase (3.3–3.8 times) in apparent mutant allele frequency in these 2 independent loci after 1 month of starvation.

Starved Yeast Isolates Show Increased Survival Compared with the Nonstarved Ancestor

The high frequency of particular karyotypes within starved populations is consistent with the possibility that beneficial traits arose within the starved cultures. We therefore tested whether starved clones were better able to survive under starvation than naive nonstarved clones. To negate the effects of possible physiological adaptation, we grew overnight in rich medium 76 starved isolates and 19 control isolates used in our previous assays. We then transferred aliquots of 1.7×10^7 cells of each regrown isolate to three 96-well plates in triplicate and starved the resulting populations for 2 weeks. After 2 weeks of "restarvation," we used the population reproductive capacity as a measure of resilience to starvation; reproductive capacity was estimated as the fraction of cells that could initiate a round of cell division following a 4-h incubation on YPD agar at 30 °C. A single isolate was considered "significantly more resilient" if its resilience to starvation fell outside the error bars ($1.58 \times$ interquartile range) for resilience to starvation of the nonstarved (control) population. Figure 2*a* shows the distribution of resilience to starvation in the original starved isolates relative to nonstarved controls.

Two important conclusions can be drawn from the data shown in figure 2. First, during restarvation, isolates from all the 3 starved populations outperformed those from nonstarved population. These differences in starvation resilience were highly significant (Mann–Whitney U test, $P < 0.005$), demonstrating that isolates from starved populations became, in general, better adapted to that condition (fig. 2*a*). Interestingly, reproductive capacity of starved isolates not undergoing starvation, such as outgrowth in fresh rich medium, was equal to or lower than that of the ancestral population (fig. 2*b*), indicating potential trade offs in adaptation to starvation.

Second, we observed that, among starved clones, the variation in starvation resilience was greater than that of their common ancestor. Values for 18 of 19 ancestral starvation values were clustered tightly around the median, whereas starved populations had a significantly wider spread, even after accounting for the higher median values in starved populations ($P < 0.005$, Kruskal–Wallis test, degrees of freedom $= 3$) (see supplementary table S1, Supplementary Material online). Thus, long-term exposure of yeast populations to starvation results not only in a general increase in starvation resilience but also in increased variability for this trait.

Specific Karyotypes Are Associated with Increased Resilience to Starvation

Certain karyotypes were more prevalent among the starved isolates, and these displayed significantly increased starvation resilience. We therefore investigated whether there was an association between particular karyotypes and this trait. First, using a Wagner maximum parsimony method, we constructed a karyotype-based dendrogram rooted to the ancestral karyotype (Eck and Dayhoff 1966). The diversity of karyotypes across starved populations was not uniform, ranging from 11 small clades (groups with identical karyotypes) in population 1 to 2 large clades and 1 small one in population 2. The majority of isolates from population 2 retained the original karyotype, whereas only 2 isolates of 19 retained the original karyotype in populations 1 and 3. The different levels of

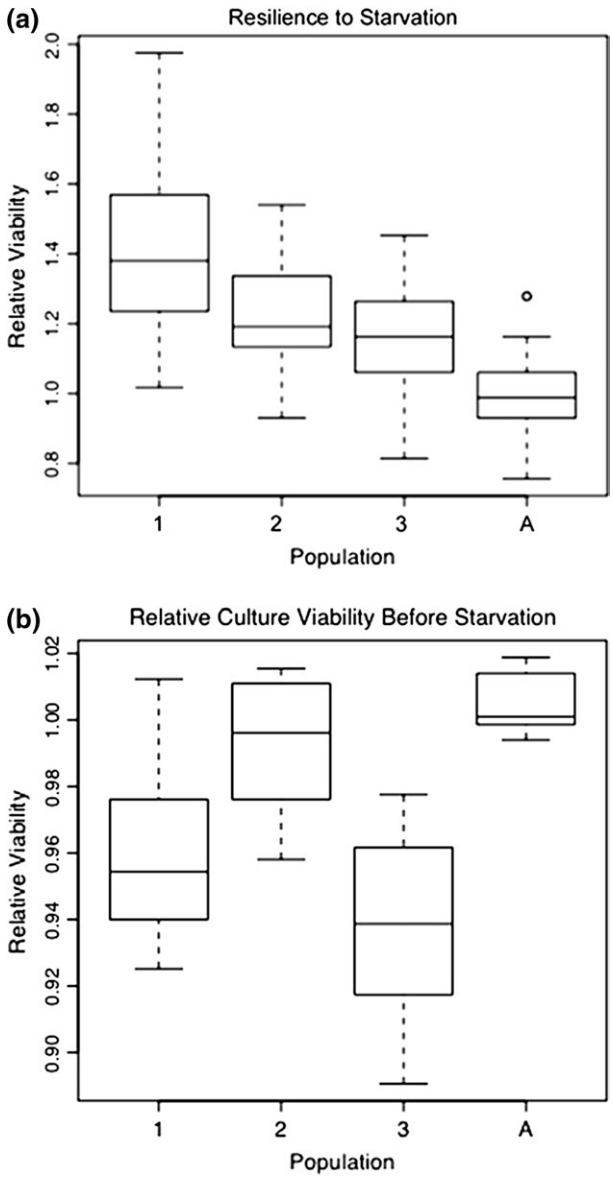

FIG. 2.—(*a*)Boxplot showing distribution of the relative resilience to starvation in starved and nonstarved cultures (1, 2, and 3) relative to the ancestral (A) population. Values were normalized to the median ancestor resilience (36%). (*b*) Boxplot showing relative (to the ancestral DB146) culture reproductive capacity (median ancestral DB146 reproductive capacity = 97%) of starved and nonstarved populations before being returned to the starvation environment. The center line in the box indicates the median value of the population, and the box is drawn to contain the middle 50% of the data; the bars extend to $+/- 1.58 \times \mathrm{IQR}/\sqrt{n}$, where IQR is the interquartile range; the point in the ancestral population is marked as an outlier as it fell outside this range (35).

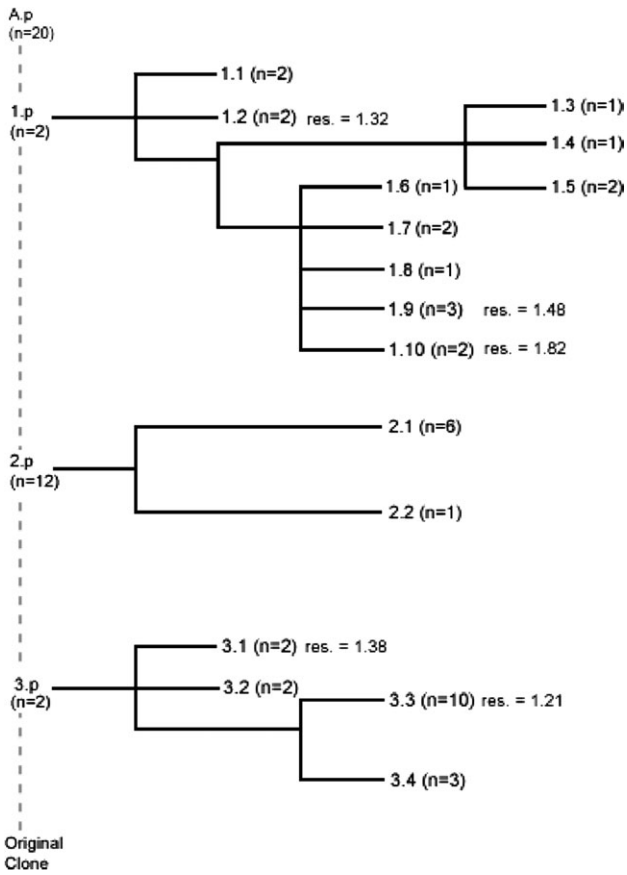

FIG. 3.—Phylogenetic tree constructed from karyotype data using a Wagner maximum parsimony method that forces the original clone to be the root of the tree (30). Branches are marked as *X.Y*, where *X* refers to the population they came from (starved cultures 1, 2, and 3 and the nonstarved culture A) and *Y* labels the clade ("p" denotes a clade that shares the ancestral karyotype of DB146). The size of each clade is indicated in parenthesis (n = #) next to each clade. Clades showing increased resilience to starvation have their mean relative resilience (res.) displayed.

karyotypic diversity observed in these populations illustrates that, despite identical starting conditions, populations take different evolutionary paths during starvation.

Next, we mapped the starvation resilience data for each isolate onto the dendrogram. We recognized "resilient clades" (see fig. 3) as those that contained isolates belonging to 5 (of 16) groups having significantly increased starvation resilience (Mann–Whitney U test, $P < 0.05$). With the exception of clade 3.1, all isolates in resilient clades were more resilient to starvation than the common ancestor. In addition, we observed 6 "partly resilient" clades that possessed a mixture of both resilient and nonresilient isolates and 5 nonresilient clades that contained no resilient isolates.

To assess the probability that chance alone could produce a tree with as many resilient clades as we observed, we constructed a computer model in which 19 isolates were marked as either "N" (not resilient) or "R" (resilient) in the same proportion as in population 1 and then randomly assigned positions on our most complex tree (of population 1). A group was resilient to starvation if and only if all of the isolates within that group were marked as R. A 1,000-cycle simulation run with 8 isolates marked R revealed that while one resilient group would arise relatively frequently by chance, we observed 2 resilient groups less than 5% of the time, and never (out of 1,000 cycles) did we observe 3 or more resilient groups as in the real experiment (fig. 3). From our simulation we conclude that chance alone (as would be the case if there was no association between karyotype and starvation resilience) could not account for the appearance of the number of resilient clades that we observed. Thus, we conclude that a significant association

must exist between some karyotypic alterations and increased resilience to starvation.

Discussion

Genomic Rearrangements Increase during Prolonged Starvation

Our experiments reproduce in the laboratory how eukaryotic microbial populations are likely to experience starvation in nature. We show that month-long starvation of a champagne vineyard yeast leads to a dramatic increase in the frequency of genomic rearrangements in replicate cultures derived from a common ancestor. Sixteen novel rearrangements are distributed in 40 of 57 starved isolates compared with 0 novel rearrangements of 20 isolates in a nonstarved, serially propagated population. As expected, replicate starved cultures exhibit different levels and kinds of karyotypic variation after starvation, indicating that rearrangement occurs during starvation. Additionally, we find that some isolates from starved populations exhibited increased, heritable resilience to starvation and that, in general, the populations derived from these starved clones fared better in subsequent starvation experiments than the common ancestor. Apparently, beneficial genome rearrangements increase in frequency during starvation owing to differential mortality and increased likelihood of chromosomal rearrangement under this type of stress. Thus, our data suggest that starvation is likely to affect the rate at which genomic rearrangements accumulate in yeast populations.

We find evidence that genomic rearrangements play a role in the acquisition of resilience to starvation. After mapping the starvation resilience phenotype onto a dendrogram based on the karyotypes of starved isolates, we find in several instances a clear connection between certain karyotypic alterations and starvation resilience.

Although here we examined a strain from a champagne vineyard, that is, a wild yeast strain that might harbor extranumerary chromosomes and thus might be more resilient to genomic rearrangements than a euploid organism, the phenomenon described here should, in our opinion, be relevant to any organism whose genome can tolerate genomic rearrangements in response to environmental conditions, even if the rates of accumulating rearrangements might vary between organisms.

The Rates for Other Types of Mutations May Also Increase

It is important to note that although we argue that large-scale genome rearrangements played a role in the acquisition of the starvation resilience phenotype, we do not dismiss the possibility that other alterations, such as point mutations, might also play an active role in the acquisition of starvation resilience. In fact, the observation that Population 2 contained no clades resilient to starvation but still showed starvation resilience at the population level suggests other modes of genetic or epigenetic adaptive change. Although we found that mutant allele frequencies were not markedly elevated during starvation, we did not estimate mutation rates by fluctuation analysis because we had to compare the effect of fine-scale mutations with that of genomic rearrangements, for which we know only frequencies (see Results) We also cannot assume that every cell in the starved populations reproduces at an equal rate; indeed, the fact that starved population underwent only 10 generations on average would exacerbate the problem of determining rates from our data. Lastly, mutation rates estimated from just 2 loci may not be representative of the whole genome, especially in stressful conditions (Hall 1998; Halme et al. 2002). Thus, it is possible that during starvation the fine-scale mutation rate is indeed elevated and that the 2 modes of genetic change act in tandem to produce the starvation-resilient phenotypes we have observed.

The Role of Genomic Rearrangements in Genome Restructuring

Large-scale genome rearrangements have played an important role in the evolution of diverse lineages across all domains of life. It has been convincingly argued that new adaptive features can arise during evolution by combinatorial reassembly of already existent traits (Gilbert 1985; Kirschner and Gerhart 1998). In enteric bacteria, genomic rearrangements can occur almost anywhere in the genome with frequencies ranging from 10^{-5} to 10^{-2} depending on the locus (Anderson and Roth 1977). Additionally, it has been recently shown by S. Rosenberg and colleagues that a subset of starving *E. coli* cells acquires reversible amplifications of a leaky *LacZ* allele, allowing for faster growth on lactose medium (Slack et al. 2006).

In contrast with bacteria, eukaryotes tend to have more complex genomes that often contain large amounts of noncoding and/or highly repeated DNA sequences. Thus, the relative contribution of point mutations to adaptive traits may be lower in eukaryotes than prokaryotes because point mutations may occur proportionally more often outside the transcribed portions of genomes. By the same token, genome rearrangements may play a greater role in eukaryotic evolution because larger genomes with duplicated chromosomes and sparsely located (and often intron containing) genes may be more tolerant to rearrangement.

Indeed, the role of genomic rearrangements in eukaryotic evolution is well established (King 1993; Nevo et al. 1994; Eichler and Sankoff 2003) and their rate has not been constant throughout evolution (Murphy et al. 2005). Evolution of the homeobox (*Hox*) gene cluster and the resulting morphological diversity of metazoans is an example of the important evolutionary role of genomic rearrangements (Bailey et al. 1997). Isolated groups of a 500-year-old population mice from the Island of Madeira contain multiple translocations associated with the subpopulation's geographical origin (Britton-Davidian et al. 2005). Agronomists routinely use chromosome aberrations in plant tissue (somaclonal variation) to produce new varieties of important domestic species, such as potato, tomato, maize, and many others (Evans 1989). Rearrangements are also highly significant in many disease processes. Tumors containing certain genomic rearrangements, or chromosome abnormalities, have been shown to be more aggressive than comparable tumors without those chromosome abnormalities (Duesberg et al. 1999; Radman et al. 1999; Zhou

et al. 2002). In particular, certain genomic rearrangements such as "Philadelphia chromosome" in certain blood cancers, the *TMPRSS2–ERG* and *TMPRSS2–ETV1* fusion genes in prostate cancer (Tomlins et al. 2005), and the *EML4–ALK* fusion gene in lung cancer (Soda 2007) can be of "adaptive value" to a tumor (Schroeder and Kurth 1971).

In yeast, retrospective studies have shown that duplications and other rearrangements have played a major role in the evolution of genome architecture in the genus *Saccharomyces* (Fischer et al. 2000; Seoighe et al. 2000; Dujon 2006). Moreover, the rate with which genomic rearrangements occur varies widely throughout time and between yeast species (Fischer et al. 2006). Lastly, prospective, experimental studies have shown that characteristic genomic rearrangements occur in yeast populations evolving under constant nutrient limitation (Adams et al. 1992; Bidenne et al. 1992; Brown et al. 1998; Dunham et al. 2002). Here, we demonstrate that genomic rearrangements occur at high frequency in yeast populations under complete starvation and that some rearrangements are adaptive because cells containing those rearrangements become more resilient to starvation.

Why then, among "wild" *S. cerevisiae* isolates, do we commonly observe the same amount of DNA similarly distributed across 16 chromosomes, in a manner so strikingly similar to *S. cerevisiae* laboratory strains? An explanation may lie in the way we define the taxon. Only those isolates whose physiological properties and genome architecture most closely resemble *S. cerevisiae* are recognized as such (*Saccharomyces* sensu stricto). Therefore asking a question of why all the wild isolates (the ones that are reminiscent of *S. cerevisiae* physiologically and genetically) have 16 chromosomes is a classic example of circularity. This simple explanation can be framed as a testable hypothesis. If, indeed, yeast chromosome number and length are highly dynamic and genomes having differing structures are not necessarily at a fitness disadvantage, we expect to find karyotypic/chromosome number diversity among starved yeast populations in nature. Furthermore, these isolates might have developed prezygotic reproductive isolation from the sensu stricto strains.

Starvation Can Be an Evolutionary Cue for Populations

A range of stressful environmental conditions can lead to starvation. Thus, organisms that face such conditions might do better (on the population level) if their population variability increases in response to starvation. Starvation might be a timely signal that an adaptation to an environmental challenge is in order. In bacteria, several mechanisms can mediate a starvation-induced adaptive responses (Hastings et al. 2004). In yeast, changes in the expression of many genes could promote genomic rearrangements (Yu and Gabriel 2004; Haber 2006; Schmidt et al. 2006). Werner-Washburne and colleagues showed that a starving yeast population contains a large proportion of cells with the increased expression of some of these genes (Allen et al. 2006); these changes may help explain mechanistically how genomic rearrangements occur in our starved cultures.

Starvation-Associated Genomic Rearrangements May Aid the Population's Evolvability

In the words of McClintock (1984), the genome "senses unusual and unexpected events and responds to them, often by restructuring." We have shown here not only that starvation induces genomic reorganization in yeast populations but also that in some cases reorganized genomes are better adapted to subsequent starvation episodes than the ancestral genome. Such a "just-in-time" supply of novel karyotypic variants may increase the adaptive potential (evolvability) of the starved population. Because starvation is a condition frequently encountered in the wild, it seems possible that starvation-associated rearrangements can be of evolutionary importance. The phenomenological framework of starvation-associated genomic reorganization described here may help direct future efforts to answer mechanistically how starvation-induced genome rearrangements arise and how they cause, or relate to, novel adaptive traits.

Supplementary Material

Supplementary table S1 and S2 and figure S4 and S5 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We would like to acknowledge the following people for providing valuable comments: K. Benjamin, S. Brenner, R. Brent, P. Coyle, B. Dunn, J. Fay, L. Ha, A. Mendelsohn, S. Moses, S. Otto, and S.P. Ridley; B. Blondin for yeast strains; and F. Rosenzweig for discussions and editing. The work was supported by the grant #0134648 from the National Science Foundation to E.K. This work was also supported by NIH-GMS R01 HG003328-01 and NIH GM79762-01A1.

Literature Cited

Abbott PJ. 1985. Stimulation of recombination between homologous sequences on carcinogen-treated plasmid DNA and chromosomal DNA by induction of the SOS response in Escherichia coli K12. Mol Gen Genet. 201:129–132.

Adams J, Puskas-Rozsa S, Simlar J, Wilke CM. 1992. Adaptation and major chromosomal changes in populations of Saccharomyces cerevisiae. Curr Genet. 22:13–19.

Allen C, Buttner S, Aragon AD, et al. (11 co-authors). 2006. Isolation of quiescent and nonquiescent cells from yeast stationary-phase cultures. J Cell Biol. 174:89–100.

Anderson RP, Roth JR. 1977. Tandem genetic duplications in phage and bacteria. Annu Rev Microbiol. 31:473–505.

Arjan JA, Visser M, Zeyl CW, Gerrish PJ, Blanchard JL, Lenski RE. 1999. Diminishing returns from mutation supply rate in asexual populations. Science. 283:404–406.

Bailey WJ, Kim J, Wagner GP, Ruddle FH. 1997. Phylogenetic reconstruction of vertebrate Hox cluster duplications. Mol Biol Evol. 14:843–853.

Bidenne C, Blondin B, Dequin S, Vezinhet F. 1992. Analysis of the chromosomal DNA polymorphism of wine strains of Saccharomyces cerevisiae. Curr Genet. 22:1–7.

Bjedov I, Tenaillon O, Gerard B, Souza V, Denamur E, Radman M, Taddei F, Matic I. 2003. Stress-induced mutagenesis in bacteria. Science. 300:1404–1409.

Britton-Davidian J, Catalan J, da Graca Ramalhinho M, Auffray JC, Claudia Nunes A, Gazave E, Searle JB, da Luz Mathias M. 2005. Chromosomal phylogeny of Robertsonian races of the house mouse on the island of Madeira: testing between alternative mutational processes. Genet Res. 86:171–183.

Brown CJ, Todd KM, Rosenzweig RF. 1998. Multiple duplications of yeast hexose transport genes in response to selection in a glucose-limited environment. Mol Biol Evol. 15:931–942.

Cairns J. 1998. Mutation and cancer: the antecedents to our studies of adaptive mutation. Genetics. 148:1433–1440.

Carle GF, Olson MV. 1985. An electrophoretic karyotype for yeast. Proc Natl Acad Sci U S A. 82:3756–3760.

Drake JW, Charlesworth B, Charlesworth D, Crow JF. 1998. Rates of spontaneous mutation. Genetics. 148:1667–1686.

Dri AM, Moreau PL. 1993. Phosphate starvation and low temperature as well as ultraviolet irradiation transcriptionally induce the Escherichia coli LexA- controlled gene sfiA. Mol Microbiol. 8:697–706.

Duesberg P, Rasnick D, Li R, Winters L, Rausch C, Hehlmann R. 1999. How aneuploidy may cause cancer and genetic instability. Anticancer Res. 19:4887–4906.

Dujon B. 2006. Yeasts illustrate the molecular mechanisms of eukaryotic genome evolution. Trends Genet. 22:375–387.

Dujon B, Sherman D, Fischer G, et al. (67 co-authors). 2004. Genome evolution in yeasts. Nature. 430:35–44.

Dunham MJ, Badrane H, Ferea T, Adams J, Brown PO, Rosenzweig F, Botstein D. 2002. Characteristic genome rearrangements in experimental evolution of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. 99:16144–16149.

Echols H. 1981. SOS functions, cancer and inducible mutation. Cell. 25:1–2.

Eck RV, Dayhoff MO. 1966. Atlas of protein sequence and structure. Silver Spring (MD): National Biomedical Research Foundation.

Eichler EE, Sankoff D. 2003. Structural dynamics of eukaryotic chromosome evolution. Science. 301:793–797.

Evans DA. 1989. Somaclonal variation–genetic basis and breeding applications. Trends Genet. 5:46–50.

Felsenstein J. 1989. PHYLIP—Phylogeny Inference Package (Version 3.2). Cladistics. 5:164–166.

Finkel SE, Kolter R. 1999. Evolution of microbial diversity during prolonged starvation. Proc Natl Acad Sci U S A. 96: 4023–4027.

Fischer G, James SA, Roberts IN, Oliver SG, Louis EJ. 2000. Chromosomal evolution in Saccharomyces. Nature. 405: 451–454.

Fischer G, Rocha EP, Brunet F, Vergassola M, Dujon B. 2006. Highly variable rates of genome rearrangements between hemiascomycetous yeast lineages. PLoS Genet. 2:e32.

Gilbert W. 1985. Genes-in-pieces revisited. Science. 228:823–824.

Haber JE. 2006. Transpositions and translocations induced by site-specific double-strand breaks in budding yeast. DNA Repair (Amst). 5:998–1009.

Hall BG. 1998. Adaptive mutagenesis: a process that generates almost exclusively beneficial mutations. Genetica. 102–103: 109–125.

Halme A, Styles C, Fink G. 2002. Morphogenetic variation via regulated mutation in yeast. Yeast Genetics and Molecular Biology Meeting. Madison (WI): University of Wisconsin.

Harris RS, Feng G, Ross KJ, Sidhu R, Thulin C, Longerich S, Szigety SK, Hastings PJ, Winkler ME, Rosenberg SM. 1999. Mismatch repair is diminished during stationary-phase mutation. Mutat Res. 437:51–60.

Hastings PJ, Slack A, Petrosino JF, Rosenberg SM. 2004. Adaptive amplification and point mutation are independent mechanisms: evidence for various stress-inducible mutation mechanisms. PLoS Biol. 2:e399.

Heller C, Pohl FM. 1990. Field inversion gel electrophoresis with different pulse time ramps. Nucleic Acids Res. 18:6299–6304.

King M. 1993. Species evolution: the role of chromosome change. Cambridge: Cambridge University Press.

Kirschner M, Gerhart J. 1998. Evolvability. Proc Natl Acad Sci U S A. 95:8420–8427.

Lea DE, Coulson CA. 1949. The distribution of the number of mutants in bacterial populations. J Genet. 49:264–285.

LeClerc JE, Li B, Payne WL, Cebula TA. 1996. High mutation frequencies among Escherichia coli and Salmonella pathogens [see comments]. Science. 274:1208–1211.

Lenski RE, Simpson SC, Nguyen TT. 1994. Genetic analysis of a plasmid-encoded, host genotype-specific enhancement of bacterial fitness. J Bacteriol. 176:3140–3147.

Li B, Tsui HC, LeClerc JE, Dey M, Winkler ME, Cebula TA. 2003. Molecular analysis of mutS expression and mutation in natural isolates of pathogenic Escherichia coli. Microbiology. 149:1323–1331.

Longo E, Vezinhet F. 1993. Chromosomal rearrangements during vegetative growth of a wild strain of Saccharomyces cerevisiae. Appl Environ Microbiol. 59:322–326.

McClintock B. 1984. The significance of responses of the genome to challenge. Science. 226:792.

Muller HJ. 1928. The measurement of gene mutation in *Drosophila*, its high variability and its dependence upon temperature. Genetics. 13:279–357.

Murphy WJ, Larkin DM, Everts-van der Wind A, et al. (25 co-authors). 2005. Dynamics of mammalian chromosome evolution inferred from multispecies comparative maps. Science. 309:613–617.

Napolitano R, Janel-Bintz R, Wagner J, Fuchs RP. 2000. All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. EMBO J. 19:6259–6265.

Nevo E, Filippucci MG, Redi C, Korol A, Beiles A. 1994. Chromosomal speciation and adaptive radiation of mole rats in Asia Minor correlated with increased ecological stress. Proc Natl Acad Sci U S A. 91:8160–8164.

Oliver A, Baquero F, Blazquez J. 2002. The mismatch repair system (mutS, mutL and uvrD genes) in Pseudomonas aeruginosa: molecular characterization of naturally occurring mutants. Mol Microbiol. 43:1641–1650.

R Foundation for Statistical. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing (www.r-project.org.).

Radman M. 1999. Enzymes of evolutionary change [news]. Nature. 401:866–869.

Radman M, Matic I, Taddei F. 1999. Evolution of evolvability. Ann N Y Acad Sci. 870:146–155.

Rosche WA, Foster PL. 2000. Determining mutation rates in bacterial populations. Methods. 20:4–17.

Schmidt KH, Pennaneach V, Putnam CD, Kolodner RD. 2006. Analysis of gross-chromosomal rearrangements in Saccharomyces cerevisiae. Methods Enzymol. 409:462–476.

Schroeder TM, Kurth R. 1971. Spontaneous chromosomal breakage and high incidence of leukemia in inherited disease. Blood. 37:96–112.

Seoighe C, Federspiel N, Jones T, et al. (20 co-authors). 2000. Prevalence of small inversions in yeast gene order evolution. Proc Natl Acad Sci U S A. 97:14433–14437.

Slack A, Thornton PC, Magner DB, Rosenberg SM, Hastings PJ. 2006. On the mechanism of gene amplification induced under stress in Escherichia coli. PLoS Genet. 2:e48.

Sniegowski PD, Gerrish PJ, Lenski RE. 1997. Evolution of high mutation rates in experimental populations of E. coli [see comments]. Nature. 387:703–705.
Soda MEA. 2007. Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. Nature. 448:561–566.
Sturtevant AH. 1937. Essays on evolution. I On the effects of selection on mutation rate. Q Rev Biol. 12:464–467.
Tenaillon O, Toupance B, Le Nagard H, Taddei F, Godelle B. 1999. Mutators, population size, adaptive landscape and the adaptation of asexual populations of bacteria. Genetics. 152:485–493.
Tomlins SA, Rhodes DR, Perner S, et al. (16 co-authors). 2005. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 310:644–648.
Vulic M, Lenski RE, Radman M. 1999. Mutation, recombination, and incipient speciation of bacteria in the laboratory. Proc Natl Acad Sci U S A. 96:7348–7351.
Woodgate R, Bridges BA, Herrera G, Blanco M. 1987. Mutagenic DNA repair in Escherichia coli. XIII. Proofreading exonuclease of DNA polymerase III holoenzyme is not operational during UV mutagenesis. Mutat Res. 183:31–37.
Yu X, Gabriel A. 2004. Reciprocal translocations in Saccharomyces cerevisiae formed by nonhomologous end joining. Genetics. 166:741–751.
Zhivotovsky LA. 1997. Environmental stress and evolution: a theoretical study. EXS. 83:241–254.
Zhou W, Goodman SN, Galizia G, et al. (17 co-authors). 2002. Counting alleles to predict recurrence of early-stage colorectal cancers. Lancet. 359:219–225.

Takashi Gojobori, Associate Editor

Accepted November 14, 2007