The spontaneous appearance rate of the yeast prion [PSI+] and its implications for the evolution of the evolvability properties of the [PSI+] system

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Abstract

Epigenetically inherited aggregates of the yeast prion \([PSI+]\) cause genome-wide readthrough translation that sometimes increases evolvability in certain harsh environments. The effects of natural selection on modifiers of \([PSI+]\) appearance has been the subject of much debate. It seems likely that \([PSI+]\) would be at least mildly deleterious in most environments, but this may be counteracted by its evolvability properties on rare occasions. Indirect selection on modifiers of \([PSI+]\) is predicted to depend primarily on the spontaneous \([PSI+]\) appearance rate, but this critical parameter has not previously been adequately measured. Here we measure this epimutation rate accurately and precisely as 5.8x10^{-7} per generation, using a fluctuation test. We also determine that genetic “mimics” of \([PSI+]\) account for up to 80% of all phenotypes involving general nonsense suppression. Using previously developed mathematical models, we can now infer that even in the absence of opportunities for adaptation, modifiers of \([PSI+]\) are only weakly deleterious relative to genetic drift. If we assume that the spontaneous \([PSI+]\) appearance rate is at its evolutionary optimum, then opportunities for adaptation are inferred to be rare, such that the \([PSI+]\) system is favored only very weakly overall. But when we account for the observed increase in the \([PSI+]\) appearance rate in response to stress, we infer much higher overall selection in favor of \([PSI+]\) modifiers, suggesting that \([PSI+]\)-forming ability may be a consequence of selection for evolvability.

INTRODUCTION

The yeast phenotype \([PSI+]\) is characterized by prion aggregates of the protein Sup35. Cells are in either a [psi-] (normal) or [PSI+] state, depending on the absence or presence of the prion aggregates (Figure 1a, 1b). Sup35 prion aggregates replicate in a similar fashion to mammalian prions but are cytoplasmic and as such, the prion state is cytoplasmically inherited (Wickner et al. 1995).

When not part of an aggregate, Sup35 helps mediate translation termination in yeast
Sup35 molecules that are incorporated into nonfunctional prion aggregates are presumably not available for translation termination, which can lead to the translation of stop codons by near-cognate tRNAs (Figure 1b; Tuite and McLaughlin 1982; Pure et al. 1985; Lin et al. 1986). This partial loss of Sup35 function leads to an increased frequency of readthrough translation of 3′ untranslated regions (3′ UTR) across all genes (Figure 1b). This increase is modest in wild-type yeast, from an average readthrough rate of 0.3% in [psi-] cells up to 1% in [PSI+] cells (Firoozan et al. 1991). Some [PSI+] yeast strains grow faster than [psi-] controls in certain harsh environments, suggesting that readthrough translation of some 3′ UTRs may be adaptive in certain conditions (True and Lindquist 2000; Joseph and Kirkpatrick 2008). This directly shows that [PSI+] -mediated capacitance may increase evolvability in the laboratory. [PSI+] -mediated phenotypes have a complex genetic basis, involving multiple loci (True et al. 2004).

As an epigenetically inherited protein aggregate, [PSI+] can easily be lost after some generations (Cox et al. 1980). This returns the lineage to its normal [psi-] state and restores translation fidelity. If a subset of revealed phenotypic variation is adaptive, it may have lost its dependence on [PSI+] by this time (True et al. 2004). This process of genetic assimilation may, for example, involve one or more point mutations in stop codons, increasing readthrough up to 100% (Figure 1e; Griswold and Masel 2009). This leaves the yeast with a new adaptive trait and with no permanent load of other, deleterious variation.

In general, stop codons can be lost either directly through point mutations, or indirectly through upstream indels. This leads to novel coding sequence coming from inframe and out-of-frame 3′ UTR, respectively. [PSI+] is expected to facilitate only the former, while mutation bias favors the latter. Yeast show a much higher ratio of inframe to out-of-frame 3′ UTR incorporation events than mammals do (Giacomelli et al. 2007), confirming a role for [PSI+] in capacitance-mediated evolvability in natural populations.

The adaptive evolution both of evolvability in general (Sniegowski and Murphy 2006; Lynch 2007; Pigliucci 2008), and of capacitance in particular (Dickinson and Seger 1999;
Wagner et al. 1999; Partridge and Barton 2000; Brookfield 2001; Pal 2001; Meiklejohn and Hartl 2002; Ruden et al. 2003), are highly controversial. In general, any costs of evolvability are borne in the present, while the benefits lie in the future, making it difficult for natural selection to favor an evolvability allele. For example, mutation rates seem to be set according to a tradeoff between metabolic cost (favoring higher mutation rates) and the avoidance of deleterious effects (favoring lower mutation rates) (Sniegowski et al. 2000). The fact that mutation creates variation, the ultimate source of evolvability, is merely a fortuitous consequence of the metabolic cost of fidelity.

Previous theoretical population genetic studies have, however, suggested that modifier alleles promoting the formation of \([\text{PSI}+]\) might, unlike mutator alleles, be favored for their evolvability properties (King and Masel 2007; Masel et al. 2007; Masel and Griswold 2009; Griswold and Masel 2009). These models depend, however, on a number of parameter estimates. In particular, a number of predictions depend on the spontaneous rate of \([\text{PSI}+]\) formation (Masel and Griswold 2009).

**\([\text{PSI}+]\) appearance rates and the fluctuation test:** The most widely cited spontaneous appearance rate for \([\text{PSI}+]\) is \(m_{\text{PSI}} \sim 10^{-7} - 10^{-5}\), based on experiments by Lund and Cox (1981). This estimate was calculated as the proportion of colonies scored as \([\text{PSI}+]\) after growth over multiple generations from a single founding \([\text{psi}-]\) clone. If \([\text{PSI}+]\) happens to appear in the first generation of growth, this leads to a “jackpot” event with only one switching event, but many \([\text{PSI}+]\) colonies. The proportion of colonies scored as \([\text{PSI}+]\) therefore yields a systematic overestimation of the \([\text{PSI}+]\) appearance rate.

Various implementations of the fluctuation test (Luria and Delbrück 1943) can address such effects. The mutation rate experiment is replicated many times using independent populations, and a Luria-Delbrück distribution is fitted to the results across all replicates. In a simulation study, Stewart (1994) examined a number of estimators of the underlying Luria-Delbrück distribution and found that the maximum likelihood estimator performed the best.
Originally developed to study mutation rates, the fluctuation test can also be used for estimating epimutation rates. Fluctuation tests have been used to estimate the rate of gene silencing in Chinese hamster ovary cells (Holliday and Ho 1998) and the yeast *Schizosaccharomyces pombe* (Singh and Klar 2002). However fluctuation tests do not appear to be used routinely for epimutation rate estimates. For example, although the rates of spontaneous appearance and disappearance of \([ISP+]\), a prion-like element in yeast, have been measured using the fluctuation test (Volkov et al. 2002), to the best of our knowledge there are no published estimates of the spontaneous rate of \([PSI+]\) appearance as measured using a fluctuation test. Although results from the fluctuation test can be confounded by reverse epimutation, or back-switching, this is only an issue if the rate of back-switching is very high, e.g. \(10^{-1}\) to \(10^{-2}\) per generation (Saunders et al. 2003). This is not the case for \([PSI+]\), for which the reverse epimutation rate (loss of \([PSI+]\)) is \(< 2 \times 10^{-4}\) (Tank et al. 2007).

**Other \([PSI+]\)-like phenotypes, including genetic mimics:** \([PSI+]\) causes partial loss of Sup35 function leading to elevated rates of translational readthrough at all stop codons (Figure 1b). There are many other spontaneous changes, presumably mutations, that also lead to elevated translational readthrough (Lund and Cox 1981). Mutations that affect readthrough at all stop codons (Figure 1c) (sometimes called “\([PSI+]\)-like”) can be considered as genetic “mimics” because they produce the same phenotype as the Sup35 aggregate, but are generally not epigenetically inherited. A specific example of such a genetic mimic was characterized by Eaglestone et al. (1999), who identified the sal3-4 point mutation in the *SUP35* gene. This leads to a defect in the Sup35 protein structure rendering the termination process less efficient (Eaglestone et al. 1999). The sal3-4 mutant can therefore be considered a partial loss-of-function genetic mimic of \([PSI+]\), since it generates the same readthrough phenotype. Translation termination could also potentially be impaired through other point mutations or deletions, for example in either the *SUP35* or *SUP45* gene (Stansfield et al. 1995a), or in a tRNA that mutates to recognize stop codons at a higher rate. The presence of genetic mimics,
whose effects are less reversible than those of \([PSI+]\), can affect the evolution of the evolvability properties of the \([PSI+]\) system such as its epimutation rate (LANCASTER and MASEL 2009). Note that genetic mimics are quite different from much rarer point mutations that convert stop codons into coding sequence (Figure 1d), resulting in readthrough at a single rather than multiple genes.

Here we performed experiments to obtain accurate and precise estimates of the baseline appearance rates of both \([PSI+]\) and \([PSI+]\)-like phenotypes in permissive laboratory conditions, excluding stop codon point mutations that affect only a single gene. Our estimates are superior to previous estimates, since we use the fluctuation test. We consider the consequences of these estimates for the evolution of the \([PSI+]\) system.
Figure 1: Comparison between the three possible modes ([PSI+], genetic mimic, point mutation revertant) of the expression of 3′ UTR sequences in yeast. (a) depicts the normal [psi−] phenotypic state; (b) the [PSI+] prion causes readthrough and low-level expression of 3′ UTRs across multiple genes, appearing at rate $m_{PSI}$; (c) depicts a genetic mimic of [PSI+] such as the sal3-4 mutant of Sup35 (EAGLESTONE et al. 1999) appearing at rate $m_{mimic}$ not reversible by the application of guanidine hydrochloride; (d) shows a point mutation in a single stop codon at rate $\mu_{point}$, leading to incorporation of formerly 3′ UTR into a single coding sequence. (e) [PSI+] can act as a “stop-gap” mechanism, buying a lineage more time to acquire one or more adaptive stop codon readthrough point mutations. When this genetic assimilation is complete, [PSI+] can revert to [psi−] (MASEL and BERGMAN 2003; GRISWOLD and MASEL 2009).
MATERIALS AND METHODS

Experiments measuring conversion from \([\psi^-]\) to \([\psi^+]\): We assessed conversion from \([\psi^-]\) to \([\psi^+]\) in strain 74-D694. 74-D694 carries the \(\text{ade}1-14\) allele that contains a premature stop codon that is suppressible by \([\psi^+]\). A \([\psi^-]\) strain of 74-D694 was generated by overexpression of Hsp104p. This \([\psi^-]\) strain was transformed with a plasmid expressing either the \([\psi^+]\) suppressible marker \(\text{ura}3-14\) (Manogaran et al. 2006), or \(\text{ura}3-197\) (Kurahashi and Nakamura 2007) both of which also contain premature stop codons suppressible by \([\psi^+]\). In addition, a transformation was performed with a plasmid containing wild type \(\text{URA}3\) as a control.

The transformed strains were grown to \(OD_{600} \sim 1.6\) and 150 \(\mu\)L of the resulting cell culture was placed onto plates of medium lacking adenine and uracil (SD-ura-ade). After four weeks of growth at 21°C on the selective medium the number of colonies was counted. These colonies derive from cells that acquired a readthrough phenotype during the growth of the cell culture. Each colony was spotted and scored as true \([\psi^+]\) if it underwent a color change from white to red following growth on guanidine hydrochloride (GdnHCl). GdnHCl cures \([\psi^+]\) permanently but only transiently affects readthrough from genetic mimics of nonsense suppression (Bradley et al. 2003).

The total number of cells at the end of growth was determined by plating 200 \(\mu\)L of a 1:10,000 dilution onto medium (SD-leu) that selects for the presence of the transformed plasmids. The total number of cells on the SD-ura-ade plates is the product of the number of colonies that grew on the SD-leu medium after 3-4 days and the dilution factor between the amount of culture plated for total cell counting and double-readthrough selective cell counting. We performed a total of 18 replicate experiments, 7 of which had a dilution factor of 22500, and 11 of which had a dilution factor of 15000. 12 of these replicates were double-marker experiments.
where colonies were simultaneously selected for readthrough at both ade and ura loci, while 6 of them were single-marker experiments using a \textit{URA3} wild-type control plasmid.

**Fluctuation test analysis:** We performed the fluctuation test on the replicate colony counts. The code originally developed by \textit{Shafer} and \textit{Sniegowski} (2005) was modified by \textit{Maughan et al.} (2006) to fix some minor issues. We modified the last version from \textit{Maughan et al.} (2006) to fit a maximum likelihood function for the Luria-Delbrück distribution for counts with two different dilution factors but the same underlying mutation rate. 95\% confidence intervals were estimated using bootstrap sampling with replacement within each of the two dilution factor subsets of the data. When bootstrapping confidence intervals on the ratio between \([PSI+]\) and \([PSI]+\)-mimic appearance rates, \([PSI+]\) and \([PSI]+\)-mimic colony counts from the same replicate experiment were resampled as a pair, as their errors may not be independent. Independent Luria-Delbrück distributions were then fitted to the sets of \([PSI+]\) and \([PSI]+\)-mimic colony counts.

**RESULTS**

**Estimating \([PSI+]\) absolute appearance rate using fluctuation test:** We selected for readthrough phenotypes and scored the number of resulting colonies. Those colonies that grew on GdnHCl were determined to be true \([PSI+]\), and the remainder were assumed to be genetic mimics of \([PSI+]\) (Table 1). Using all 18 replicates we performed the fluctuation test described previously to compute the maximum likelihood estimate (MLE) and 95\% confidence intervals (CIs) for the spontaneous \([PSI+]\) appearance rate \(m_{PSI}\) and mimic appearance rate \(m_{mimic}\). The MLE for \(m_{PSI}\) is 5.79x10\(^{-7}\) with a CI of (4.59–7.46)x10\(^{-7}\); the MLE for \(m_{mimic}\) is 2.34x10\(^{-6}\), with a CI of (2.04–2.77)x10\(^{-6}\).

We expect a single-locus stop codon point mutation reversion rate of only around 10\(^{-9}\), which is expected to make an insignificant contribution to the total. In agreement with this, using only
the 12 double selection replicates, which should almost completely eliminate the possibility of including single-locus revertants, we obtained $m_{\text{mimic}} = 2.23 \times 10^{-6}$ [CI: 2.1–3.2x$10^{-6}$], while the 6 single marker replicates gave us $m_{\text{mimic}} = 1.93 \times 10^{-6}$ [CI: 1.68–2.17x$10^{-6}$]. Since these confidence intervals overlap, and the estimates are in fact in the opposite direction to that which would be expected if single-locus revertants made a significant contribution, we used all replicates (both the single and double marker experiments) interchangeably in subsequent analyses. Note that there may be weak $[\text{PSI}+]$ variants in the population that are unable to overcome the auxotrophic selection: this would make our spontaneous appearance rate an underestimate.

**Large fraction of readthrough phenotypes are mimics of $[\text{PSI}+]$:** Based on the MLEs for $m_{\text{PSI}}$ and $m_{\text{mimic}}$ from these experiments, we can now estimate the quantity

$$R = \frac{m_{\text{PSI}}}{m_{\text{PSI}} + m_{\text{mimic}}} = 0.198$$

Using the bootstrap analysis described in Materials and Methods we obtained a 95% confidence interval for $R$ of 0.166 to 0.246.

This agrees with a meta-analysis of previous data. Lund and Cox (1981) scored the proportion of spontaneous $ADE2$ readthrough colonies that were GdnHCl-curable. In our meta-analysis, we include only 6 replicates treated by KCl and 4 replicates treated by dimethyl sulphoxide as the other treatments were mutagens, which could overestimate the number of genetic mimics. We also include similar spontaneous $ADE2$ readthrough experiments of Shewmaker and Wickner (2006) (2 replicates in each of two yeast strains). For this meta-analysis, we estimate $R$ independently for each replicate experiment using raw colony counts, and average this estimate across replicates. Systematic overestimation due to jackpots affects both the numerator and the denominator and so may be relatively unimportant for measuring relative rather than absolute rates. When including only the previous 14 replicates from Lund and Cox
(1981) and Shewmaker and Wickner (2006) we obtain a mean, median, and standard error of the mean of $R$ of 0.194, 0.186 and 0.034, respectively.

Using instead the 18 replicates from our new experiments in the same way for the purposes of comparison, we estimate the mean, median, and standard error of the mean of $R$ as 0.170, 0.186 and 0.023, respectively. Assuming the estimator of $R$ is normally distributed, an approximate 95% CI for $R$ can be obtained from the mean plus or minus twice the standard error of the mean, yielding a CI of [0.124, 0.216]. This corresponds fairly well to the more reliable 95% CI [0.166, 0.246] estimated using the bootstrap method. Our new data are in good agreement with our meta-analysis of literature data, which yield a CI of [0.126, 0.262]. Since we do not have the raw data necessary to do a more accurate and precise fluctuation test on this literature data, here we use the ML estimate for data in this paper only. We do note, however, the consistency between previous literature and our data, giving broad support to the conclusion that around 80% of all spontaneous [$PSI^+$]-like phenotypes are actually genetic mimics.
Table 1: Population and mutant counts to estimate rate of appearance of readthrough phenotype in yeast.

| Replicate | URA3 allele | Number of Dilution GdnHCl- Grown Non- Population | Mutant counts |
|-----------|-------------|-------|--------|-----------------|----------------|-----------------|
|           |             | plates factor curable (PSI+) SD-ura- (total) [PSI+] (total - | counts | counts |
| 1         | ura3-14     | 3     | 22500  | 8               | 45             | 37             | 207 |
| 2         | ura3-14     | 2     | 15000  | 4               | 36             | 32             | 214 |
| 3         | ura3-14     | 3     | 22500  | 1               | 44             | 43             | 188 |
| 4         | ura3-14     | 2     | 15000  | 4               | 17             | 13             | 285 |
| 5         | ura3-14     | 2     | 15000  | 5               | 22             | 17             | 228 |
| 6         | ura3-14     | 2     | 15000  | 4               | 79             | 75             | 281 |
| 7         | ura3-197    | 3     | 22500  | 4               | 44             | 40             | 250 |
| 8         | ura3-197    | 3     | 22500  | 16              | 61             | 45             | 232 |
| 9         | ura3-197    | 2     | 15000  | 37              | 76             | 39             | 210 |
| 10        | ura3-197    | 2     | 15000  | 3               | 14             | 11             | 210 |
| 11        | ura3-197    | 2     | 15000  | 2               | 22             | 20             | 160 |
| 12        | ura3-197    | 2     | 15000  | 9               | 40             | 31             | 314 |
| 13        | URA3        | 3     | 22500  | 7               | 42             | 35             | 222 |
| 14        | URA3        | 3     | 22500  | 1               | 74             | 73             | 158 |
| 15        | URA3        | 3     | 22500  | 2               | 35             | 33             | 181 |
| 16        | URA3        | 2     | 15000  | 5               | 23             | 18             | 234 |
| 17        | URA3        | 2     | 15000  | 2               | 14             | 12             | 261 |
| 18        | URA3        | 2     | 15000  | 5               | 23             | 18             | 413 |

Implications for natural selection on modifiers of the [PSI+] system: Evolvability will be favored by natural selection so long as \( N_e \Omega > 1 \) (King and Masel 2007; Griswold and Masel 2009), where \( \Omega \) is the probability per generation that [PSI+] becomes adaptive. We estimate the yeast effective population size \( N_e \) as \( \sim 3 \times 10^6 - 6 \times 10^6 \), computed from \( N_e = (\Theta(1 + F))/(4\mu) \) using average pairwise divergence of sequences \( \Theta \sim 0.0032 - 0.0038 \).
(Tsai et al. 2008), inbreeding coefficient $F = 0.98$ (Tsai et al. 2008) and a genome-wide per-base pair point mutation rate around $\mu = 3.3\times10^{-10}$ (Lynch et al. 2008) to $\mu = 5\times10^{-10}$ (Lang and Murray 2008). At first sight, however, $\Omega$ seems an unknowable quantity.

If, however, we assume optimality, then the rate of spontaneous $[PSI+]$ appearance $m_{PSI} = 4.59\times10^{-7} - 7.46\times10^{-7}$ that we have measured here can be used to infer $\Omega$. Without genetic mimics, $[PSI+]$ is expected to evolve towards an optimal rate of $[PSI+]$ appearance equal to the probability $\Omega$ that $[PSI+]$ appearance will be adaptive (Lachmann and Jablonka 1996; Kussell and Leibler 2005; Kussell et al. 2005; Wolf et al. 2005; King and Masel 2007).

When genetic mimics are common, a higher rate of $[PSI+]$ appearance is expected (Lancaster and Masel 2009).

The modifier model of Lancaster and Masel (2009) allows the calculation of the evolved rate of $[PSI+]$ appearance $m_{PSI}$ as a function of the rate of genetic mimic mutations $m_{mimic}$, the effective population size $N_e$ and the rate $\Omega$ at which environmental change makes $[PSI+]$-like phenotypes adaptive.

The final parameter of this model is the amount of population structure $N_em_k$, where $m_k$ is the proportion of individuals that migrate between demes. Recent genome-wide polymorphism surveys of yeast suggest that there is substantial population structure in both the wild yeast Saccharomyces paradoxus (Liti et al. 2009) and Saccharomyces cerevisiae (Liti et al. 2009; Schacherer et al. 2009). Almost all the strains of $S.\ paradoxus$ surveyed appear to fall into one of three subpopulations (Liti et al. 2009). $S.\ cerevisiae$ population structure appears to be more complex, with the first survey finding 5 subpopulations: Malaysian, “sake”, North African, West African and wine/European (Liti et al. 2009), and the second finding 3 subpopulations: “sake”, wine/European and laboratory strains (Schacherer et al. 2009). Subpopulations appear to group by ecological niche rather than strict geographical location (Schacherer et al. 2009), consistent with another $S.\ cerevisiae$ study (AA et al. 2006) where significant population structure was found between oak tree strains and vineyard strains, independent of location.

Taken together these data show that wild yeast populations are structured, although this
structure is possibly more driven by ecological specialization than by geographical proximity. Structured populations mean that gene flow between subpopulations is in the range $0 < N_e m_k < 1$. We confirmed this by estimating $N_e m_k$ based on $F_{ST}$ values from AA et al. (2006) for a limited number of genes (data not shown).

Using the model of Lancaster and Masel (2009), we calculated optimal $m_{PSI}$ as a function of $N_e$, $\Omega$, $N_e m_k$, and $m_{mimic}$. We explored the 95% confidence interval for $m_{mimic}$ of $(2.04−2.77)x10^{-6}$ and an effective population size $N_e$ in the range $(3−6)x10^6$. By assuming that observed $m_{PSI}$ is equal to the calculated optimal, we were able to infer $N_e\Omega$ (Figure 2). Most of the uncertainty in the inferred range of $N_e\Omega$ estimates is due to the uncertainty in $N_e$ rather than in $m_{mimic}$ or $N_e m_k$. Assuming optimality implies extremely low values of $\Omega \approx (3.3−5)x10^{-7}$ leading to very weak selection for the ability to form $[PSI+]$, with marginal values of $N_e\Omega \approx 1−3$. Evolvability properties will only drive the evolution of the $[PSI+]$ system if $N_e\Omega > 1$ (King and Masel 2007; Griswold and Masel 2009). This undermines the assumption of optimality, and seems to suggest that $[PSI+]$ has not evolved because of its evolvability.

However, $[PSI+]$ appearance rates are not constant, but instead are strongly dependent on environmental conditions. Stressful conditions that affect the $[PSI+]$ appearance rate include low temperatures (up to 100-fold increase) (Derkatch et al. 2000; Chernoff et al. 1995), and high salt concentrations, oxidative stress and high temperatures (up to 60-fold increase) (Tyedmers et al. 2008). Stressful conditions may induce a higher $[PSI+]$ appearance rate at times when an opportunity for $[PSI+]$-mediated adaptation exists. The model of Lancaster and Masel (2009) considers only a single $[PSI+]$ appearance rate, rather than one with and one without stress. Optimal switching is a tradeoff between the benefits of switching when needed and the costs of switching when not. However, the optimal switching rate is determined primarily by the benefits rather than the costs (King and Masel 2007; Lancaster and Masel 2009). The model is therefore a predictor of optimal induced rather than optimal baseline $[PSI+]$ appearance rate. In Figure 2 we therefore explore a range of values of induced
$m_{PSI}$ from our measured baseline “unstressed” rate, up to a 100-fold elevation of the baseline. If we consider induced values of $m_{PSI}$ up to 60-fold higher at times when $[PSI+]$ is most likely to be adaptive, we infer much higher values of $N_e\Omega \sim 3 - 50$ (Figure 2). These values are compatible with evolvability properties driving the evolution of the $[PSI+]$ system.

Most of the remaining uncertainty in the shaded regions of Figure 2 derives from uncertainty in the measurement of $N_e$ in *Saccharomyces*. Further uncertainty comes from the fact that $N_e$ may not have been constant over the evolutionary history of yeast. We therefore explore sensitivity to the parameter $N_e$ more fully in Figure 3. Evolvability is still inferred so long as $N_e >\sim 10^5$. 

Figure 2: Model of Lancaster and Masel (2009) predicts the parameter range (shaded) for which \( m_{PSI} \) (expressed as a function of the elevation from the unstressed baseline observed value of \( 5.8 \times 10^{-7} \)) is compatible with both a 95% CI of \( m_{mimic} \) of \((2.04 - 2.77) \times 10^{-6}\) and \( N_e = (3 - 6) \times 10^6 \). The upper bound is given by the combination \( \{ m_{mimic} = 2.03 \times 10^{-6}, N_e = 6 \times 10^6 \} \) and the lower bound by \( \{ m_{mimic} = 2.77 \times 10^{-6}, N_e = 3 \times 10^6 \} \).
Figure 3: Sensitivity of our inference to the effective population size, $N_e$. For $N_e \sim 5\times10^6$, as estimated for *Saccharomyces*, we infer a role for natural selection in favoring $[PSI+]$-mediated evolvability if and only if $m_{PSI}$ is elevated by stress, as has been observed. (a) For $N_e \lesssim 10^5$ we would not infer evolvability, even with realistically elevated switching rates. (b) Selection for evolvability would be inferred even in absence of stress-mediated induction for $N_e \gtrsim 4\times10^7$. Higher values of $N_e$ are not shown because their computation requires excessive memory, but the result is still clear.

**DISCUSSION**

A previous study estimated the spontaneous appearance rate of $[PSI+]$ ($m_{PSI}$) as $10^{-7} - 10^{-5}$ ([Lund and Cox 1981](#)). Here we provide a more accurate and precise measurement in unstressed conditions of baseline $m_{PSI}$ as $5.8\times10^{-7}$ with a 95% confidence interval of $4.6\times10^{-7} - 7.5\times10^{-7}$, correcting for “jackpot” effects of the Luria-Delbrück distribution. The same previous study found many non-$[PSI+]$ colonies with elevated rates of stop codon readthrough at a single locus ([Lund and Cox 1981](#)). We have confirmed that these colonies typically show readthrough at multiple loci, and are best thought of as genetic mimics of $[PSI+]$ rather than as single locus revertants. Only 20% of phenotypically $[PSI+]$-like colonies represent true $[PSI+]$. Both of
these new quantitative estimates have implications for understanding the selective forces operating on $[\text{PSI}+]$.

**Deleterious selection against $[\text{PSI}+]$ modifiers under usual conditions is very weak:** $[\text{PSI}+]$ is rare in natural yeast populations, despite the fact that outcrossed sex should cause it to spread, suggesting that it is deleterious (NAKAYASHIKI *et al.* 2005). If so, modifier alleles that permit $[\text{PSI}+]$ formation will also be subject to indirect negative selection with strength $m_{\text{PSI}}N_e$ under usual environmental circumstances during which $[\text{PSI}+]$ is deleterious (MASEL and GRISWOLD 2009). With a yeast effective population size $N_e$ between 3x10$^6$ and 6x10$^6$ (TSAI *et al.* 2008) and the confidence intervals for $m_{\text{PSI}}$ measured here, we compute $m_{\text{PSI}}N_e$ as lying between 1.38 and 4.48, i.e. $m_{\text{PSI}}N_e > 1$. This means that selection against $[\text{PSI}+]$ modifiers at times when $[\text{PSI}+]$ is deleterious is only weakly effective relative to genetic drift.

**Overall positive selection for $[\text{PSI}+]$ modifiers is only strong enough if $[\text{PSI}+]$ is induced by stress:** The $[\text{PSI}+]$ system will be favored due to its evolvability properties if $N_e\Omega > 1$ (KING and MASEL 2007; GRISWOLD and MASEL 2009). By assuming that observed $[\text{PSI}+]$ appearance rates are optimal, we have inferred the frequency $\Omega$ with which $[\text{PSI}+]$ is adaptive. Based on our measured $[\text{PSI}+]$ appearance rate of 5.8x10$^{-7}$, we infer only a marginal parameter range of $N_e\Omega \sim 1 - 3$. Higher rates of $[\text{PSI}+]$ appearance consistent with stress-mediated induction give $N_e\Omega \sim 3 - 50$. Stress induction is a necessary and sufficient condition for the inference that $[\text{PSI}+]$-forming ability has been favored by natural selection for evolvability.

**Mutational degradation:** MASEL *et al.* (2007) found that in order for the $[\text{PSI}+]$ system to avoid mutational degradation during long intervals when $[\text{PSI}+]$ is not adaptive, the condition $\bar{\tau} > 1.3/\Omega$ must be met. Calculating $\bar{\tau}$ as a function of $m_{\text{PSI}} = 5.8x10^{-7}$ and $N_e = 5x10^6$ according to the formulae of MASEL *et al.* (2007), we calculate an upper bound $m$ on the mutation rate with which the ability to form $[\text{PSI}+]$ is lost. $N_e\Omega = 3$ requires $m < 10^{-6}$ while
Ne = 50 requires $m < 4.6 \times 10^{-5}$ to avoid the mutational degradation of switching ability. It seems unlikely that the mutational degradation rate would exceed these thresholds.

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