DOI: 10.1534/genetics.109.110213

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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Manuscript received October 15, 2009
Accepted for publication November 4, 2009

ABSTRACT

Epigenetically inherited aggregates of the yeast prion [*PSI+*] cause genomewide readthrough translation that sometimes increases evolvability in certain harsh environments. The effects of natural selection on modifiers of [*PSI+*] appearance have 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 5.8×10^{-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.

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 1, a and b). 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 (Stansfield *et al.* 1995b; Zhouravleva *et al.* 1995). 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 in-frame and out-of-frame 3′-UTRs, respectively. [*PSI+*] is expected to facilitate only the former, while mutation

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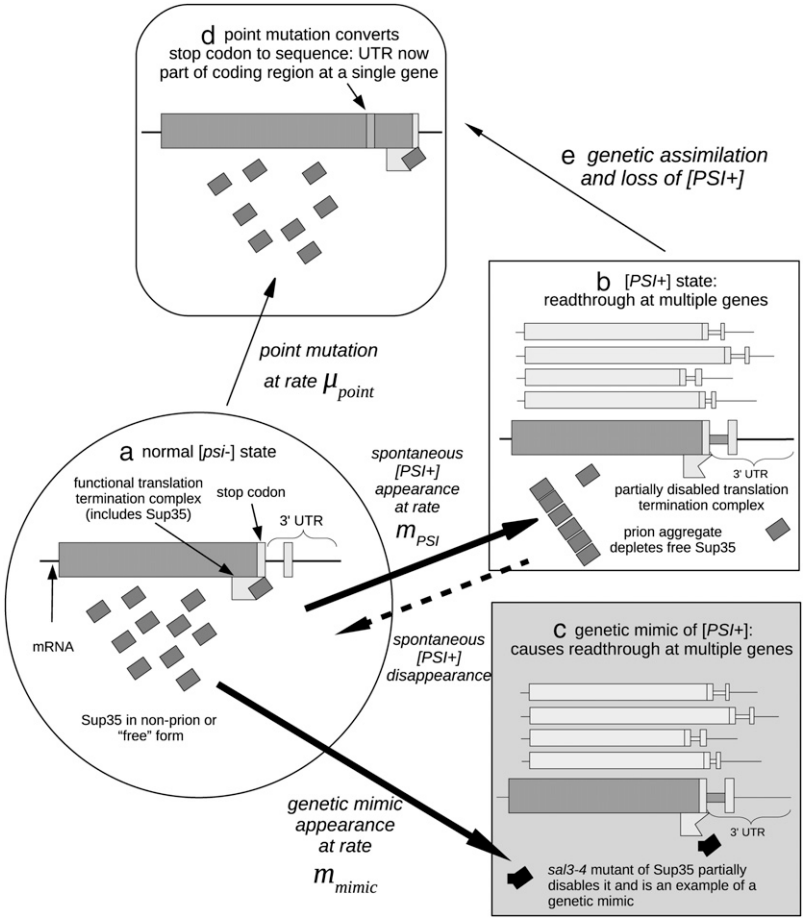

Figure 1.—Comparison between the three possible modes ([*PSI*+], *genetic mimic*, *point mutation revertant*) of the expression of 3′-UTR sequences in yeast. (a) The normal [*psi*−] phenotypic state; (b) the [*PSI*+] prion causes readthrough and low-level expression of 3′-UTRs across multiple genes, appearing at rate m_{PSI}; (c) 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) a point mutation in a single stop codon at rate μ_{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).

bias favors the latter. Yeasts show a much higher ratio of in-frame 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) is 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 trade-off 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 [*PSI*+] might, unlike mutator alleles, be favored for their evolvability properties (King and Masel 2007; Masel *et al.* 2007; Griswold and Masel 2009; Masel and Griswold 2009). These models depend, however, on a number of parameter estimates. In particular, a number of predictions depend on the spontaneous rate of [*PSI*+] formation (Masel and Griswold 2009).

[*PSI*+] appearance rates and the fluctuation test: The most widely cited spontaneous appearance rate for [*PSI*+] is $m_{PSI} \sim 10^{-7}$–10^{-5}, on the basis of experiments by Lund and Cox (1981). This estimate was calculated as the proportion of colonies scored as [*PSI*+] after growth over multiple generations from a single founding [*psi*−] clone. If [*PSI*+] happens to appear in the first generation of growth, this leads to a "jackpot" event with only one switching event, but many [*PSI*+] colonies. The proportion of colonies scored as [*PSI*+] therefore yields a systematic overestimation of the [*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 in 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 an issue only if the rate of back-switching is very high, *e.g.*, 10^{-1}–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 the *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 gene 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.

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 *ade1-14* 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 *ura3-14* (Manogaran *et al.* 2006) or *ura3-197* (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 *URA3* as a control.

The transformed strains were grown to $OD_{600} \sim 1.6$ and 150 μl of the resulting cell culture was placed onto plates of synthetic defined medium (SD) lacking adenine and uracil (SD −Ura −Ade). After 4 weeks of growth at 21° 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 μl 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 22,500 and 11 of which had a dilution factor of 15,000. Twelve 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 *URA3* wild-type control plasmid.

Fluctuation test analysis: We performed the fluctuation test on the replicate colony counts. The code originally developed by Shaver and Sniegowski (2005) was modified by Maughan *et al.* (2006) to fix some minor issues. We modified the last version from 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. Ninety-five percent 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 a 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

TABLE 1

Population and mutant counts to estimate rate of appearance of readthrough phenotype in yeast

Replicate	*URA3* allele	No. of plates	Dilution factor	Mutant counts: GdnHCl curable ([*PSI*+])	Mutant counts: Grown in SD −Ura −Ade (total)	Mutant counts: Non-[*PSI*+] (total − [*PSI*+])	Population counts (grown in SD −Leu)
1	*ura3-14*	3	22,500	8	45	37	207
2	*ura3-14*	2	15,000	4	36	32	214
3	*ura3-14*	3	22,500	1	44	43	188
4	*ura3-14*	2	15,000	4	17	13	285
5	*ura3-14*	2	15,000	5	22	17	228
6	*ura3-14*	2	15,000	4	79	75	281
7	*ura3-197*	3	22,500	4	44	40	250
8	*ura3-197*	3	22,500	16	61	45	232
9	*ura3-197*	2	15,000	37	76	39	210
10	*ura3-197*	2	15,000	3	14	11	210
11	*ura3-197*	2	15,000	2	22	20	160
12	*ura3-197*	2	15,000	9	40	31	314
13	*URA3*	3	22,500	7	42	35	222
14	*URA3*	3	22,500	1	74	73	158
15	*URA3*	3	22,500	2	35	33	181
16	*URA3*	2	15,000	5	23	18	234
17	*URA3*	2	15,000	2	14	12	261
18	*URA3*	2	15,000	5	23	18	413

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 (C.I.'s) for the spontaneous [*PSI*+] appearance rate m_{PSI} and mimic appearance rate m_{mimic}. The MLE for m_{PSI} is 5.79×10^{-7} with a C.I. of $(4.59–7.46) \times 10^{-7}$; the MLE for m_{mimic} is 2.34×10^{-6}, with a C.I. of $(2.04–2.77) \times 10^{-6}$.

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

A large fraction of readthrough phenotypes are mimics of [*PSI*+]: On the basis of the MLEs for m_{PSI} and m_{mimic} from these experiments, we can now estimate the quantity

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

Using the bootstrap analysis described in MATERIALS AND METHODS, we obtained a 95% confidence interval for R of 0.166–0.246.

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 sulfoxide 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

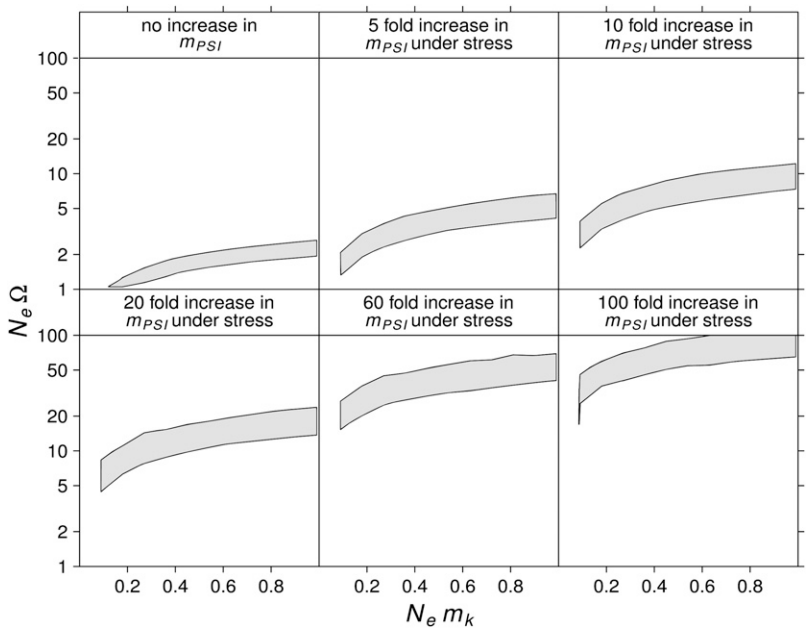

FIGURE 2.—The model of LANCASTER and MASEL (2009) predicts the parameter range (shaded) for which m_{PSI} (expressed as a function of the elevation from the unstressed baseline observed value of 5.8×10^{-7}) is compatible with both a 95% C.I. of m_{mimic} of $(2.04–2.77) \times 10^{-6}$ and $N_e = (3–6) \times 10^6$. The upper bound is given by the combination $\{m_{\text{mimic}} = 2.04 \times 10^{-6}, N_e = 6 \times 10^6\}$ and the lower bound by $\{m_{\text{mimic}} = 2.77 \times 10^{-6}, N_e = 3 \times 10^6\}$.

approximate 95% C.I. for R can be obtained from the mean plus or minus twice the standard error of the mean, yielding a C.I. of [0.124, 0.216]. This corresponds fairly well to the more reliable 95% C.I. of [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 C.I. of [0.126, 0.262]. Since we do not have the raw data necessary to do a more accurate and precise fluctuation test on these literature data, here we use the ML estimate for data in this article only. We do note, however, the consistency between previous literature and our data, giving broad support to the conclusion that ~80% of all spontaneous [*PSI*+]-like phenotypes are actually genetic mimics.

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 Ω is the probability per generation that [*PSI*+] becomes adaptive. We estimate the yeast effective population size N_e as $\sim 3 \times 10^6$–6×10^6, 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 genomewide per-base pair point mutation rate $\sim\mu = 3.3 \times 10^{-10}$ (LYNCH *et al.* 2008) to $\mu = 5 \times 10^{-10}$ (LANG and MURRAY 2008). At first sight, however, Ω seems an unknowable quantity.

If, however, we assume optimality, then the rate of spontaneous [*PSI*+] appearance $m_{PSI} = 4.59 \times 10^{-7}$–$7.46 \times 10^{-7}$ that we have measured here can be used to infer Ω. Without genetic mimics, [*PSI*+] is expected to evolve toward an optimal rate of [*PSI*+] appearance equal to the probability Ω 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 Ω at which environmental change makes [*PSI*+]-like phenotypes adaptive.

The final parameter of this model is the amount of population structure $N_e m_k$, where m_k is the proportion of individuals that migrate between demes. Recent genomewide polymorphism surveys of yeast suggest that there is substantial population structure in both the wild yeast *S. paradoxus* (LITI *et al.* 2009) and *S. 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 five subpopulations, Malaysian, "sake," North African, West African, and wine/European (LITI *et al.* 2009), and the second finding three 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 $N_e m_k$ on the basis of 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, Ω, $N_e m_k$, and m_{mimic}. We explored the 95% confidence interval for m_{mimic} of (2.04–2.77) $\times 10^{-6}$ and an effective population size N_e in the range (3–6) $\times 10^6$. 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 $\Omega \approx (3.3–5) \times 10^{-7}$, leading to very weak selection for the ability to form [*PSI*+], with marginal values of $N_e\Omega \approx 1–3$. Evolvability properties will drive the evolution of the [*PSI*+] system only 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) (Chernoff *et al.* 1995; Derkatch *et al.* 2000) 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 trade-off 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 $N_e > \sim 10^5$.

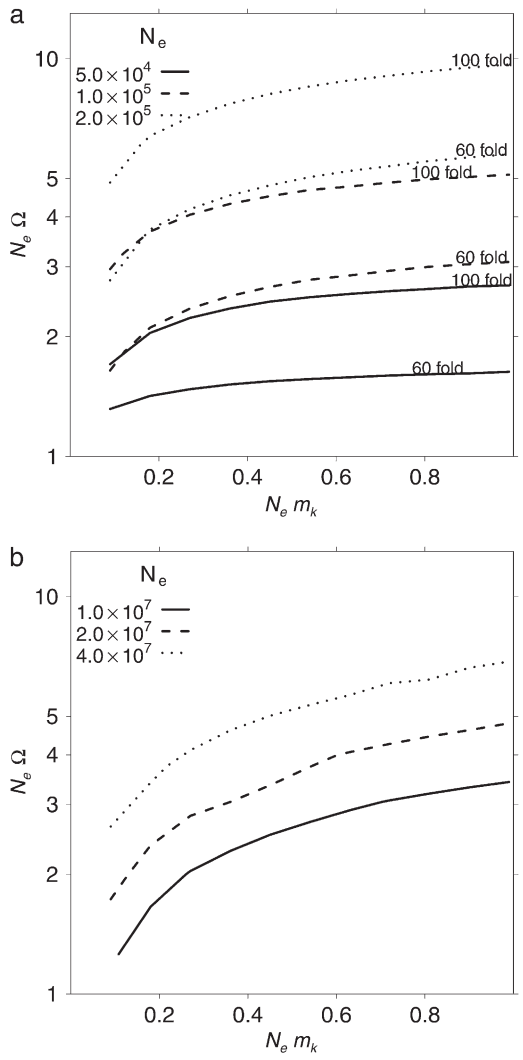

Figure 3.—Sensitivity of our inference to the effective population size, N_e. For $N_e \sim 5 \times 10^6$, as estimated for Saccharomyces, we infer a role for natural selection in favoring [*PSI*+]-mediated evolvability if and only if m_{PSI} is elevated by stress, as has been observed. (a) For $N_e \lesssim 10^5$ we would not infer evolvability, even with realistically elevated switching rates. (b) Selection for evolvability would be inferred even in the absence of stress-mediated induction for $N_e \gtrsim 4 \times 10^7$. 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 5.8×10^{-7} with a 95% confidence interval of 4.6×10^{-7}–7.5×10^{-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 [*PSI*+].

Deleterious selection against [*PSI*+] modifiers under usual conditions is very weak: [*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 [*PSI*+] formation will also be subject to indirect negative selection with strength $m_{PSI}N_e$ under usual environmental circumstances during which [*PSI*+] is deleterious (Masel and Griswold 2009). With a yeast effective population size N_e between 3×10^6 and 6×10^6 (Tsai *et al.* 2008) and the confidence intervals for m_{PSI} measured here, we compute $m_{PSI}N_e$ as lying between 1.38 and 4.48; *i.e.*, $m_{PSI}N_e > 1$. This means that selection against [*PSI*+] modifiers at times when [*PSI*+] is deleterious is only weakly effective relative to genetic drift.

Overall positive selection for [*PSI*+] modifiers is only strong enough if [*PSI*+] is induced by stress: The [*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 [*PSI*+] appearance rates are optimal, we have inferred the frequency Ω with which [*PSI*+] is adaptive. On the basis of our measured [*PSI*+] appearance rate of 5.8×10^{-7}, we infer only a marginal parameter range of $N_e\Omega \sim 1$–3. Higher rates of [*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 [*PSI*+]-forming ability has been favored by natural selection for evolvability.

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

We thank Oliver D. King for C code; Cortland Griswold, John Novembre, Michael Hickerson, Naoki Takebayashi, Grant Peterson, and Jessica Garb for helpful discussions; and the National Institutes of Health for funding (R01 GM076041 to J.M. and R01 GM072228 to H.L.T.). J.M. is a Pew Scholar in the Biomedical Sciences and an Alfred P. Sloan Research Fellow.

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Communicating editor: M. W. Feldman