The Strength of Selection Against the Yeast Prion [*PSI*⁺]

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ABSTRACT

The [*PSI*⁺] prion causes widespread readthrough translation and is rare in natural populations of *Saccharomyces*, despite the fact that sex is expected to cause it to spread. Using the recently estimated rate of *Saccharomyces* outcrossing, we calculate the strength of selection necessary to maintain [*PSI*⁺] at levels low enough to be compatible with data. Using the best available parameter estimates, we find selection against [*PSI*⁺] to be significant. Inference regarding selection on modifiers of [*PSI*⁺] appearance depends on obtaining more precise and accurate estimates of the product of yeast effective population size N_e and the spontaneous rate of [*PSI*⁺] appearance m . The ability to form [*PSI*⁺] has persisted in yeast over a long period of evolutionary time, despite a diversity of modifiers that could abolish it. If $mN_e < 1$, this may be explained by insufficiently strong selection. If $mN_e > 1$, then selection should favor the spread of [*PSI*⁺] resistance modifiers. In this case, rare conditions where [*PSI*⁺] is adaptive may permit its persistence in the face of negative selection.

[*P*^{*SI*+}] is the prion form of the protein Sup35 (WICKNER *et al.* 1995). Sup35 is involved in stop codon recognition during gene translation (STANSFIELD *et al.* 1995; ZHOORAVLEVA *et al.* 1995). In prion form, Sup35 is sequestered in aggregates, depleting the availability of functional Sup35 (WICKNER *et al.* 1995). This leads to an elevated rate of readthrough error at every stop codon in the *Saccharomyces* genome (FIROOZAN *et al.* 1991).

Given the likely cost of these translation readthrough errors, one might expect [*PSI*⁺] to have substantial deleterious effects. Nevertheless, in standard lab conditions [*PSI*⁺] strains containing prions seem to grow just as well as [*psi*⁻] strains that lack them (TRUE and LINDQUIST 2000). While it is difficult to detect small differences in fitness in the lab, wild yeast species have large effective population sizes of $\sim 10^7$ (TSAI *et al.* 2008), making natural selection extremely sensitive to tiny differences in fitness.

In Figure 1 we show the logic of all possible scenarios of selection on [*PSI*⁺], including the necessary conditions for them to hold, as derived in this article, and our assessment of the plausibility of those conditions. [*PSI*⁺] must be adaptive, neutral, or deleterious under the majority of normal conditions found in the wild. A predominantly adaptive role for [*PSI*⁺] is obviously

not compatible with its rarity (CHERNOFF *et al.* 2000; NAKAYASHIKI *et al.* 2005).

A predominantly deleterious role for [*PSI*⁺] would raise the question as to why the ability to form [*PSI*⁺] has not been eliminated by natural selection, but instead been conserved over long periods of yeast evolution (CHERNOFF *et al.* 2000; KUSHNIROV *et al.* 2000a; SANTOSO *et al.* 2000; NAKAYASHIKI *et al.* 2001). Consider a modifier locus called *prf* [prion-forming (MASEL and BERGMAN 2003)] with allele *prf*⁺ permitting [*PSI*⁺] formation and allele *prf*⁰ preventing it. When [*PSI*⁺] is deleterious, then *prf*⁺ lineages are also at a disadvantage, as they repeatedly give rise to [*PSI*⁺] progeny: this is known as indirect selection. If indirect selection is strong enough, this should lead to the fixation of the [*PSI*⁺]-resistant *prf*⁰ allele in the population. Similarly, rare positive selection on [*PSI*⁺] would lead to indirect positive selection on the *prf*⁺ allele that generated the favored [*PSI*⁺] lineage.

A variety of modifiers exist whose mutation could impart resistance to [*PSI*⁺], including the [*PIN*⁺] prion (DERKATCH *et al.* 1997) and chaperone molecules (CHERNOFF *et al.* 1999; KUSHNIROV *et al.* 2000b; SHARMA and MASISON 2008). One particular modifier is an oligopeptide repeat region within Sup35 that is required for [*PSI*⁺] formation and propagation (PARHAM *et al.* 2001). Although this region (HARRISON *et al.* 2007), together with the ability of the Sup35 protein to form [*PSI*⁺] (CHERNOFF *et al.* 2000; KUSHNIROV *et al.* 2000a; SANTOSO *et al.* 2000; NAKAYASHIKI *et al.* 2001), is conserved in yeast evolution, some natural populations of *Saccharomyces cerevisiae* carry a deletion within this oligopeptide repeat region of Sup35 that eliminates

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FIGURE 1.—Logical breakdown of all possible scenarios of direct and indirect selection on $[PSI^+]$, together with an assessment of their plausibility in explaining the data. $[PSI^+]$ must be adaptive, neutral, or deleterious under the majority of normal conditions found in the wild, as shown by the three direct selection possibilities on the left. If direct negative selection against $[PSI^+]$ is appreciable, the next question is whether indirect selection against modifiers of $[PSI^+]$ is also appreciable. Appreciable selection is equivalent to the standard population genetics criterion $sN_e > 1$. Conditions for appreciable selection being compatible with data on $[PSI^+]$ rarity are derived in terms of the spontaneous rates of $[PSI^+]$ appearance m and disappearance m' , as well as the frequency ϵ of $[PSI^+]$ within a $[PIN^+]$ population and the effective population size in the wild N_e . The conditions under which each scenario is compatible with data are summarized along each branch.

$[PSI^+]$ -forming ability (RESENDE *et al.* 2003). If this region has some inseparable pleiotropic function apart from promoting $[PSI^+]$ formation, then this function cannot be essential over short evolutionary timescales.

Although a variety of candidate modifier loci exist, in our analysis we consider an abstract modifier locus in the tradition of theoretical population genetics, rather than a specific, empirically identified modifier. We assume two alleles at this locus, a prf^0 allele that completely suppresses *de novo* $[PSI^+]$ formation and a prf^+ allele that allows for it.

Any specific modifier of $[PSI^+]$, including but not limited to changes in the sequence of Sup35, could of course be subject to pleiotropic constraint, but given the diversity of available modifiers and the long period of evolutionary time, we would expect resistance to $[PSI^+]$ to have fixed were it advantageous. Instead, the relevant pleiotropic constraint may be a single adaptive function of $[PSI^+]$ itself, rather than a series of different pleiotropic functions constraining each and every one of the possible modifier loci. It is possible that $[PSI^+]$ -formation ability has not been lost because $[PSI^+]$ occasionally has advantageous effects by exposing cryptic genetic variation in novel environments (C. K. GRISWOLD and J. MASEL, unpublished results; TRUE and LINDQUIST 2000; MASEL and BERGMAN 2003; MASEL 2005; GIACOMELLI *et al.* 2007; KING and MASEL 2007; JOSEPH and KIRKPATRICK

2008). This relatively rare role in promoting adaptation would explain its persistence over long evolutionary timescales, despite not being essential on short timescales.

An alternative hypothesis states that $[PSI^+]$ is better regarded as a sexually transmitted disease of yeast (NAKAYASHIKI *et al.* 2005). A Mendelian genetic element is inherited by only half the meiotic products and remains at a constant frequency in the absence of other forces such as selection or drift. In contrast, $[PSI^+]$ is inherited cytoplasmically during both asexual and sexual reproduction. Since $[PSI^+]$ is inherited by all four meiotic products, it will therefore increase in frequency when outcrossing occurs, giving rise to the analogy of a sexually transmitted disease (NAKAYASHIKI *et al.* 2005). $[PSI^+]$ is not found at high frequency in natural populations, so either negative selection against $[PSI^+]$ or high epigenetic reversion rates must be invoked to counter the fact that outcrossing will increase $[PSI^+]$ frequency. The extent of selection (and/or reversion) needed depends on the amount of outcrossing that spreads $[PSI^+]$. The rate of outcrossed sex in *S. paradoxus* was recently estimated as only 10^{-5} per reproductive event (TSAI *et al.* 2008). Here we quantify (through Equation 3) the parameter range necessary to keep $[PSI^+]$ at its observed low frequency, given realistic parameters describing wild yeast sex.

If $[PSI^+]$ is deleterious, then given the variety of modifiers and the long evolutionary period during which the ability to form $[PSI^+]$ has persisted, one might expect yeast to have evolved resistance to the $[PSI^+]$ “disease” through the evolution of modifiers of $[PSI^+]$ formation. For most transmissible diseases, pathogen coevolution makes this difficult, but the evolution of $[PSI^+]$ resistance needs only to adapt to a stationary target and so should be straightforward. However, indirect selection may be too weak to overcome genetic drift. With direct selection, selective costs are incurred in every generation. With indirect selection, the same cost is incurred only infrequently and is therefore less effective as an evolutionary force, since not all prf^+ individuals incur the cost of a $[PSI^+]$ phenotype. So it is possible that although direct selection against the deleterious effects of $[PSI^+]$ is strong enough to overcome genetic drift and keep $[PSI^+]$ at low frequency, indirect selection may not be strong enough to fix modifier or resistance alleles against $[PSI^+]$. In a finite population, arguments concerning the strength of selection amount to an assessment of whether or not $sN_e > 1$, where s is the selection coefficient and N_e is the effective population size.

The focus of this article is to determine the conditions for the different scenarios described above and in Figure 1 by (1) calculating the strength of direct selection $s_{PSI^+}N_e$ against $[PSI^+]$ and (2) calculating the strength of indirect selection $s_{prf^+}N_e$ against a modifier allele prf^+ that permits $[PSI^+]$ formation. Our inferences are based on the TSAI *et al.* (2008) data on sexual frequencies in *S. paradoxus* and a spontaneous rate of epigenetic conversion from $[psi^-]$ to $[PSI^+]$ of $\sim 10^{-7}$ – 10^{-5} in lab strains of *S. cerevisiae* (N. KOLOTEVA-LEVINE, G. H. MERRIT and M. F. TUIITE, personal communication; LUND and COX 1981; CHERNOFF *et al.* 1999). The reversion rate from $[PSI^+]$ to $[psi^-]$ is known to be $< 2 \times 10^{-4}$ (TANK *et al.* 2007) and is widely believed to be similar to the rate of $[PSI^+]$ appearance, although it is less studied: we explore a substantial range for this parameter. The effective population size N_e in *S. paradoxus* can be estimated as $\theta(1 + F)/(4\mu)$, where θ is the pairwise sequence divergence estimated as 0.0032–0.0038 (TSAI *et al.* 2008), the inbreeding coefficient $F = 0.98$ (TSAI *et al.* 2008), and the genomewide per-base-pair point mutation rate μ is $\sim 3.3 \times 10^{-10}$ (LYNCH *et al.* 2008) to 5×10^{-10} (LANG and MURRAY 2008). This yields $N_e \approx 3 \times 10^6$ – 6×10^6 . We use $N_e = 5 \times 10^6$ in Figures 2–4. We also use an upper bound of 1% on the frequency of $[PSI^+]$ within wild prf^+ populations of a variety of *Saccharomyces* species (NAKAYASHIKI *et al.* 2005; see below).

MODEL AND RESULTS

$[PSI^+]$ frequency in the wild: $[PSI^+]$ is rarely found at high frequency within natural populations (CHERNOFF *et al.* 2000; NAKAYASHIKI *et al.* 2005). Nevertheless, in the

presence of a second prion $[PIN^+]$ (DERKATCH *et al.* 1997), $[PSI^+]$ forms *de novo* at a rate of $\sim m = 10^{-7}$ – 10^{-5} per generation (N. KOLOTEVA-LEVINE, G. H. MERRIT and M. F. TUIITE, personal communication; LUND and COX 1981; CHERNOFF *et al.* 1999). Although it may be subject to both negative selection and reversion to $[psi^-]$, $[PSI^+]$ reappears every generation and is expected to spread by sex. Its frequency within a $[PIN^+]$ population may therefore be low, but will not be zero.

Prion presence was assessed by the visible presence of GFP-fusion protein aggregates, and 11 of 70 natural populations of various *Saccharomyces* species were positive for $[PIN^+]$ and competent to form $[PSI^+]$ following Sup35 overexpression (NAKAYASHIKI *et al.* 2005). Although none were composed primarily of $[PSI^+]$ cells, 4 of these 11 $[PIN^+]$ populations had a low but detectable frequency ($< 5\%$) of cells positive for Sup35 aggregates almost immediately after transformation with a Sup35-GFP fusion product (NAKAYASHIKI *et al.* 2005). This may (ZHOU *et al.* 2001) or may not (SALNIKOVA *et al.* 2005) be a reliable indicator of the preexisting presence of $[PSI^+]$. What is clear is that this sets an upper bound on the $[PSI^+]$ frequency within these populations. Taking into account likely limits of detection in the other 7 $[PIN^+]$ populations, we estimate a maximum frequency of 1% of $[PSI^+]$ in $[PIN^+]$ populations and use this value of $\varepsilon = 0.01$ to illustrate our calculations. To determine the sensitivity of our calculations to $[PSI^+]$ false positives, we also consider the implications of a frequency of 0.01%.

Selection against $[PSI^+]$: Consider a $[PIN^+]$ population that can form $[PSI^+]$. We assume the population is fixed for $[PIN^+]$ together with any other modifiers necessary for $[PSI^+]$ formation. Fixation is consistent with the finding of NAKAYASHIKI *et al.* (2005) that $[PIN^+]$ was either completely absent or strongly present for any given population.

We model discrete generations of reproduction followed by selection. We approximate the haploid life stage as instantaneous, so that a sexual generation takes no longer than an asexual one. We assume that $[PSI^+]$ loss occurs with probability m' during cell division when a daughter cell fails to inherit any prion aggregates. We assume *de novo* $[PSI^+]$ appearance occurs with probability m during cell growth in diploids. These assumptions mean that $[PSI^+]$ loss can be followed by $[PSI^+]$ gain within a single generation, but not vice versa.

Let z be the frequency of $[PSI^+]$ individuals. In each generation, yeast undergo meiosis with probability p_{sex} ; otherwise they reproduce clonally. Following meiosis, yeast reproduction may involve within-tetrad fertilization with probability p_{auto} , random mating with probability p_{amphi} , or haplo-selfing (haploid mother–daughter mating enabled by mating-type switching) with probability p_{haplo} . Note that $p_{\text{auto}} + p_{\text{amphi}} + p_{\text{haplo}} = 1$. Taking into account reproductive strategy and spontaneous $[PSI^+]$ appearance and disappearance as described above, the frequency of $[PSI^+]$ after reproduction (z') is

FIGURE 2.—Strength of selection against $[PSI^+]$ vs. the rate of spontaneous $[PSI^+]$ loss m' : (A) $\epsilon = 0.0001$ and (B) $\epsilon = 0.01$. TSAI *et al.* (2008) estimates of the probabilities of sex, automixis, amphimixis, and haplo-selfing are assumed. Above the dotted line the strength of selection is $>1/N_e$, where $N_e = 5 \times 10^6$. The vertical dotted line corresponds to TANK *et al.*'s (2007) upper limit on m' (m' is constrained to fall to the left of this line).

$$z' = (1 - p_{\text{sex}})(z(1 - m' + m'm) + (1 - z)m) + p_{\text{sex}} \left(p_{\text{auto}}(z(1 - m'^2 + m'^2m) + (1 - z)m) + p_{\text{amphi}}(z^2(1 - m'^2 + m'^2m) + 2z(1 - z)(1 - m' + m'm) + (1 - z)^2m) + p_{\text{haplo}}(z(1 - m' + m'm) + (1 - z)m) \right). \quad (1)$$

Equation 1 captures the increase in $[PSI^+]$ frequency during sex, as well as its appearance and disappearance by epimutation. Note that the rate of outcrossing $p_{\text{sex}}p_{\text{amphi}}$ is the most important sexual parameter in Equation 1, with the other forms of sex having more subtle quantitative effects on the effective epimutation rates.

If the relative fitness of $[PSI^+]$ individuals is $(1 - s_{PSI+})$, then after selection the frequency of $[PSI^+]$ (z'') is

$$z'' = \frac{z'(1 - s_{PSI+})}{z'(1 - s_{PSI+}) + (1 - z')}. \quad (2)$$

The equilibrium frequency of $[PSI^+]$ ($\hat{z}$) satisfies $z'' - z = 0$. Given an observed equilibrium frequency of $[PSI^+]$ $\hat{z} = \epsilon$, we estimate s_{PSI+} as

$$s_{PSI+} = \frac{m(1 - \epsilon + \epsilon m') + \epsilon p_{\text{sex}}(1 - m)(1 - m')(m'p_{\text{auto}} + (1 - \epsilon + \epsilon m')p_{\text{amphi}}) - \epsilon m'}{(1 - \epsilon)(m + \epsilon(1 - m)(1 - m') + \epsilon p_{\text{sex}}(1 - m)(1 - m')(m'p_{\text{auto}} + (1 - \epsilon + \epsilon m')p_{\text{amphi}}))}. \quad (3)$$

Substituting in the estimates $m = 10^{-7}$ – 10^{-5} (N. KOLOTEVA-LEVINE, G. H. MERRIT and M. F. TUIE, personal communication; LUND and COX 1981; CHERNOFF *et al.* 1999), $p_{\text{sex}} = 0.001$, $p_{\text{auto}} = 0.94$, $p_{\text{amphi}} = 0.01$, and $p_{\text{haplo}} = 0.05$ (TSAI *et al.* 2008), we find that the strength of selection against $[PSI^+]$ is significant (*i.e.*, $s_{PSI+}N_e > 1$) as long as $m' < m/\epsilon$ (Figure 2). If the

FIGURE 3.—Strength of selection against the modifier allele prf^+ vs. the rate of spontaneous $[PSI^+]$ loss m' . The dotted lines and conditions are the same as in Figure 2.

spontaneous rate of $[PSI^+]$ loss m' is larger, back epimutation alone is sufficient to keep $[PSI^+]$ at low levels, and we do not need to invoke selection against $[PSI^+]$ to explain why sex does not cause $[PSI^+]$ to rise to high frequency.

Selection against prion-forming modifiers: We determine the effective strength of indirect selection against prf^+ (s_{prf+}) by comparing the long-term growth rates of populations fixed for one or the other allele. Assume that $[psi^-]$ individuals have R offspring on average while $[PSI^+]$ individuals have $R(1 - s_{PSI+})$. In the population fixed for prf^+ , let $Y_1(t)$ and $Y_2(t)$ be the numbers of $[PSI^+]$ and $[psi^-]$ individuals, respectively, so $[PSI^+]$ is present at equilibrium frequency $\hat{z} = \epsilon = \lim_{t \rightarrow \infty} Y_1(t)/(Y_1(t) + Y_2(t))$. In the prf^0 population, $[PSI^+]$ is absent. The population dynamics of a prf^+ population, given mutation, selection, and the reproductive modes described in the previous section, now follow

$$\begin{pmatrix} Y_1(t+1) \\ Y_2(t+1) \end{pmatrix} = R \begin{pmatrix} A & m \\ B & (1 - m) \end{pmatrix} \begin{pmatrix} Y_1(t) \\ Y_2(t) \end{pmatrix}. \quad (4)$$

$$A = (1 - s_{PSI+})((1 - p_{\text{sex}})(1 - m' + m'm) + p_{\text{sex}}(p_{\text{auto}}(1 - m'^2 + m'^2m) + p_{\text{amphi}}(\hat{z}(1 - m'^2 + m'^2m) + (1 - \hat{z})(1 - m' + m'm)) + p_{\text{haplo}}(1 - m' + m'm)))$$

$$B = (1 - s_{PSI+})((1 - p_{\text{sex}})(m' - m'm) + p_{\text{sex}}(p_{\text{auto}}(m'^2 - m'^2m) + p_{\text{amphi}}(\hat{z}(m'^2 - m'^2m) + (1 - \hat{z})(m' - m'm)) + p_{\text{haplo}}(m' - m'm))).$$

FIGURE 4.—The solid and dashed curves give the boundaries between nearly neutral (top) and deleterious (bottom) parameter regions for $[PSI^+]$ and prf^+ , respectively, based on $N_e = 5 \times 10^6$. $[PSI^+]$ is deleterious while its modifier prf^+ is nearly neutral only in the very restricted shaded region in the middle for both $\epsilon = 0.0001$ and $\epsilon = 0.01$. The horizontal dotted line corresponds to TANK *et al.*'s (2007) upper limit on m' (m' is constrained to fall below this line).

The long-term prf^+ population growth rate is determined by the leading eigenvalue (λ_1) of (4), while the long-term growth rate of prf^0 populations is R . The strength of selection against prf^+ is

$$s_{prf^+} = 1 - \frac{\lambda_1}{R} \quad (5a)$$

$$s_{prf^+} = 1 - \frac{1}{2} \left(1 + A - m + \sqrt{1 - 2A + A^2 - 2m + 2Am + 4Bm + m^2} \right). \quad (5b)$$

The strength of selection against prf^+ is negligible (*i.e.*, $s_{prf^+} N_e \ll 1$) for $m' > m/\epsilon$ and equal to the rate of $[PSI^+]$ appearance m for $m' < m/(10\epsilon)$ (Figure 3), using the same parameters as before. The latter selection strength indicates that $[PSI^+]$ lineages are effectively “doomed” from the moment of their appearance.

For selection of any kind to be effective, we need $sN_e > 1$. The disease hypothesis requires selection on $[PSI^+]$ to be effective while selection on prf^+ is not. When $mN_e > 1$, the small shaded region in Figure 4 indicates values of m and m' where the conditions for the disease hypothesis are met. Outside this narrow shaded region, either both $[PSI^+]$ and prf^+ are deleterious or both are nearly neutral. Pending more precise experimental estimates of ϵ , m , and m' , the condition $m' > m/(10\epsilon)$ seems unlikely to be satisfied. This implies that if $mN_e > 1$, then both $[PSI^+]$ and prf^+ are likely deleterious.

Our analysis does, however, support the disease hypothesis if $mN_e < 1$. N_e is currently estimated as $\approx 3 \times 10^6$ – 6×10^6 in the wild (see calculation in the Introduction) and m as 10^{-7} – 10^{-5} in the lab (N. KOLOTEVA-LEVINE, G. H. MERRIT and M. F. TUIE, personal communication; LUND and COX 1981; CHERNOFF *et al.* 1999; H. L.

TRUE, personal communication). This yields $mN_e \approx 0.3$ – 60 , a range that is not sufficiently precise either to accept or to reject the disease hypothesis. Note, however, that conversion assays may return only a subset of particularly strong and stable $[PSI^+]$ colonies (H. L. TRUE, personal communication), suggesting that the true value of m may be higher: if this result is borne out, the disease hypothesis could be rejected in favor of relatively weak but still appreciable selection against prf^+ . On the other hand, previous conversion assays have not accounted for the possibility that multiple colonies may arise from the same conversion event: such a correction can be made by using a fluctuation test based on fitting a Luria–Delbrück distribution and may lead to a lower estimate of m . Accurate measurement of m at the lower end of the current parameter range would support the disease hypothesis, in which indirect selection is too weak to eliminate the ability to form the generally deleterious $[PSI^+]$ prion.

DISCUSSION

Our calculations imply that $[PSI^+]$ is usually deleterious if $m' < m/\epsilon$, and we infer that effective selection also extends to modifiers of $[PSI^+]$ if both $m' < m/(10\epsilon)$ and $mN_e > 1$. Spontaneous conversion from $[psi^-]$ to $[PSI^+]$ has been estimated in lab strains to have a rate of $\sim 10^{-7}$ – 10^{-5} (N. KOLOTEVA-LEVINE, G. H. MERRIT and M. F. TUIE, personal communication; LUND and COX 1981; CHERNOFF *et al.* 1999) and a reverse rate $m' < 2 \times 10^{-4}$ (TANK *et al.* 2007). Pending more precise measurements, it seems likely that the condition $m' < m/(10\epsilon)$ is easily met. This argument is even stronger if not all the Sup35 aggregates observed by NAKAYASHIKI *et al.* (2005) correspond to $[PSI^+]$ and ϵ is therefore even < 0.01 . If $mN_e > 1$, we can therefore infer that selection against both $[PSI^+]$ and its modifiers is effective. If instead $mN_e < 1$, then this would support the disease hypothesis in which indirect selection is too weak to eliminate a propensity to form the generally deleterious element $[PSI^+]$.

Improved measurement of the parameters, particularly the product mN_e , is critical to understanding selective forces on $[PSI^+]$. Inference ultimately depends on the range of parameters in effect over long periods of yeast evolution, so extrapolation of population genetic measurements of N_e and of sexual frequencies in *S. paradoxus* to cover the history of a clade of closely related species is not unreasonable. However, it is hard to rule out the important possibility that lab strains have substantially different epimutation rates m and m' than wild species, especially when those lab strains have been extensively used to study $[PSI^+]$. Our population genetic model is very general in its treatment of well-characterized yeast and $[PSI^+]$ biology. Correct inference from the model ultimately depends on accurate parameter estimates.

Note that the rate of $[PSI^+]$ appearance increases in response to stress (TYEDMERS *et al.* 2008). Negative selection against $[PSI^+]$ -forming ability, as calculated here, depends largely on the value of mN_e under normal conditions, while only the adaptive potential of $[PSI^+]$ -forming ability increases with m under stress.

If it turns out that $mN_e > 1$, and both $[PSI^+]$ and prf^+ are therefore inferred to be significantly deleterious under most conditions, then something else must be acting to maintain prion-forming ability over long periods of yeast evolution. Insurmountable pleiotropic constraint for each of the many possible modifiers, each enduring over long periods of evolutionary time, seems an unlikely explanation. Indeed, the data directly show that prion-forming ability can be dispensable in wild populations on short evolutionary timescales (RESENDE *et al.* 2003). Instead of multiple insurmountable constraints at each possible modifier, $[PSI^+]$ itself could have a single, mechanistic inseparable adaptive pleiotropic function that counters its other deleterious effects. With this view, long periods of relatively weak selection against $[PSI^+]$ and its modifiers would be balanced by short, intense periods of positive selection for them (MASEL and BERGMAN 2003; KING and MASEL 2007; MASEL *et al.* 2007). During the long periods of selection against $[PSI^+]$, the rarity of outbreeding, combined with weak negative selection, explains why meiosis does not drive $[PSI^+]$ to high frequency. $[PSI^+]$ is predicted to occur at high frequency during occasional episodes of $[PSI^+]$ -mediated adaptation. These episodes are likely to be both rare and brief, however, and so theoretical considerations suggest that it is not surprising that none were seen in a sample of 70 natural populations (MASEL *et al.* 2007).

Note that while improved measurements showing that $mN_e > 1$ would support a rare adaptive role for $[PSI^+]$, a finding that $mN_e < 1$ would not rule adaptation out. Indeed, it should be easier for an adaptive modifier allele prf^+ to evolve when only negligible selection acts against it at other times. The appearances of $[PSI^+]$ as a disease at some times and as an adaptation at others are not mutually exclusive.

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LITERATURE CITED

- CHERNOFF, Y. O., G. P. NEWNAM, J. KUMAR, K. ALLEN and A. D. ZINK, 1999 Evidence for a protein mutator in yeast: role of the Hsp70-related chaperone Ssb in formation, stability, and toxicity of the $[PSI]$ prion. *Mol. Cell. Biol.* **19**: 8103–8112.
- CHERNOFF, Y. O., A. P. GALKIN, E. LEWITIN, T. A. CHERNOVA, G. P. NEWNAM *et al.*, 2000 Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein. *Mol. Microbiol.* **35**: 865–876.
- DERKATCH, I. L., M. E. BRADLEY, P. ZHOU, Y. O. CHERNOFF and S. W. LIEBMAN, 1997 Genetic and environmental factors affecting the de novo appearance of the $[PSI^+]$ prion in *Saccharomyces cerevisiae*. *Genetics* **147**: 507–519.
- FIROOZAN, M., C. M. GRANT, J. A. B. DUARTE and M. F. TUITTE, 1991 Quantitation of readthrough of termination codons in yeast using a novel gene fusion assay. *Yeast* **7**: 173–183.
- GIACOMELLI, M. G., A. S. HANCOCK and J. MASEL, 2007 The conversion of 3' UTRs into coding regions. *Mol. Biol. Evol.* **24**: 457–464.
- HARRISON, L. B., Z. YU, J. E. STAJICH, F. S. DIETRICH and P. M. HARRISON, 2007 Evolution of budding yeast prion-determinant sequences across diverse fungi. *J. Mol. Biol.* **368**: 273–282.
- JOSEPH, S. B., and M. KIRKPATRICK, 2008 Effects of the $[PSI^+]$ prion on rates of adaptation in yeast. *J. Evol. Biol.* **21**: 773–780.
- KING, O., and J. MASEL, 2007 The evolution of bet-hedging adaptations to rare scenarios. *Theor. Popul. Biol.* **72**: 560–575.
- KUSHNIROV, V. V., N. KOCHNEVA-PERVUKHOVA, M. B. CHECHENOVA, N. S. FROLOVA and M. D. TER-AVANESYAN, 2000a Prion properties of the Sup35 protein of yeast *Pichia methanolica*. *EMBO J.* **19**: 324–331.
- KUSHNIROV, V. V., D. S. KRYNDUSHKIN, M. BOGUTA, V. N. SMIRNOV and M. D. TER-AVANESYAN, 2000b Chaperones that cure yeast artificial $[PSI^+]$ and their prion-specific effects. *Curr. Biol.* **10**: 1443–1446.
- LANG, G. I., and A. W. MURRAY, 2008 Estimating the per-base-pair mutation rate in the yeast *Saccharomyces cerevisiae*. *Genetics* **178**: 67–82.
- LUND, P. M., and B. S. COX, 1981 Reversion analysis of $[psi^-]$ mutations in *Saccharomyces cerevisiae*. *Genet. Res.* **37**: 173–182.
- LYNCH, M., W. SUNG, K. MORRIS, N. COFFEY, C. R. LANDRY *et al.*, 2008 A genome-wide view of the spectrum of spontaneous mutations in yeast. *Proc. Natl. Acad. Sci. USA* **105**: 9272–9277.
- MASEL, J., 2005 Evolutionary capacitance may be favored by natural selection. *Genetics* **170**: 1359–1371.
- MASEL, J., and A. BERGMAN, 2003 The evolution of the evolvability properties of the yeast prion $[PSI^+]$. *Evolution* **57**: 1498–1512.
- MASEL, J., O. D. KING and H. MAUGHAN, 2007 The loss of adaptive plasticity during long periods of environmental stasis. *Am. Nat.* **169**: 38–46.
- NAKAYASHIKI, T., K. EBHARA, H. BANNAI and Y. NAKAMURA, 2001 Yeast $[PSI^+]$ “prions” that are cross-transmissible and susceptible beyond a species barrier through a quasi-prion state. *Mol. Cell* **7**: 1121–1130.
- NAKAYASHIKI, T., C. P. KURTZMAN, H. K. EDSKES and R. B. WICKNER, 2005 Yeast prions $[URE3]$ and $[PSI^+]$ are diseases. *Proc. Natl. Acad. Sci. USA* **102**: 10575–10580.
- PARHAM, S. N., C. G. RESENDE and M. F. TUITTE, 2001 Oligopeptide repeats in the yeast protein Sup35p stabilize intermolecular prion interactions. *EMBO J.* **20**: 2111–2119.
- RESENDE, C. G., T. F. OUTEIRO, L. SANDS, S. LINDQUIST and M. F. TUITTE, 2003 Prion protein gene polymorphisms in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **49**: 1005–1017.
- SALNIKOVA, A. B., D. S. KRYNDUSHKIN, V. N. SMIRNOV, V. V. KUSHNIROV and M. D. TER-AVANESYAN, 2005 Nonsense suppression in yeast cells overproducing Sup35 (eRF3) is caused by its non-heritable amyloids. *J. Biol. Chem.* **280**: 8808–8812.
- SANTOSO, A., P. CHIEN, L. Z. OSHEROVICH and J. S. WEISSMAN, 2000 Molecular basis of a yeast prion species barrier. *Cell* **100**: 277–288.
- SHARMA, D., and D. C. MASISON, 2008 Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different anti-prion effects. *Genetics* **179**: 1301–1311.
- STANSFIELD, I., K. M. JONES, V. V. KUSHNIROV, A. R. DAGKESAMANSKAYA, A. I. POZNYAKOVSKI *et al.*, 1995 The products of the sup45 (eRF1) and sup35 genes interact to mediate translation termination in *Saccharomyces cerevisiae*. *EMBO J.* **14**: 4365–4373.
- TANK, E. M. H., D. A. HARRIS, A. A. DESAI and H. L. TRUE, 2007 Prion protein repeat expansion results in increased aggregation and reveals phenotypic variability. *Mol. Cell. Biol.* **27**: 5445–5455.
- TRUE, H. L., and S. L. LINDQUIST, 2000 A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* **407**: 477–483.
- TSAL, I. J., D. BENSASSON, A. BURT and V. KOUFOPANOU, 2008 Population genomics of the wild yeast *Saccharomyces paradoxus*: quantifying the life cycle. *Proc. Natl. Acad. Sci. USA* **105**: 4957–4962.
- TYEDMERS, J., M. L. MADARIAGA and S. LINDQUIST, 2008 Prion switching in response to environmental stress. *PLoS Biol.* **6**: e294.

- WICKNER, R. B., D. C. MASISON and H. K. EDSKES, 1995 [PSI] and [URE3] as yeast prions. *Yeast* **11**: 1671–1685.
- ZHOU, P., I. L. DERKATCH and S. W. LIEBMAN, 2001 The relationship between visible intracellular aggregates that appear after overexpression of Sup35 and the yeast prion-like elements [PSI⁺] and [PIN⁺]. *Mol. Microbiol.* **39**: 37–46.
- ZHOURAVLEVA, G., L. FROLOVA, X. LEGOFF, R. LEGUELLEC, S. INGE-VECHTOMOV *et al.*, 1995 Termination of translation in eukaryotes is governed by two interacting polypeptide-chain release factors, eRF1 and eRF3. *EMBO J.* **14**: 4065–4072.

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