Published in final edited form as:

Theor Popul Biol. 2011 December ; 80(4): 317–322. doi:10.1016/j.tpb.2011.08.004.

The consequences of rare sexual reproduction by means of selfing in an otherwise clonally reproducing species

Joanna Masel^{*} and David N. Lyttle[§]

^{*}Dpt. Ecology & Evolutionary Biology, University of Arizona, Tucson AZ 85721, USA

[§]Applied Math, University of Arizona, Tucson AZ 85721, USA

Abstract

Clonal reproduction of diploids leads to an increase in heterozygosity over time. A single round of selfing will then create new homozygotic genotypes. Given the same allele frequencies, heritable genetic variation is larger when there are more extreme, i.e. homozygotic, genotypes. So after a long clonal expansion, one round of selfing increases heritable genetic variation, but any fully or partially recessive deleterious alleles simultaneously impose a fitness cost. Here we calculate that the cost of selfing in the yeast *Saccharomyces* is experienced only by a minority of zygotes. This allows a round of selfing to act as an evolutionary capacitor to unlock genetic variation previously found in a cryptic heterozygous form. We calculate the evolutionary consequences rather than the evolutionary causes of sex. We explore a range of parameter values describing sexual frequencies, focusing especially on the parameter values known for wild *Saccharomyces*. Our results are largely robust to many other parameter value choices, so long as meiosis is rare relative to the strength of selection on heterozygotes. Results may also be limited to organisms with a small number of genes. We therefore expect the same phenomenon in some other species with similar reproductive strategies.

Keywords

cyclic parthenogenesis; inbreeding depression; evolvability; automixis; intratetrad mating; genome size

INTRODUCTION

Yeast in the genus *Saccharomyces*, as well as other taxa, use a little-studied reproductive strategy that alternates mitotic expansion with selfing. Here we explore some of the population genetic consequences of this reproductive strategy. This strategy is known to have evolved, and our focus in this paper is on its evolutionary consequences, rather than its evolutionary causes.

Wild *Saccharomyces* spend most of their life cycle as mitotically dividing diploids. Occasional episodes of meiosis usually lead to intratetrad mating between different products

© 2011 Elsevier Inc. All rights reserved.

Corresponding author: Joanna Masel, Dpt. Ecology & Evolutionary, 1041 E. Lowell St., Tucson AZ 85721, USA. Ph. +1 520 626 9888 Fax. +1 520 621 9190, masel@u.arizona.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of the same meiosis, with occasional haplo-selfing deriving from a single meiotic product, and still rarer outcrossing (Tsai et al., 2008). We take advantage of *Saccharomyces* data that has allowed the inference of life cycle parameter values (Tsai et al., 2008).

Other species, such as the plants *Iris versicolor* (Kron et al., 1993) and *Botrychium dissectum* (McCauley et al., 1985), also alternate primarily between asexuality and selfing, but their parameter values are unknown. The alternation of some form of occasional sex with clonal reproduction is found in many other taxa, including *Daphnia*, aphids, gall wasps, and an enormous variety of non-metazoan species. Here we calculate the consequences of a preponderance of selfing rather than outcrossing within the context of such an alternation between clonal expansion and sex. For many species, especially unicellular species, that are known to undergo meiosis rarely, there is as yet no data concerning the frequency of outcrossing vs. selfing (Halkett et al., 2005), and so life cycles resembling that of *Saccharomyces* could be common. We therefore also explore the life cycle parameter space, to test the generality of our results to other species that undergo meiosis rarely.

Consider one locus with two alleles with frequencies p and q . Assume additive effects, so that the 3 possible genotypes add 0, 1 or 2 to a phenotypic value and/or to fitness. The genotype frequencies are p^2+2Fpq , $2pq-2Fpq$ and q^2+2Fpq , where F is the inbreeding coefficient (Wright, 1951 Table 1). In the absence of epistasis, this locus adds $2q(1-q)(1+F)$ to genetic variance (Wright, 1951 p.324). This illustrates the way in which genetic variance per locus increases with the frequency of extreme genotypes (i.e. with homozygosity) and hence with inbreeding. When we consider the fact that most rare mutations are at least partially recessive, inbreeding may increase genetic variance still further by increasing the frequency of recessive homozygote genotypes.

In obligately sexual diploids it has been shown both theoretically (Robertson, 1952; Wang et al., 1998; Willis and Orr, 1993) and empirically (Briggs and Goldman, 2006; Bryant et al., 1986; Fernández et al., 1995; López-Fanjul and Villaverde, 1989; Van Buskirk and Willi, 2006; van Heerwaarden et al., 2008; Wade et al., 1996) that the effects of inbreeding can more than offset the reduction in additive genetic variance caused by increased genetic drift during population bottlenecks. However, although additive genetic variance may increase with bottleneck-driven inbreeding, the accompanying reduction in mean fitness via inbreeding depression means that the increased additive genetic variance is unlikely to be converted into an improved response to selection (López-Fanjul et al., 2000; van Heerwaarden et al., 2008).

Here we examine the consequences of selfing in the absence of population bottlenecks, with facultative rather than obligate sex, and with sex taking the form of selfing. Most mutations are deleterious, at least in the historical environment to which a population is presumably already well-adapted. During long intervals between meioses, mutations, especially those that are partially recessive with $h < 0.5$, are expected to accumulate in heterozygotes. Following selfing, their new appearance in homozygotic genotypes will have a significant impact on the population. Many of these homozygotes are likely to be deleterious and hence be evolutionary dead ends. The substantial portion of the population that lacks deleterious recessive alleles will, however, experience an increase in heritable variation. This idea has previously been proposed verbally and in brief as “genome renewal” in *Saccharomyces* (Mortimer et al., 1995; Mortimer et al., 1994): here we examine its plausibility in the light of a more rigorous model.

MODEL

Wild *Saccharomyces* spend most of their life cycle as diploids, with occasional episodes of meiosis and sporulation usually rapidly followed by fusion. Consider first a one-locus

model. Let X , Y and Z be the numbers of wild-type, heterozygous and homozygous mutant individuals at a locus with no genetic linkage to the mating type locus. Considering mutation, differential reproduction, and death, we have the replicator equations

$$\begin{aligned}\frac{dX}{dt} &= \ln 2((1 - \delta)X - 2\mu X) \\ \frac{dY}{dt} &= \ln 2((1 - hs - \delta)Y + 2\mu X - \mu Y) \\ \frac{dZ}{dt} &= \ln 2((1 - s - \delta)Z + \mu Y)\end{aligned}\quad (1)$$

where μ is the mitotic mutation rate. Selection, with strength s against homozygotes and dominance h , can be interpreted as differential reproduction, with death occurring at a constant rate δ . When $\delta=0$, a wild-type population would double in size every generation in the absence of mutation: population size expansion during the asexual phase is common in facultatively sexual taxa. The $\ln 2$ scaling factor converts rates from their usual discrete, per-generation formulation into instantaneous rates for our continuous differential equation approach.

Working with genotype frequencies is more convenient than working with absolute numbers. Let A be the frequency rather than the number of wild-type homozygotes, and B be the frequency of heterozygotes, so the frequency of homozygous mutant individuals is $1 - A - B$. Using the quotient rule (see Supplementary Information for complete derivation), the parameter δ disappears, and the system can now be reduced to only two equations

$$\begin{aligned}\frac{dA}{dt} &= A \ln 2(-2\mu + hsB + s(1 - A - B)) \\ \frac{dB}{dt} &= \ln 2(B((1 - h)s(1 - B) - \mu - sA) + 2\mu A)\end{aligned}\quad (2)$$

Yeast undergo sex approximately once every 1000 generations in wild populations of *S. paradoxus* (Tsai et al., 2008). This also affects genotype frequencies. Sex starts with the production of four haploid products of a single meiosis within the ascus. It is estimated that in wild *S. paradoxus*, 94% of meioses result in within-tetrad mating, 5% in haploid propagation leading to mating-type switching and mother-daughter mating, and only 1% result in outcrossing (Tsai et al., 2008). Mating within a tetrad produced by a heterozygote yields 2/3 heterozygotic progeny and 1/6 homozygotic progeny of each of the two types. Haploselfing yields all homozygotic progeny. Both of these kinds of selfing are known as automixis, since they involve the fusion of two haploid cells that originated from the same meiosis (Mogie, 1986). In contrast, selfing between the products of different meioses (autogamy) from the same heterozygotic parent yields 1/2 heterozygotic progeny and 1/4 homozygotic progeny of each of the two types.

Using the estimated sexual frequencies for *Saccharomyces*, genotype frequencies after sex A' and B' are given by

$$\begin{aligned}A' &= 0.94(A + B/6) + 0.05(A + B/2) + 0.01(A + B/2)^2 \\ B' &= 0.94(2B/3) + 0.02(A + B/2)(1 - A - B/2)\end{aligned}\quad (3)$$

While Equations (1) and hence (2) have messy but still obtainable solutions, we were not able to solve the composition of this solution with Eq. (3). Using Mathematica 7, we therefore numerically solve for the presex and postsex values of A and B for the composition

of (2) with $\Delta t=1000$ and (3) in order to predict genotype frequencies for each gene in the population. In other words, the limit of many cycles is described by a cyclical attractor, and we solve for two informative points along this attractor.

Here we ignore selection during the brief haploid phase, but note that it is straightforward to extend our modeling approach to explore separately the population dynamics of the mating-specific genes likely to be under strongest selection in the yeast haploid phase. Some models of selection during a brief haploid stage suggest that its impact on diploid fitness is small (Charlesworth and Charlesworth, 1992), although see Mulcahy et al. (1996) for an alternative view with regard to plants.

In order to apply this deterministic one-locus model to multiple loci, we need to assume complete independence between the loci, i.e. we must assume that there is neither physical linkage nor linkage disequilibrium. Physical linkage becomes important not as a function of the total number of genes (e.g., 1179 for genes essential to *Saccharomyces*) and their proximity, but rather as a function of the number of genes that are heterozygotic in one individual prior to meiosis, and hence as the average genetic distance between each such pair. With inbreeding much more common than outcrossing, very few genes are heterozygotic in one individual prior to meiosis (see Results), allowing us to ignore physical linkage.

Linkage disequilibrium can, however, occur even without physical linkage. While equal quantities of positive and negative disequilibria arise by chance, positive disequilibria are rapidly eliminated by natural selection (Barton and Otto, 2005). Linkage disequilibrium is therefore likely to be negative on average in asexual populations (Barton and Otto, 2005; Keightley and Otto, 2006; Kouyos et al., 2007). This means that double mutants, primarily heterozygotes, will be slightly less common than in our deterministic approximation, where we multiply individual allele frequencies derived from a one-locus model. On the other hand, treating loci independently also neglects identity disequilibrium due to the common history of two loci of selfing vs. outcrossing (Haldane, 1949; Weir and Cockerham, 1973); this is expected to make double homozygotes slightly more common.

Since the magnitude and balance between these two effects is not empirically known, we performed multi-locus Wright-Fisher simulations that include linkage disequilibrium, and compare results to those of our deterministic solution that assumes independent sites. Population size was set by default to $N=100,000$, and we tracked each individual as a complete genotype at all loci. At least twenty replicates of each condition were simulated to calculate the median and 60% confidence interval. Each replicate simulated multiple cycles of a long asexual phase followed by a single sexual generation. To allow burn-in, results were taken from the tenth sexual cycle of each replicate. For each asexual replication, we first simulated mutation at each wild-type site. Fitness is assumed to be multiplicative across sites. Rejection sampling was then used to select individuals for the next generation on the basis of their fitness. For the sexual phase, we assumed for simplicity that all sex consists of intra-tetrad mating, such that individuals heterozygotic for a given gene will be replaced by either two heterozygotic offspring (with probability $\frac{2}{3}$), or one wild type and one homozygous mutant (with probability $\frac{1}{3}$). The outcome of mating is independent across genes, representing the absence of physical linkage. Selection is not implemented in the sexual phase. Instead, half of the population is chosen at random to reproduce.

Given their computational cost, simulations were performed only for a small selection of parameter values, in order to validate our deterministic methods. Deterministic calculations explored a much greater variety of parameter values.

RESULTS

Saccharomyces Genome Database lists 1179 essential genes. With $s=1$, $h=0.2$ and the mitotic loss of function mutation rate $\mu=10^{-6}$ per gene (Lynch et al., 2008), mutants at a given locus are rare. If we treat each site independently, and solve the composition of Eqs. (2) and (3) for each gene, we find that after sex, only 0.2% of zygotes will be inviable due to homozygous loss of function of at least one of these 1179 genes. Even with $h=0.02$, we still have only 2% of zygotes inviable.

Saccharomyces also has around 4700 non-essential genes. Although occasional gene loss is observed, especially in subtelomeric genes involved in diverse carbon metabolism (Schacherer et al., 2009), generally we expect homozygous loss of function of one of these non-essential genes to be deleterious. We set $h=0.2$ (Agrawal and Whitlock, 2011; Szafraniec et al., 2003) and the mitotic loss of function mutation rate $\mu=10^{-6}$ per gene. Before sex, we calculate the mean number of loci heterozygous for loss of function as 2.3 for $s=0.02$: this low number across the entire genome allows us to ignore physical linkage.

Figure 1A shows how the proportion of zygotes with homozygous loss of neither essential ($s=1$) nor non-essential (one value of $0 \leq s \leq 1$ across all non-essential genes) genes depends on s . As an illustration of the distribution, with $s=0.02$ and $h=0.2$, 65.8% of zygotes have no homozygous loss of function, 27.4% have lost one non-essential gene, 5.7% have lost two and 0.8% have lost three. Figure 1B shows how there would be far fewer individuals free of low- s mutations in an organism with the same life-cycle but a larger number of genes. In our multi-locus simulations of finite populations, which allow for linkage disequilibrium and identity disequilibrium, results are very similar, with confidence intervals shown in Figure 1.

Since loss of function of one or more genes seems, *a priori*, an unlikely path to future adaptation, we consider also a class of mutations of smaller effect. Although each has only a small probability of being adaptive, in a manner dependent on the environment and genetic background, collectively they are the raw material of future adaptation.

Assume that each unique and potentially adaptive mutation appears by mitotic mutation at a low rate, set as 10^{-10} in calculations here. Figure 2 results are proportional to this mutation rate, and Figure 3 results are independent of it, up to values as high as 10^{-6} : calculations on lower alternative values are subject to numerical instability. Assume that each such mutation has historically been deleterious with selection coefficient s , and has $h=0.2$. Assume that one such mutation is adaptive in a new environment, and that this environmental change happens at approximately the same time as sex. This is a reasonable assumption in *Saccharomyces* where, as in many other species, facultative sex is closely associated both with stress and with dispersal.

In Figure 2 we see that sex dramatically increases the expected number of individuals in a population of size 5×10^6 that are both free of gene knockouts and homozygous for a specified mutation of small effect. In finite populations, even large ones, homozygotes are practically absent prior to sex. For subsequent adaptation to occur via clonal expansion, at least one individual in the population needs to be carrying a genotype of interest. If multiple loci contribute but are found in different individuals, clonal expansion of each followed by an eventual round of outcrossing allows their combination. Simulations of (smaller) finite populations yield similar results to the deterministic case, with lower absolute numbers of individuals per population (not shown).

Note that yeast are highly inbred even before sex: for comparison, with the same allele frequency as calculated according to our Model, the expected absolute number of homozygous mutant in a population under Hardy-Weinberg equilibrium would be far lower

than given in the results of Figure 2, e.g. 2×10^{-7} for $s=0.001$ or 3×10^{-9} for $s=0.02$. However, although the degree of inbreeding is always high, it is not constant. Each round of clonal expansion and mutation slightly decreases the inbreeding coefficient F , and each round of meiosis increases it again.

In Figure 3 we explore how this translates into a quantitative change in heritable genetic variation with sex. We allow for dominance, but assume independent and additive effects across loci. Since most replication events are mitotic, dominance effects are easily inherited. Total genetic variance per site is therefore calculated directly from the three genotype frequencies (Wright, 1951 Table 1) and non-epistatic genotype effects, and is equivalent to $V_A + V_D$ (see Figure 3 legend for details). This, rather than V_A alone, is the relevant quantity describing variance that can be inherited and hence selected during asexual reproduction, which forms the main part of the life cycle. We compare this genetic variance before and after a round of sex.

In Figure 3A we see that when sex is rare relative to the magnitude of s , each sexual episode will increase heritable phenotypic variation. When sex is common relative to the magnitude of s , each episode of sex has no effect on heritable phenotypic variation in the deterministic calculations. Since our results are in other ways independent of the precise sexual frequency, they therefore do not require our simplifying assumption of synchronous sex, with meiosis always occurring after exactly 1000 rounds of mitosis. Stochastic simulations show that for rare sex, our deterministic calculations exaggerate the increase in variation, but only slightly (Figure 3A). Stochastic simulations also show that there is usually a small increase in variation even for common sex (Figure 3A).

The extent of the increased variation is primarily a function of the dominance coefficient h . Very small values of h close to zero lead to dramatic increases in variance, while very large values of h close to 1 lead only to a very mild decrease in variance (Figure 3B). Since Figure 3A shows that the mean effect of an allele does not depend on s once it is above a threshold set by the frequency of sex, the expected aggregate effect of inbred sex can be obtained by integrating the composition of the probability distribution of h across relevant alleles with the appropriate curve from Figure 3B. Given the shape of these curves, we therefore expect an increase in variance, even if alleles that are likely to be adaptive tend to have larger h than other mutations. Even for $h=0.5$, we see a small increase in variance. However, the quantitative extent of the overall increase in variance will be dominated by the frequency of the tail of potentially adaptive alleles with the lowest values of h .

In Figure 3B we see that the effect of the mix of sexual strategies found in *Saccharomyces* is dominated by the most frequent strategy (intra-tetrad mating). Similar results are found for autogamous selfing, i.e. the fusion of the products of separate meioses. Variance increases still more with a haplo-selfing strategy. With an outcrossing strategy, there is no non-epistatic increase in variance per sexual episode except with respect to completely recessive alleles. In this paper we have shown the effects of episodes of selfing against a background of clonal reproduction: episodes of outcrossing have quite different effects, and have been treated elsewhere (e.g., Hastings, 1991; Lynch and Gabriel, 1983; Pálsson, 2001).

DISCUSSION

Here we have calculated that the sexual strategy of clonal expansion followed by selfing leads to substantial increases in heritable phenotypic variation following each sexual episode. Since facultative sex is often associated with stress and dispersal, this variation appears at times when it is most likely to be useful. In this sense, heterozygosity in diploid clonal lineages can be thought of as a form of “evolutionary capacitance” (Masel and Siegal,

2009; Masel and Trotter, 2010; Rutherford and Lindquist, 1998), storing up partially cryptic heterozygous alleles to be released later in a non-cryptic homozygous form through selfing. Heterozygosity of recessive alleles is a form of crypticity. Crypticity promotes evolvability by shifting the ratio between unconditionally deleterious mutations of large effect, which are purged by selection even while partially cryptic, and other mutations of small effect that are effectively neutral, and hence accumulate while partially cryptic (Masel, 2006; Rajon and Masel, 2011). The latter, by a logical process of elimination, must be the source of future adaptations.

Episodes of inbreeding have long been known to increase heritable phenotypic variation per locus, by creating otherwise rare homozygous genotypes out of existing allelic diversity (Wright, 1951 p.324). But episodes of inbreeding will also decrease mean fitness when deleterious alleles tend to be recessive, an effect that is particularly pronounced following a period of asexual propagation (Muirhead and Lande, 1997). In the case of population bottlenecks in obligate sexuals, the increase in genetic variance in fitness does not overcome the decrease in mean, yielding no benefit in terms of evolvability (López-Fanjul et al., 2000; van Heerwaarden et al., 2008).

Here we take a novel approach to the partition of these two forces, in a different context of large population size and episodes of selfing against a background of clonal reproduction. We consider the case of a large excess of inbreeding over outcrossing, so that most heterozygosity results from mutation in the asexual phase rather than from outcrossing. Models that assume infinitesimal gene effects and calculate only means and variances implicitly assume that changes affect all members of a population. Here we instead treat loss of function mutation using a multi-locus discrete approach. If the vast majority of individuals carry at least one deleterious recessive loss of function mutation, then it is clear that increased variance will be of little use from this low fitness baseline. Instead we find that for realistic parameter values, the majority of the population is completely free from deleterious recessive loss of function mutations (Fig. 1A). The decreased mean fitness due to inbreeding depression can therefore be thought of as affecting only a minority rather than all members of the population. If zygote production is not limiting and reproductive compensation exists (i.e. if a dying zygote of low fitness rapidly releases available resources in favor of close relatives that lack its genetic load), then the affected minority create even less of a burden (Porcher and Lande, 2005). The knockout-free majority do, however, still benefit from increased variance following selfing. The majority status of the knockout-free population depends on the number of genes in the genome (Fig. 1B), which may create a limit to genome size for this life cycle.

A previous discrete model of deleterious mutations during a similar life cycle assumed truncation selection above a maximum K deleterious mutations (Kondrashov, 1984). Our approach is equivalent to setting $K=1$ for the long-term (but not always short-term) fate of more dramatic loss of function mutations, while assuming that compensatory mutations mean that there is no limit K to mutations that tinker with rather than destroy function. $K=1$ is supported by the rarity of loss-of-function mutations in natural populations (Schacherer et al., 2009). Our approach stems from the observation that the distribution of fitness effects of new mutations is strongly bimodal (Fudala and Korona, 2009; Wylie and Shakhnovich, 2011).

Switches that tap into cryptic genetic stocks to increase heritable phenotypic variation and hence evolvability can be thought of as a form of bet-hedging against unpredictable environments, and may be adaptive (King and Masel, 2007). Selfing can play this role against a background of usually clonal reproduction. It has even been proposed that the appearance of certain recessive loss of function homozygotes in a significant minority of

zygotes may further increase selectable variation, by a process of decanalization leading to the release of cryptic genetic variation (Levy and Siegal, 2008). Decanalization can be captured in the context of our model as an increase in the effective quantity of mutations with the potential to contribute to adaptation and/or an increase in the magnitude of the effects of each of these mutations. This further-increased variance would, however, be set against the likely substantial deleterious effects of gene loss of function. Here we have found a significant increase in selectable variation even without paying this price.

Dramatic decanalization via loss of function is merely an extreme instance of the possible epistatic consequences of an episode of selfing. We have accounted for dominance in our model, but have ignored epistasis, assuming additivity across loci. With outcrossing, this would not be justifiable: outcrossing brings together alleles in combinations that selection may have rarely or never seen before, with unpredictable effects. With selfing, however, all alleles have already been present in combination in the mother cell. Homozygosity at one site may not have been seen by an allele at another; this form of epistasis seems likely to be dominated by the scenario discussed above, namely recessive loss of function leading to decanalization, causing formerly neutral alleles to have an effect.

Our results address the consequences of a life cycle that alternates between clonal reproduction and selfing; it is distinct from the larger body of related work whose primary focus is the evolutionary cause of reproductive strategies, including sex in general and selfing in particular. Sex, including selfing, can have benefits both by eliminating deleterious mutations and by promoting more rapid adaptation (Otto, 2009). The advantages of even a single, rare round of sex in eliminating deleterious mutations under seasonal truncation selection have been discussed elsewhere (Kondrashov, 1984), including in the context of yeast selfing (Mortimer et al., 1994). Here we have illustrated the potential of selfing to promote adaptation.

Note that the recent developmental of single-cell analysis of morphometric traits in *Saccharomyces* (Levy and Siegal, 2008) should make it possible to verify the capacitance effect by measuring viability and phenotypic variance before and after selfing, following different durations of asexual experimental evolution. If the appropriate lab techniques can be extended to non-model fungal species, the theory could also be tested in wild fungi of different ages isolated from fairy rings of different sizes.

While perhaps harder to parameterize than yeast, selfing plants may also make good empirical systems to test our theory. Approximately 70% of all flowering plants, including some that self, are capable of asexual reproduction (Klimeš et al., 1997), which can decrease genetic load (Marriage and Kelly, 2009; Marriage and Orive, manuscript submitted). Asexual evolution can also occur in mitotic cell-lineages (Fagerström et al., 1998), interrupted by selfing events. In plants, heterozygotic mutations accumulate, despite selective purging, during vegetative growth (Klekowski, 2003). Even in the absence of selection, mutational degradation can be slow to reach completion (Masel et al., 2007). In support of the applicability of our calculations to plants, it has been shown for some ferns that the proportion of clones free of heterozygotic mutations of strong recessive effect (Klekowski, 1988; Klekowski, 1984) is comparable to those we predict here for yeast. This proportion can increase with the age of a plant (Smith, 1943 Table 10), which may explain why large statured species tend to have low selfing rates (Schofield and Schultz, 2006). The principal prediction of the theory presented here, which to the best of our knowledge has not yet been tested, is a relationship between the number of mitotic divisions within a normally selfing plant (i.e. plant tissue age) and selectable phenotypic variance among those seedlings free of mutations of strong effect following selfing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Mike Barker, Lilach Hadany, Richard Neher and Mark Siegal for helpful discussions and the anonymous reviewers for their suggestions on the manuscript. Work was supported by National Institutes of Health grant R01 GM076041. J.M. is a Pew Scholar in the Biomedical Sciences.

References

- Agrawal AF, Whitlock MC. Inferences About the Distribution of Dominance Drawn from Yeast Gene Knockout Data. *Genetics*. 2011; 187:553–566. [PubMed: 21098719]
- Barton NH, Otto SP. Evolution of Recombination Due to Random Drift. *Genetics*. 2005; 169:2353–2370. [PubMed: 15687279]
- Briggs WH, Goldman IL. Genetic variation and selection response in model breeding populations of *Brassica rapa* following a diversity bottleneck. *Genetics*. 2006; 172:457–465. [PubMed: 16157675]
- Bryant EH, McCommas SA, Combs LM. The effect of an experimental bottleneck upon quantitative genetic variance in the housefly. *Genetics*. 1986; 114:1191–1211. [PubMed: 17246359]
- Charlesworth D, Charlesworth B. The Effects of Selection in the Gametophyte Stage on Mutational Load. *Evolution*. 1992; 46:703–720.
- Fagerström T, Briscoe DA, Sunnucks P. Evolution of mitotic cell-lineages in multicellular organisms. *Trends Ecol Evol*. 1998; 13:117–120. [PubMed: 21238227]
- Fernández A, Toro MA, López-Fanjul C. The effect of inbreeding on the redistribution of genetic variance of fecundity and viability in *Tribolium castaneum*. *Heredity*. 1995; 75:376–381.
- Fudala A, Korona R. Low frequency of mutations with strongly deleterious but nonlethal fitness effects. *Evolution*. 2009; 63:2164–2171. [PubMed: 19473394]
- Haldane JBS. The association of characters as a result of inbreeding and linkage. *Ann Eugen*. 1949; 15:15–23. [PubMed: 15403124]
- Halkett F, Simon J-C, Balloux F. Tackling the population genetics of clonal and partially clonal organisms. *Trends Ecol Evol*. 2005; 20:194–201. [PubMed: 16701368]
- Hastings IM. Germline Selection: Population Genetic Aspects of the Sexual/Asexual Life Cycle. *Genetics*. 1991; 129:1167–1176. [PubMed: 1783297]
- Keightley PD, Otto SP. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature*. 2006; 443:89–92. [PubMed: 16957730]
- King O, Masel J. The evolution of bet-hedging adaptations to rare scenarios. *Theor Popul Biol*. 2007; 72:560–575. [PubMed: 17915273]
- Klekowski EJ. Progressive cross- and self-sterility associated with aging in fern clones and perhaps other plants. *Heredity*. 1988; 61:247–253.
- Klekowski EJ. Plant clonality, mutation, diplontic selection and mutational meltdown. *Biol J Linnean Soc*. 2003; 79:61–67.
- Klekowski EJ Jr. Mutational Load in Clonal Plants: A Study of Two Fern Species. *Evolution*. 1984; 38:417–426.
- Klimeš, L.; Klimešová, J.; Hendriks, R.; van Groenendael, J. Clonal plant architecture: a comparative analysis of form and function. In: de Kroon, H.; van Groenendael, J., editors. *The Ecology and Evolution of Clonal Plants*. Backhuys; 1997. p. 1-29.
- Kondrashov AS. A possible explanation of cyclical parthenogenesis. *Heredity*. 1984; 52:307–308.
- Kouyos RD, Silander OK, Bonhoeffer S. Epistasis between deleterious mutations and the evolution of recombination. *Trends Ecol Evol*. 2007; 22:308–315. [PubMed: 17337087]
- Kron P, Stewart SC, Back A. Self-compatibility, autonomous self-pollination, and insect-mediated pollination in the clonal species *Iris versicolor*. *Can J Bot*. 1993; 71:1503–1509.
- Levy SF, Siegal ML. Network hubs buffer environmental variation in *Saccharomyces cerevisiae*. *PLoS Biol*. 2008; 6:e264. [PubMed: 18986213]

- López-Fanjul C, Fernández A, Toro MA. Epistasis and the conversion of non-additive to additive genetic variance at population bottlenecks. *Theor Popul Biol.* 2000; 58:49–59. [PubMed: 10944475]
- López-Fanjul C, Villaverde A. Inbreeding increases genetic variance for viability in *Drosophila melanogaster*. *Evolution.* 1989; 43:1800–1804.
- Lynch M, Gabriel W. Phenotypic evolution and parthenogenesis. *Am Nat.* 1983; 122:745–764.
- Lynch M, Sung W, Morris K, Coffey N, Landry CR, Dopman EB, Dickinson WJ, Okamoto K, Kulkarni S, Hartl DL, Thomas WK. A genome-wide view of the spectrum of spontaneous mutations in yeast. *Proc Natl Acad Sci USA.* 2008; 105:9272–9277. [PubMed: 18583475]
- Marriage TN, Kelly JK. Inbreeding depression in an asexual population of *Mimulus guttatus*. *J Evol Biol.* 2009; 22:2320–2331. [PubMed: 19824930]
- Marriage TN, Orive ME. Mutation-selection balance and mixed mating with asexual reproduction. manuscript submitted.
- Masel J. Cryptic genetic variation is enriched for potential adaptations. *Genetics.* 2006; 172:1985–1991. [PubMed: 16387877]
- Masel J, King OD, Maughan H. The loss of adaptive plasticity during long periods of environmental stasis. *Am Nat.* 2007; 169:38–46. [PubMed: 17206583]
- Masel J, Siegal ML. Robustness: mechanisms and consequences. *Trends Genet.* 2009; 25:395–403. [PubMed: 19717203]
- Masel J, Trotter MV. Robustness and evolvability. *Trends Genet.* 2010; 26:406–414. [PubMed: 20598394]
- McCauley DE, Whittier DP, Reilly LM. Inbreeding and the Rate of Self-Fertilization in a Grape Fern, *Botrychium dissectum*. *American Journal of Botany.* 1985; 72:1978–1981.
- Mogie M. Automixis: its distribution and status. *Biol J Linnean Soc.* 1986; 28:321–329.
- Mortimer R, Romano P, Suzzi G, Polsinelli M. Genome renewal: a natural selective mechanism for providing new phenotypes in *Saccharomyces cerevisiae*. *Oenologie 95: 5e symposium international d'oenologie.* 1995; 95:284–286.
- Mortimer RK, Romano P, Suzzi G, Polsinelli M. Genome renewal: A new phenomenon revealed from a genetic study of 43 strains of *Saccharomyces cerevisiae* derived from natural fermentation of grape musts. *Yeast.* 1994; 10:1543–1552. [PubMed: 7725789]
- Muirhead CA, Lande R. Inbreeding depression under joint selfing, outcrossing, and asexuality. *Evolution.* 1997; 51:1409–1415.
- Mulcahy D, Sari-Gorla M, Mulcahy G. Pollen selection — past, present and future. *Sexual Plant Reproduction.* 1996; 9:353–356.
- Otto, Sarah P. The evolutionary enigma of sex. *Am Nat.* 2009; 174:S1–S14. [PubMed: 19441962]
- Pálsson S. The effects of deleterious mutations in cyclically parthenogenetic organisms. *J Theor Biol.* 2001; 208:201–214. [PubMed: 11162064]
- Porcher E, Lande R. Reproductive compensation in the evolution of plant mating systems. *New Phytol.* 2005; 166:673–684. [PubMed: 15819929]
- Rajon E, Masel J. The evolution of molecular error rates and the consequences for evolvability. *Proc Natl Acad Sci USA.* 2011; 108:1082–1087. [PubMed: 21199946]
- Robertson A. The effect of inbreeding on the variation due to recessive genes. *Genetics.* 1952; 37:189–207. [PubMed: 17247385]
- Rutherford SL, Lindquist S. Hsp90 as a capacitor for morphological evolution. *Nature.* 1998; 396:336–342. [PubMed: 9845070]
- Schacherer J, Shapiro JA, Ruderfer DM, Kruglyak L. Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. *Nature.* 2009; 458:342–345. [PubMed: 19212320]
- Scofield DG, Schultz ST. Mitosis, stature and evolution of plant mating systems: low- Φ and high- Φ plants. *Proc R Soc Lond Ser B-Biol Sci.* 2006; 273:275–282.
- Smith EC. A study of cytology and speciation in the genus *Populus* L. *Journal of the Arnold Arboretum.* 1943; 24:275–305.

- Szafranec K, Wloch DM, Sliwa P, Borts RH, Korona R. Small fitness effects and weak genetic interactions between deleterious mutations in heterozygous loci of the yeast *Saccharomyces cerevisiae*. *Genet Res.* 2003; 82:19–31. [PubMed: 14621268]
- Tsai IJ, Bensasson D, Burt A, Koufopanou V. Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. *Proc Natl Acad Sci USA.* 2008; 105:4957–4962. [PubMed: 18344325]
- Van Buskirk J, Willi Y. The change in quantitative genetic variation with inbreeding. *Evolution.* 2006; 60:2428–2434. [PubMed: 17263106]
- van Heerwaarden B, Willi Y, Kristensen TN, Hoffmann AA. Population bottlenecks increase additive genetic variance but do not break a selection limit in rain forest *Drosophila*. *Genetics.* 2008; 179:2135–2146. [PubMed: 18689893]
- Wade MJ, Shuster SM, Stevens L. Inbreeding: Its effect on response to selection for pupal weight and the heritable variance in fitness in the flour beetle, *Tribolium castaneum*. *Evolution.* 1996; 50:723–733.
- Wang JL, Caballero A, Keightley PD, Hill WG. Bottleneck effect on genetic variance: A theoretical investigation of the role of dominance. *Genetics.* 1998; 150:435–447. [PubMed: 9725859]
- Weir BS, Cockerham CC. Mixed selfing and random mating at two loci. *Genet Res.* 1973; 21:247–262. [PubMed: 4731639]
- Willis JH, Orr HA. Increased heritable variation following population bottlenecks: the role of dominance. *Evolution.* 1993; 47:949–957.
- Wright S. The genetical structure of populations. *Ann Eugen.* 1951; 15:323–354.
- Wylie CS, Shakhnovich EI. A biophysical protein folding model accounts for most mutational fitness effects in viruses. *Proc Natl Acad Sci USA.* 2011; 108:9916–9921. [PubMed: 21610162]

Figure 1.

Proportion of zygotes free of homozygous loss of function mutations in both the 1179 essential genes and the 4700 or more non-essential genes. The x-axis represents the strength of selection s against the loss of non-essential genes. Curves are shown for different values of dominance h (A) and for different numbers of non-essential genes (B). The same dominance coefficient h is assumed for both essential and non-essential genes: using realistically lower values of h for strongly deleterious mutations (Agrawal and Whitlock, 2011) has a negligible effect, since these mutations remain effectively purged regardless. There are 4700 non-essential genes in part A, and $h=0.2$ in part B. Meiosis occurs every 1000 generations, and results were found not to be sensitive to the frequency of sex (not shown). 60% confidence intervals are shown for simulations with finite population size set by default to 100,000. In part A, 3 simulations were done for $s=0.02$, and staggered for clarity of presentation: these correspond to the lower values of $N=5000$, $N=10,000$, and $N=50,000$. For still smaller values of N such as 1000, results have very high variance with neither mean nor median consistent with the deterministic solution.

Figure 2.

Number of individuals homozygous for a mutation of interest, while lacking a gene knockout. Results are shown as the absolute number of individuals expected in a population of size $N_e=5 \times 10^6$. This absolute scale illustrates that any one homozygote is likely absent when it appears at the low mutation rate of 10^{-10} . However, many different mutations are possible. In the environment encountered so far, the focal mutation had $h=0.2$ and s as shown on the x-axis. Simulation results are not shown in this Figure because results depend on the population size, and the computations necessary for realistically large yeast effective population sizes are prohibitive. For smaller population sizes, simulations agree with deterministic calculations (not shown).

Figure 3.

Fold-change in heritable phenotypic variance during each episode of sex. Part A illustrates the quantitative effects of the dominance coefficient and of the frequency of sex, while part B illustrates the effect of the precise kind of sex. Variance is at one locus with a dominance coefficient h , subject to selection s in the past. Variance was calculated by assuming without loss of generality a phenotypic effect size (this parameter cancels out when the ratio of variances is taken), and using the dominance coefficient h to calculate the product of the three genotype frequencies with their phenotypic value's squared deviation from the mean. Where not specified, meiosis occurs every 1000 generations, consisting of 94% intra-tetrad mating, 5% haploselfing and 1% outcrossing. Results are superimposable for different mutation rates for potentially adaptive alleles, from 10^{-10} to 10^{-6} , with lower values likely also superimposable but numerically unstable. In B, results are essentially superimposable for autogamy compared to a realistic mix of *Saccharomyces* sexual frequencies. Median and 60% confidence intervals of simulations with $N=100,000$ are shown in both Figure parts. When selection is strong relative to the frequency of sex, deterministic calculations slightly exaggerate the increase in variance shown in simulations. For weak selection, simulations provide support for a small increase in variance not seen in deterministic calculations.