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Mate choice among yeast gametes can purge deleterious mutations

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Abstract

Meiosis in *Saccharomyces* yeast produces four haploid gametes that usually fuse with each other, an extreme form of self-fertilization among the products of a single meiosis known as automixis. The gametes signal to each other with sex pheromone. Better-quality gametes produce stronger signals and are preferred as mates. We suggest that the function of this signalling system is to enable mate choice among the four gametes from a single meiosis and so to promote the clearance of deleterious mutations. To support this claim, we construct a mathematical model that shows that signalling during automixis (i) improves the long-term fitness of a yeast colony and (ii) lowers its mutational load. We also show that the benefit to signalling is greater with larger numbers of segregating mutations.

Introduction

Saccharomyces haploid cells signal to one another before mating. Both mating types (MAT**a** and MAT**α**) court each other, produce attractive pheromones and respond to the pheromone of the other mating type. Stronger signallers are more attractive (Jackson & Hartwell, 1990), and all cells produce more pheromones than is required for mating (Jackson & Hartwell, 1990; Pagel, 1993; Rogers & Greig, 2009), suggesting that the signals have been exaggerated by sexual selection. It has been proposed that the signals evolved under the handicap principle (Zahavi, 1975; Pomiankowski, 1988; Grafen, 1990) and act as honest indicators of genetic quality (Pagel, 1993; Nahon *et al.*, 1995). This hypothesis now has strong experimental support, with the pheromone having been shown to be costly to produce and this cost being relatively smaller for higher-quality individuals than it is for lower-quality individuals (Smith & Greig, 2010).

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However, the application of the handicap principle to yeast is not straightforward because of its life cycle and mating system. Although little is known about the ecology of wild *Saccharomyces*, it is believed that individuals usually reproduce asexually as diploids. However, under starvation conditions, diploid cells undergo meiosis, each forming a tetrad of haploid spores within a sac called the ascus (Greig & Leu, 2009). When starvation conditions cease, the spores germinate and usually mate with others from the same tetrad, forming two diploid offspring cells (we refer to this process as automixis; it is sometimes called 'intratetrad mating', Knop, 2006). Due to segregation and recombination between loci during meiosis, the resulting offspring cells are genetically distinct from the mother cell (and each other). Although outcrossing between haploid cells from different asci is also possible, it occurs at very low rates in natural populations (Ruderfer *et al.*, 2006; Zeyl & Otto, 2007; Tsai *et al.*, 2008; though see also Murphy & Zeyl, 2010), and

automixis is considered to be the normal mode of sexual reproduction in *Saccharomyces*. This requires two modifications to the application of the handicap principle as it is usually considered.

Firstly, in many sexual signalling systems, the costly handicap mating signal evolves in one sex (usually the males), and the preference trait evolves in the other sex (females). Here, however, both mating types signal and show preference for stronger signallers of the other type. Secondly, handicap mating signals between individuals (or gametes from different individuals) are believed to be beneficial to the signaller as they increase the chance of obtaining a mate. But in *Saccharomyces*, the signallers are gametes from the same meiosis, which are bound together by the ascus and thus only signal to one another. So, it seems unlikely that the purpose of the costly signal is to increase the probability of achieving a mating. Thus, we are forced to ask what is the benefit of costly signalling during automixis.

In this study, we develop a theoretical model to investigate the reason for mate choice in *Saccharomyces*. We assume that deleterious mutations accumulate during the asexual diploid growth phase and segregate randomly during meiosis into gametes. Under these conditions, signalling and mate choice, acting within the meiotic tetrad, could have important effects on the fitness of the offspring produced by automixis. The model shows how automixis alters the mutational load of offspring relative to their parents and how this process is enhanced by mate choice based on the pheromone signal.

The model

Our model is a game-theoretical treatment in that the effects of the differing strategies are considered, although it includes population genetic elements in considering the segregation and effects of deleterious mutations. The model is based upon a simplified version of the *Saccharomyces cerevisiae* life cycle (Fig. 1). We assume the diploid parent cell is experiencing starvation conditions and undergoes meiosis to form a tetrad of four haploid spores, two of which are **α** type and two of which are **a** type (Spencer & Spencer, 1997). When favourable growth conditions return, the **α** and **a** haploid spores will germinate and mate, forming two nonidentical diploids. Mating can only occur between **α** and **a** haploids. We assume that haploid spores indicate their genetic quality, as the strength of pheromone production correlates with the number of deleterious alleles carried in the haploid genome (Smith & Greig, 2010). The haploids can either fuse randomly or fuse selectively, according to the strength of the pheromone signal they produce. Our focus is therefore the degree to which the haploid cells select their mating partner, which we refer to as the mating strategy. Whatever the mating strategy, two diploid offspring are produced. These then reproduce by asexual budding, producing a colony of cells whose rate of increase depends on the distribution of mutations between the two offspring.

As we are interested in the effect of deleterious mutations, we consider cases where the parent yeast cell is heterozygous for deleterious alleles at a number of loci in its genome and assume that these loci are not linked to the mating-type locus. We start with the one-locus case and then proceed to consider higher numbers of loci.

One locus

We analyse the effect of the reproductive strategy followed by the parent yeast cell in the simplest case where it carries a single deleterious mutation as a heterozygote. We denote the wild-type allele as + and the deleterious mutant as *m*. Then, the genotype of the parent yeast cell is denoted by (+*m*). After automixis, the possible offspring genotypes are (++), (+*m*) and (*mm*). When the parent cell undergoes meiosis, the deleterious mutations will segregate so as to produce one of the three different types of ascus (after accounting for symmetry) with probabilities as seen in Table 1.

Only those asci in which the + and *m* alleles segregate into both **α** and **a** mating types give opportunity for selection, so we concentrate on them (ascus type 2 in Table 1). We assume that each haploid signals the number of deleterious mutations it has in its genome through the amount of **α** and **a** pheromone it releases. We refer to the haploid of each mating type with the least number of deleterious alleles as the strongest and call the other haploid of that type the weakest. For example, in the one-locus model, the **a** haploid with genotype (+) is the strongest, whereas the **a** haploid with genotype (*m*) is the weakest.

We denote the probability that the strongest **a** haploid fuses with the strongest **α** haploid (and thus also the weakest **a** haploid fuses with the weakest **α** haploid) by p. This is our measure of mating strategy. We suppose that p is genetically determined, and we investigate the effect on the future colony that differing values of p would have. The model can account for any value of p. Three p-values are of particular interest: $p = 1$ represents fusion of the strongest **a** haploid with the strongest **α** haploid (and thus also the weakest with the weakest), hereafter called 'strongest-to-strongest' mating, $p = 1/2$ represents random mating (i.e. no haploid selection) and $p = 0$ represents fusion of the strongest **a** haploid with the weakest **α** haploid (and thus also weakest **a** with strongest **α**), hereafter called 'weakest-to-strongest' mating. Although there is no experimental evidence that we know of that suggests haploid cells exhibit strategies with $p < 1/2$, and the mechanisms by which such processes could occur are difficult to imagine, we include such strategies out of completeness. For a parent yeast cell with strategy p, the expected proportion of offspring cells of each of the possible genotypes are shown in Table 2.

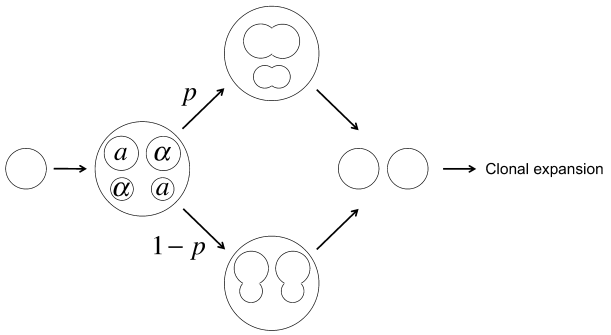

Fig. 1 A simplified yeast life cycle: The diploid parent cell, under starvation conditions, undergoes automixis and forms four spores. These then germinate to result in two **a** and two **α** haploid cells. The haploids are not genetically identical, and here, we have depicted the genetically stronger of each mating type as larger. Mating then occurs. With probability p, the strongest **a** haploid and the strongest **α** haploid mate to form one offspring, and the two weaker haploids mate to form the other offspring (top path). By contrast, with probability $(1-p)$, the strongest **a** haploid and the weakest **α** haploid mate to form one offspring, with the weakest **a** and strongest **α** forming the other (bottom path). Either way, two diploid offspring are formed, and the colony then grows via clonal expansion.

Note that the value p is a parental diploid trait, determining the probability that a strongest-to-strongest mating occurs among the gametes in a single ascus.

Regardless of the strategy p, two diploid offspring are produced, and these then reproduce by asexual budding, forming a colony. We assume that the (++) genotype is the fittest, and therefore, one discrete timestep is defined as the expected time it takes for a cell with genotype (++) to complete an asexual budding event and become two (++) cells. This amount of time is insufficient for the other genotypes to completely divide. We parameterize this by defining values $0 < h < 1$, $0 < s < 1$, such that in one timestep, each $(+m)$ cell will partially split to become $(1 + (1-hs))$ $(+m)$ cells and each (mm) cell will partially split to become $(1 + (1-s))$ (mm) cells. Thus, s is the measure of homozygous fitness loss and h the dominance coefficient.

Starting at timestep 0 with a single diploid offspring yeast cell of known genotype, after k timesteps of budding, the expected number $W[p\,|\,k,h,s]$ of cells in a colony descended from a parent cell with strategy p is

$$W[p\,|\,k,h,s] = \frac{p}{3}2^k + \left(1-\frac{2p}{3}\right)(1+(1-hs))^k + \frac{p}{3}(1+(1-s))^k, \quad (1)$$

which is the probability of occurrence of each genotype after mating multiplied by the number of that type expected after k timesteps. We can also calculate a measure Λ of the mutational load of the colony after a given number of timesteps: the proportion of deleterious alleles at the locus of interest across the colony of descendant cells, depending upon the strategy p,

$$\Lambda[p\,|\,k,h,s] = \frac{(1/2-p/3)(1+(1-hs))^k + p/3(1+(1-s))^k}{W[p\,|\,k,h,s]}.$$

This is the number of each genotype multiplied by the proportion of that genotype that is deleterious, divided by the total number of offspring.

Multiple loci

Having investigated one locus, we now consider n loci. For simplicity, we assume that each locus is unlinked to the *MAT* locus or to other deleterious heterozygote loci. This assumption becomes problematic as n becomes large, because there are only 16 chromosomes (Spencer & Spencer, 1997), but as mentioned in the Discussion, the qualitative findings still hold. With n loci, the number of possible results of meiosis is 3^n, as is the number of possible genotypes that the offspring can take.

We assume that fitness is multiplicative and that all deleterious mutations have the same effect upon fitness, so that an offspring cell with a genotype that is heterozygous $(+m)$ at x loci, homozygous (mm) at y loci and homozygous (++) at $n-x-y$ loci will produce $(1 + (1-hs)^x (1-s)^y)$ offspring per timestep. The results are calculated numerically (see Appendix 3).

Variation in the duration of clonal expansion

Our model features a period of clonal expansion (Fig. 1). This can be thought of as the number of timesteps before the food source runs out and the yeast cells again enter meiosis. The duration of the clonal expansion period, in terms of number of timesteps, is an important parameter. Using the one-locus model, we now consider what happens if the duration of the clonal expansion period is a random variable. We suppose that in every timestep, there is a small probability that the food runs out and the clonal expansion period ends. The length of the clonal expansion period is therefore a geometrically distributed random variable. If we denote the expected length by κ, we can express the probability that the clonal expansion period ends after exactly k timesteps as

$$f(k) = \frac{\kappa^k}{(\kappa+1)^{k+1}}.$$

Given strategy p, the absolute population size of a colony after k generations is eqn (1) above. As this grows exponentially with k, it has no well-defined expectation with respect to the distribution given by $f(k)$. Therefore, we instead consider the population size relative to the maximum possible population size, which is 2^k,

Table 1 The three possibilities for the segregation of genes at the locus of interest when forming a tetrad of spores, after accounting for symmetry.

Ascus type	α		a		Probability
1	+	+	*m*	*m*	1/6
2	+	*m*	+	*m*	2/3
3	*m*	*m*	+	+	1/6

Table 2 Expected proportions of offspring of each genotype as a function of reproductive strategy p of the parent cell. The values are obtained considering the possible outcomes of each of the ascus types given in Table 1, given the probability p of strongest-to-strongest matings.

	Probability of genotype		
Strategy	(++)	(+*m*)	(*mm*)
Strategy p	$p/3$	$1\text{-}2p/3$	$p/3$
Weakest-to-strongest ($p = 0$)	0	1	0
Random ($p = 1/2$)	1/6	2/3	1/6
Strongest-to-strongest ($p = 1$)	1/3	1/3	1/3

$$W_{\mathrm{rel}}[p \mid k,h,s] = \frac{1}{2^k} W[p \mid k,h,s] = \frac{p}{3} + \left(1 - \frac{2p}{3}\right)\left(1 - \frac{hs}{2}\right)^k + \frac{p}{3}\left(1 - \frac{s}{2}\right)^k.$$

This expression has a well-defined expected value with respect to the distribution $f(k)$,

$$\bar{W}_{\mathrm{rel}}[p \mid h,s] = \sum_{k=0}^{\infty} f(k) W_{\mathrm{rel}}[p \mid k,h,s] = \frac{p}{3} + \left(1 - \frac{2p}{3}\right)\sum_{k=0}^{\infty} f(k)\left(1 - \tfrac{1}{2}hs\right)^k + \frac{p}{3}\sum_{k=0}^{\infty} f(k)\left(1 - \tfrac{1}{2}s\right)^k \quad (2)$$

We can then calculate the expected size of the colony at the end of the clonal expansion period given strategy p and the distribution $f(k)$ of clonal expansion period length.

Results

One locus

To find out which strategic choice p gives the biggest colony size after k timesteps, we differentiate the fitness function (1) with respect to p, to get

$$\frac{\partial W}{\partial p}[k,h,s] = \frac{1}{3}\left(2^k - 2(1 + (1 - hs))^k + (1 + (1 - s))^k\right). \quad (3)$$

As the right-hand side of (3) does not contain any terms in p, we know that at any timestep k, colony size will be either an increasing or decreasing linear function of p (or constant). Using (3), we can write the following function:

$$V[k,h,s] = 1 - 2\left(1 - \frac{hs}{2}\right)^k + \left(1 - \frac{s}{2}\right)^k. \quad (4)$$

Then, given parameter values k, h, and s, if $V[k, h, s] < 0$, the strategy $p = 0$ will have colonies with the most cells. If $V[k, h, s] = 0$, all strategy choices produce colonies of equal size. Finally, if $V[k, h, s] > 0$, the strategy $p = 1$ will produce the largest colonies.

The results are dependent on the values of s, the selective disadvantage of being homozygous for the mutant deleterious allele, and h, the dominance coefficient governing how much this disadvantage also applies to heterozygotes. Assuming both h and s are greater than zero (i.e. the deleterious mutation has a negative effect on fitness in both heterozygotes and homozygotes), colonies descended from parent cells following the $p = 1$ strongest-to-strongest strategy will eventually contain more cells than those descending from parent cells following any other strategy (i.e. we can find a value z such that for all timesteps, $k > z$, $V[k, h, s] > 0$). Higher values of h and s give lower values of z. In particular, for deleterious mutants that are to some degree dominant with $h \geq 1/2$, $V[k, h, s] > 0$ for all $k \geq 1$, and so the $p = 1$ strategy wins immediately (Appendix 1). However, this is not the case for deleterious recessive mutants with $h < 1/2$, and we can plot z as a function of h for a given s (Fig. 2). This shows that the number of timesteps before the $p = 1$ strategy does better increases exponentially as recessivity increases ($h \to 0$).

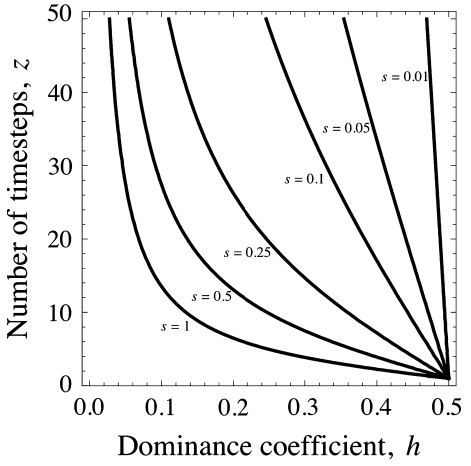

Fig. 2 Number of timesteps z after which the strongest-to-strongest sexual selection strategy outcompetes all others, plotted against h. The six curves are for $s = 1$, 0.5, 0.25, 0.1, 0.05 and 0.01 all labelled appropriately. As s and h increase, the strongest-to-strongest strategy requires fewer timesteps before becoming victorious.

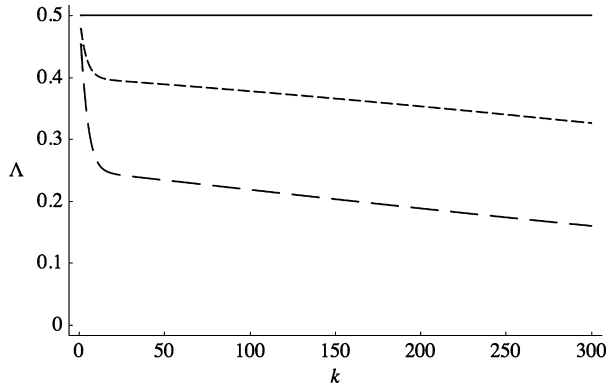

Fig. 3 Proportion Λ of the loci in the population that have the deleterious mutant allele m at the locus of interest after k timesteps for three different strategies: weakest-to-strongest selection (solid line, $p = 0$, for which Λ is fixed at 0.5), random mating (short-dashed line, $p = 0.5$) and strongest-to-strongest selection (long-dashed line, $p = 1$), with parameter values $h = 0.01$ and $s = 0.5$. The functions have been plotted to $k = 300$ as the strongest-to-strongest strategy ($p = 1$) outperforms the others by this stage.

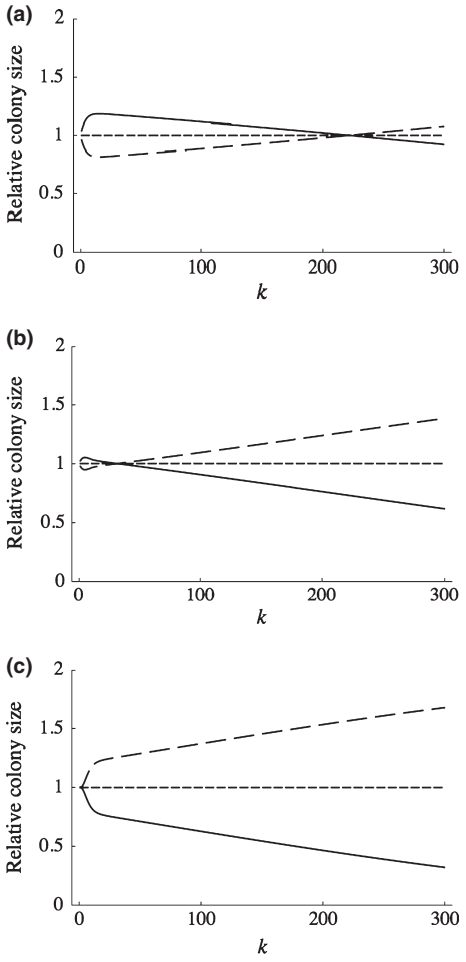

Fig. 4 Colony sizes following a strategy of weakest-to-strongest selection (solid line), random mating (short-dashed line, $p = 0.5$) and strongest-to-strongest selection (long-dashed line, $p = 1$) relative to the size of a colony that underwent random mating, after k timesteps, with selection parameters $h = 0.01$, $s = 0.5$. The number of loci n for which the parent cell was heterozygous for a deleterious mutation is 2 (a), 5 (b) and 10 (c). For $n = 2$ and $n = 5$, the weakest-to-strongest mating strategy starts out superior but is eventually surpassed by strongest-to-strongest. For $n = 10$, the strongest-to-strongest strategy is better immediately. The random mating strategy is only ever equally as good as the other two. Note that we have plotted up to $k = 300$ in each figure because by then the strongest-to-strongest strategy can be clearly seen to have won in (a), and we wanted the same axes for each graph for ease of comparison. We do not expect that in reality, there will be this many generations between starvation events.

In addition, we can compare the three mating strategies to see how they perform in suppressing the proportion Λ of deleterious mutations at the locus of interest (Fig. 3). Reductions in Λ occur with the strongest-to-strongest strategy ($p = 1$) and with random mating ($p = 1/2$), with strongest-to-strongest mating performing considerably better. As the weakest-to-strongest strategy ($p = 0$) results in the production of two heterozygote offspring, it does not suppress Λ. It can be shown analytically that $\Lambda[p \mid k, h, s]$ is a decreasing function of p for all appropriate k, h and s (Appendix 2).

Thus, in the one-locus case, sexual selection leads to a higher long-term fitness by creating more offspring that lack deleterious mutations, which eventually more than makes up for the fact that it also creates more offspring homozygous for deleterious mutations. Sexual selection also results in a colony with a lower proportion of deleterious mutations at the locus of interest. These effects are stronger if selection against the mutant (s) is higher, and the deleterious mutant causes larger selective disadvantage in heterozygotes (higher h).

Multiple loci

We numerically calculated expected colony population size over time for each of our three strategies for different numbers of segregating mutants from $n = 2$ to 10 (Appendix 3). As colony size increases in all cases, we compared the population size of the strongest-to-strongest strategy ($p = 1$) and weakest-to-strongest strategy ($p = 0$) with the population size given the random mating strategy ($p = 1/2$). We have only displayed the results from $n = 2$, 5 and 10 (Fig. 4), with $h = 0.01$ and $s = 0.5$. In all three cases, the $p = 1$ strategy outperforms the other two strategies over the long term. As the number of loci increases, the $p = 1$ strategy outperforms the others more and more rapidly, until by $n = 10$, it results in a larger colony immediately.

We also modelled the proportion Λ of deleterious alleles at n loci for each strategy, investigating the same

range of n-values, and found, as expected, automixis suppressed mutational load by exposing the deleterious loci to selection, and we found that this process was enhanced by mate choice. The findings were qualitatively similar to those found with a single locus (Fig. 3). For $n > 1$, the $p = 0$ strategy does suppress Λ slightly because for multiple loci, it is no longer true that weakest-to-strongest mating always leads to the production of heterozygote offspring genetically identical to the parent (Appendix 3). However, suppression of Λ is still an increasing function of p.

Variation in the duration of clonal expansion

Differentiating eqn (2) with respect to p gives

$$\frac{\partial}{\partial p}\bar{W}_{\mathrm{rel}}[p\,|\,h,s] = \frac{1}{3} - \frac{2}{3}\sum_{k=0}^{\infty} f(k)\left(1-\tfrac{1}{2}hs\right)^k + \frac{1}{3}\sum_{k=0}^{\infty} f(k)\left(1-\tfrac{1}{2}s\right)^k.$$

As the right-hand side of this expression is independent of p, it must be either positive or negative. If positive, then eqn (2) is maximized at $p = 1$ and the strongest-to-strongest mating strategy has the highest expected colony size. If negative, then eqn (2) is maximized at $p = 0$ and the strongest-to-weakest mating strategy has the highest expected colony size. If $h \geq 1/2$, then $p = 1$ is the evolutionary stable strategy (ESS; Smith & Price, 1973). Otherwise, we can show that the outcome depends on the expected length of the clonal expansion period, κ (Appendix 4). With $h < 1/2$, $p = 1$ is the ESS if and only if

$$\kappa > \frac{\frac{1}{2} - h}{\frac{1}{4}hs} \tag{5}$$

Therefore, strongest-to-strongest signalling will evolve, with the ESS at $p = 1$, if $h \geq 1/2$ or $h < 1/2$ and the expected length of the clonal expansion period κ is sufficiently large. If this interval is not large enough at $p = 0$, strongest-to-weakest signalling is the ESS.

Discussion

In this work, we have modelled a simplified version of the yeast reproductive cycle, to investigate the benefits *S. cerevisiae* derives from sexual selection during automixis. In our model, we consider a single meiosis followed by automixis and a period of clonal expansion by asexual budding. Under the simplest form of automixis, there is a random fusion of gametes. Alternatively, nonrandom assortment can result from mate choice based on pheromone signals that are assumed to accurately reflect genetic quality.

The immediate impact of preference for stronger signallers is that there is a higher frequency of homozygous (++) and (*mm*) loci in offspring. Thus, strongest-to-strongest mating produces the largest number of homozygotes. Alternatively, mate preference that favours strongest-to-weakest couplings produces the largest number of heterozygotes. This is seen most clearly in the one-locus model (Table 2), in which all of the offspring of a heterozygous parent will have genotype (+*m*) through weakest-to-strongest mating, compared to two-thirds through random mating and only one-third of the offspring through strongest-to-strongest mating. Given that deleterious alleles are recessive, the surfeit of homozygotes reduces fitness in the short term compared to colonies with more (+*m*) offspring, as the fitness loss due to homozygous mutant genotypes (*mm*) outweighs the fitness benefit due to homozygous wild-type genotypes (++). However, in the mid-to-long term, because the growth of the colony is exponential, the offspring stemming from (++) zygotes have a reproductive advantage and will eventually outnumber those from (+*m*) zygotes.

So, the strongest-to-strongest strategy can outcompete all others if there is sufficient time for the advantage to be felt. How long this takes depends on heterozygote fitness (Fig. 2). If selection is weak ($s \sim 0$) and/or deleterious mutants are nearly completely recessive ($h \sim 0$), heterozygotes have fitness approximately equal to that of the wild-type homozygote (i.e. $hs \sim 0$), and the expected number of timesteps of asexual budding needed for the strongest-to-strongest strategy to outcompete the others becomes very large. However, even with quite small loss of heterozygote fitness (i.e. $hs \sim 0.01$–0.03), the number of timesteps falls significantly and strongest-to-strongest mate choice becomes the favoured strategy in 30–100 timesteps (Fig. 2, Appendix 1). The length of the period of clonal expansion is likely to vary, but we have shown that its expected value is the key parameter (eqn 5). If this is long enough, strongest-to-strongest mating will be favoured, with the optimal strategy being $p = 1$. In addition, the time it takes before the strongest-to-strongest strategy outcompetes all others falls rapidly as the number of deleterious loci present on the genome rises (Fig. 4), and quickly reaches a point where the strongest-to-strongest strategy outcompetes all others immediately.

Sexual selection can also cause a lowering of the proportion of deleterious alleles in a colony of descendent yeast cells (Fig. 3), if it results in more frequent matings between stronger haploids. The more likely the strongest **a** and the strongest $\boldsymbol{\alpha}$ are to mate, the more the proportion of deleterious alleles will be lowered (Fig. 3, Appendix 2).

These results support a novel explanation for the evolution and function of the yeast pheromone signal. Strongest-to-strongest mate choice during automixis provides a clear fitness advantage by creating offspring with fewer deleterious mutations. This is particularly the case when the parental cell carries higher numbers of deleterious loci, as this increases the fitness advantage of strongest-to-strongest mating (Fig. 4). Direct estimates of the genome-wide deleterious mutation rate for haploid yeast cells show that it could be as high as approximately 0.32 per cell division (Wloch *et al.*, 2001; Zeyl & DeVisser,

2001; Lynch *et al.*, 2008; although other estimates have suggested lower values), with average dominance $h \sim 0.2$ and smaller values associated with larger homozygous effects (though see also Bell, 2010; Agrawal & Whitlock, 2011). Given that meiosis and automixis only occur at the end of periods of clonal expansion (Greig & Leu, 2009), there is the potential for significant numbers of deleterious mutations to build up between recombination events, meaning that the enhanced purging of deleterious alleles provided by mate choice is likely to be important.

Our model takes the number of mutations segregating within the parent cell to be an exogenous parameter n. In reality, of course, this number will arise from the mutation–selection balance in *Saccharomyces*. In future, we intend to produce a fuller model incorporating multiple rounds of meiosis and clonal expansion, which will feature mutation explicitly. A key question will be whether selection during clonal expansion will act to keep the accumulation of new deleterious mutations down to a low frequency. Our intention here, however, was only to show the benefits of mate choice within *Saccharomyces*, for which this simpler model is sufficient.

Our model greatly simplifies the *Saccharomyces* mating system. We ignore the possibilities of haploid clonal expansion after germination by mitosis, before fusion to form diploid cells. Should this occur, it is reasonable to suppose that the haploid cells with the least deleterious mutations (the strongest cells according to our terminology) would be able to reproduce faster. The net effect (ignoring spatial structure) would be that the probability of fusing strongest-to-strongest would be higher. If this phenomenon is confirmed, it would be interesting to incorporate it into the model. Related to this is mating-type switching that can occur when haploid cells divide by mitosis and then switch from **a** to **α** (or vice versa) (Haber, 1998). We expect the selective value of mating-type switching and intraclone mating to be heavily dependent on the number and severity of deleterious mutations, as the resultant offspring would be a totally homozygous diploid cell (apart from at the *MAT* locus). It also may be that this only occurs when a haploid cell has no mating partner and so serves the more traditional role ascribed to inbreeding of assuring reproduction (Jarne & Charlesworth, 1993).

Our model also ignores outcrossing, the fusion of haploids from different asci, derived from two different diploid parent cells. This can occur if haploids from different asci are mixed, for example in the guts of insect dispersal vectors (Reuter *et al.*, 2007), and could be important in the purging of deleterious alleles or the generation of novel combinations with advantageous epistasis. In general, these processes are believed to be rare in *S. cerevisiae* (Zeyl & Otto, 2007; Tsai *et al.*, 2008; though see Murphy & Zeyl, 2010), but as little is known about the reproductive biology of *Saccharomyces* yeast under natural conditions (Greig & Leu, 2009), it is difficult to gauge their evolutionary importance. It may be that outcrossing events provide additional benefits for gametic mate choice, but we have shown that even in the absence of outcrossing, such choice is adaptive.

We also neglect the possibility of deleterious loci being linked with one another. This must increasingly be taken into account as the number of loci at which such alleles appear goes up. If there is linkage between deleterious alleles, the effective number of segregating deleterious mutations is reduced (although the mean severity of the mutations is effectively increased because we assume fitness is multiplicative). This means that n is effectively smaller than in reality. This is likely to result in the threshold number of timesteps after which the $p = 1$ strategy gives the largest colony being greater than that predicted in Fig. 4, but we expect that the qualitative finding that increasing the number of mutations decreases the value of this threshold to still hold.

We have also not considered any costs of signalling. Signalling is performed by the mating of haploid cells (equivalent to gametes in higher organisms) during automixis, prior to the clonal expansion of the resulting diploids. During clonal expansion, the diploid offspring cells making up the colony do not perform any signalling, and therefore, no costs will affect them. The only place the cost could affect the outcome is in the initial mating. This can be incorporated into the model by suggesting that, as a result of these costs, strategies other than random mating ($p = 1/2$) take longer to complete automixis, so that a $p = 1/2$ strategy gets a headstart of a few timesteps worth of clonal expansion. The delay in completion of automixis can be modelled as an increasing function of the deviation of p from $p = ½$. This will quantitatively affect the results, but due to the exponential nature of colony growth, the $p = 1$ strategy will still result in larger colonies in the long term, and so our basic findings remain the same. Costs of this nature do not lead to intermediate ESS values of p.

We have demonstrated a genetic benefit of mate preference for stronger signallers during yeast automixis. Pheromone signalling and mate choice in yeast probably originated simply as a system to identify and locate mates of the opposite mating type. This remains a basic function of the system: haploids that produce no pheromone cannot mate at all (Kurjan, 1985; Michaelis & Herskowitz, 1988). If this was its only function, pheromone production should have the minimum possible cost. Instead, we suggest that the signal has evolved under sexual selection to become stronger and more costly than that required for finding mates, because such an exaggerated sexual signal advertises the genetic quality of the signaller (Smith & Greig, 2010), allowing those with the best genotypes to choose each other as mates. Intratetrad sex in yeast could function to eliminate the recessive deleterious alleles that accumulate by mutation within a diploid clone, and signalling and mate choice enhance this process. Given the genetic knowledge of yeast and its

tractability for experiments, this unique and unusual case has the potential to become one of the best examples of sexual selection in action.

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References

Agrawal, A.F. & Whitlock, M.C. 2011. Inferences about the distribution of dominance drawn from yeast gene knockout data. *Genetics* **187**: 553–566.

Bell, G. 2010. Experimental genomics of fitness in yeast. *Proc. Biol. Sci.* **277**: 1459–1467.

Grafen, A. 1990. Biological signals as handicaps. *J. Theor. Biol.* **144**: 517–546.

Greig, D. & Leu, J.Y. 2009. Natural history of budding yeast. *Curr. Biol.* **19**: R886–R890.

Haber, J.E. 1998. Mating-type gene switching in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* **32**: 561–599.

Jackson, C.L. & Hartwell, L.H. 1990. Courtship in *Saccharomyces cerevisiae* – both cell-types choose mating partners by responding to the strongest pheromone signal. *Cell* **63**: 1039–1051.

Jarne, P. & Charlesworth, D. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu. Rev. Ecol. Syst.* **24**: 441–466.

Knop, M. 2006. Evolution of the hemiascomyclete yeasts: on life styles and the importance of inbreeding. *BioEssays* **28**: 696–708.

Kurjan, J. 1985. Alpha-factor structural gene-mutations in *Saccharomyces cerevisiae* – effects on alpha-factor production and mating. *Mol. Cell. Biol.* **5**: 787–796.

Lynch, M., Sung, W., Morris, K., Coffey, N., Landry, C.R., Dopman, E.B. *et al.* 2008. A genome-wide view of the spectrum of spontaneous mutations in yeast. *Proc. Nat. Acad. Sci. USA.* **105**: 9272–9277.

Maynard Smith, J. & Price, G.R. 1973. The logic of animal conflict. *Nature* **246**(5427): 15–18.

Michaelis, S. & Herskowitz, I. 1988. The a-factor pheromone of *Saccharomyces cerevisiae* is essential for mating. *Mol. Cell. Biol.* **8**: 1309–1318.

Murphy, H.A. & Zeyl, C.W. 2010. Yeast sex: surprisingly high rates of outcrossing between asci. *PLoS ONE* **5**: e10461.

Nahon, E., Atzmony, D., Zahavi, A. & Granot, D. 1995. Mate selection in yeast – a reconsideration of the signals and the message encoded by them. *J. Theor. Biol.* **172**: 315–322.

Pagel, M. 1993. Honest signalling among gametes. *Nature* **363**: 539–541.

Pomiankowski, A. 1988. The evolution of female mate preferences for male genetic quality. In: *Oxford Surveys in Evolutionary Biology, Vol. 5* (P.H. Harvey & L. Partridge, eds), pp. 136–184. Oxford University Press, New York, NY, USA.

Reuter, M., Bell, G. & Greig, D. 2007. Increased outbreeding in yeast in response to dispersal by an insect vector. *Curr. Biol.* **17**: R81–R83.

Rogers, D.W. & Greig, D. 2009. Experimental evolution of a sexually selected display in yeast. *Proc. Biol. Sci.* **276**: 543–549.

Ruderfer, D.M., Pratt, S.C., Seidel, H.S. & Kruglyak, L. 2006. Population genomic analysis of outcrossing and recombination in yeast. *Nat. Genet.* **38**: 1077–1081.

Smith, C. & Greig, D. 2010. The cost of sexual signaling in yeast. *Evolution* **64**: 3114–3122.

Spencer, J.F.T. & Spencer, D.M. 1997. The yeasts: sex and nonsex. life cycles, sporulation and genetics. In: *Yeasts in Natural and Artificial Habitats* (D.M. Spencer & J.F.T. Spencer, eds), pp. 133–152. Springer-Verlag, Berlin.

Tsai, I.J., Bensasson, D., Burt, A. & Koufopanou, V. 2008. Population genomics of the wild yeast *Saccharomyces paradoxus*: quantifying the life cycle. *Proc. Nat. Acad. Sci. U.S.A.* **105**: 4957–4962.

Wloch, D.M., Szafraniec, K., Borts, R.H. & Korona, R. 2001. Direct estimate of the mutation rate and the distribution of fitness effects in the yeast *Saccharomyces cerevisiae*. *Genetics* **159**: 441–452.

Wolfram 2007. *Mathematica. Wolfram Research*. Wolfram Research Inc, Champaign, IL.

Zahavi, A. 1975. Mate selection – selection for a handicap. *J. Theor. Biol.* **53**: 205–214.

Zeyl, C. & DeVisser, J. 2001. Estimates of the rate and distribution of fitness effects of spontaneous mutation in *Saccharomyces cerevisiae*. *Genetics* **157**: 53–61.

Zeyl, C.W. & Otto, S.P. 2007. A short history of recombination in yeast. *Trends Ecol. Evol.* **22**: 223–225.

Appendix 1: Calculation of the number of timesteps before selective intratetrad mating strategy becomes the best

We want to find out when $V[k, h, s] > 0$. To determine when this is, let $x = s/2$ and consider the function

$$f(h) = 1 + (1-x)^z - 2(1-hx)^z \qquad \text{(S1)}$$

where $0 < h < 1$, $0 < x < 1/2$ and $z \geq 1$. Then, $f(h) > 0$ if and only if $V[k, h, s] > 0$. Note that we do not assume z is an integer.

We first show that $f(h) \geq 0$ for $0 < x < 1/2$, $z \geq 1$ and $h \geq 1/2$, with equality only when $z = 1$ and $h = 1/2$. From eqn (S1), when $z = 1$, we have

$$f(h)|_{z=1} = 2x(h - 1/2),$$

and thus that $f(1/2)|_{z=1} = 0$, and $f(h)|_{z=1} > 0$ for $h > 1/2$.

Now assume that $z > 1$. Differentiating (S1) with respect to h gives $f'(h) = 2xz(1-hx)^{z-1} > 0$, so $f(h)$ is a monotonically increasing function of h for fixed x and z, and hence $f(h) \geq f(1/2)$ for $h \geq 1/2$. Now,

$$\frac{\partial f(1/2)}{\partial x} = z\left((1-x/2)^{z-1} - (1-x)^{z-1}\right) > 0$$

(because $z > 1$ and $1-x/2 > 1-x$), so $f(1/2)$ is monotonically increasing in x. Therefore, $f(h) \geq f(1/2)$

$> f(1/2)|_{x=0} = 0$ for all $h \geq 1/2$ and $0 < x < 1/2$, and we have proved our result.

We now consider the range $0 < h < 1/2$. Given a fixed x and z, we can solve the inequality $f(h) \geq 0$ explicitly to get a condition on h,

$$h \geq \frac{1}{x}\left(1 - \left(\frac{1+(1-x)^z}{2}\right)^{\frac{1}{z}}\right). \qquad \text{(S2)}$$

Define the function $h(z)$ by equality in (S2). Then, $h(z)$ is a monotonically decreasing function of z (see Fig. 2). It follows that the equation $h = h(z)$ can be solved uniquely to obtain z as a monotonically decreasing function $z(h)$ of h for fixed x. This function will give the value z for which $1 + (1-x)^z - 2(1-hx)^z = 0$, and thus for all integers, $k > z(h)$, $V[k, h, s] > 0$.

Appendix 2: The proportion Λ of deleterious mutations at the locus of interest across a colony decreases as p increases

We can write $\Lambda[p \mid k, h, s]$ as

$$\Lambda[p|k,h,s] = \frac{1/2(1+(1-hs))^k + p/3\left((1+(1-s))^k - (1+(1-hs))^k\right)}{\frac{p}{3}2^k + \left(1-\frac{2p}{3}\right)(1+(1-hs))^k + \frac{p}{3}(1+(1-s))^k}$$

and differentiate with respect to p to get

$$d\Lambda/dp = \frac{3\left((2-s)^k - 2^k\right)(2-hs)^k}{2\left((2p-3)(2-hs)^k - p\left(2^k + (2-s)^k\right)\right)^2}.$$

This is negative because $\left((2-s)^k - 2^k\right) < 0$.

Appendix 3: Numerical procedure for multiple-loci model

When there are multiple deleterious loci, the strategy $p = 0$ does not necessarily always produce offspring genetically identical to the parent (unlike in the one-locus case). For example, in the $n = 2$ case, meiosis could lead to the ascus containing two **a** haploids with genotypes $(+_1, +_2)$ and (m_1, m_2) and two **α** haploids with genotypes $(+_1, m_2)$ and $(m_1, +_2)$, so that the resultant pair of diploid offspring will have either genotypes $(+_1+_1, +_2m_2)$ and $(m_1m_1, +_2m_2)$, or genotypes $(+_1m_1, +_2+_2)$ and $(+_1m_1, m_2m_2)$.

To calculate the results, we used the open-source programming environment Processing 1.09 (http://www.processing.org) to provide the genotypic probabilities for number of loci n, from 2 to 10, and then Mathematica 7 (Wolfram, 2007) to numerically calculate the number of cells in colonies following the three different strategies after k generations, from $k = 1$ to 300. As the growth of the colonies is exponential, we represented the number of cells relative to the number in a colony that followed the asexual budding strategy. The code is available on request.

Appendix 4: Analysis of varying clonal expansion periods

The right-hand side of

$$\frac{\partial}{\partial p}\bar{W}_{\text{rel}}[p \mid h, s] = \frac{1}{3} - \frac{2}{3}\sum_{k=0}^{\infty} f(k)\left(1-\tfrac{1}{2}hs\right)^k + \frac{1}{3}\sum_{k=0}^{\infty} f(k)\left(1-\tfrac{1}{2}s\right)^k$$

is positive if and only if

$$1 + \sum_{k=0}^{\infty} f(k)\left(1-\tfrac{1}{2}s\right)^k > 2\sum_{k=0}^{\infty} f(k)\left(1-\tfrac{1}{2}hs\right)^k.$$

Substitution for $f(k)$ from the main text above gives

$$\kappa + 1 + \sum_{k=0}^{\infty}\left(\frac{\kappa}{\kappa+1}\right)^k\left(1-\tfrac{1}{2}s\right)^k > 2\sum_{k=0}^{\infty}\left(\frac{\kappa}{\kappa+1}\right)^k\left(1-\tfrac{1}{2}hs\right)^k.$$

The two infinite series are both geometric series and can be summed to give

$$\kappa + 1 + \frac{1}{1-\left(\frac{\kappa}{\kappa+1}\right)\left(1-\frac{1}{2}s\right)} > \frac{2}{1-\left(\frac{\kappa}{\kappa+1}\right)\left(1-\frac{1}{2}hs\right)}$$

This can be rearranged to give the following condition that $p = 1$ is the ESS:

$$h - \frac{1}{2} + \frac{1}{4}h\kappa s > 0.$$

This will always hold if $h \geq 1/2$. If $h < ½$, it will hold if and only if the condition in eqn (5) holds. If this inequality is reversed, then $p = 0$ is the unique ESS.

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