Fitness Epistasis among 6 Biosynthetic Loci in the Budding Yeast Saccharomyces cerevisiae

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Abstract

We generated all possible haploid and homozygous diploid genotypes at 6 biosynthetic loci in yeast and scored their fitness to examine whether there was any pattern of weak synergistic epistasis, which is a requirement of the deterministic mutation model for the evolution of sex. We measured 4 components of fitness: haploid growth rate, haploid mating efficiency, diploid growth rate, and diploid sporulation efficiency. We found that in agreement with previous work in yeast, epistasis tended to be small in magnitude and variable in sign, regardless of the fitness component measured. The number of background mutations had either no effect or no consistent effect on epistasis distributions. For every combination of 2 loci in a mutation-free background, we also generated all heterozygous genotypes so that we could partition diploid epistasis into additive × additive, additive × dominance, and dominance × dominance epistasis. Our main interest was in determining whether dominance by dominance epistasis was large and negative, which is a requirement of diploid models with inbreeding to explain high levels of recombination. Dominance by dominance epistasis estimates obtained by partitioning diploid epistasis for growth rates were both positive and negative. With the caveat that our results are based on only 6 biosynthetic loci, epistasis for fitness is not supported as an explanation for the maintenance of sex or the high rate of meiotic recombination in yeast.

Key words: dominance by dominance epistasis, evolution of sex, fitness, synergistic epistasis, yeast

Understanding the evolution of sex and recombination remains an unanswered question. Although several hypotheses have been examined theoretically, a general explanation has proved elusive (see Otto and Lenormand 2002 and deVisser and Elena 2007 for recent reviews). Several of the hypotheses that have been advanced to explain the evolution of sex and recombination require particular interactions among loci affecting fitness. Perhaps the most influential of these is Kondrashov’s deterministic mutation hypothesis (Kondrashov 1984, 1988) which states that sexual reproduction will have an advantage when deleterious alleles at different loci exhibit weak synergistic (negative) epistasis.

The situations in which particular signs and magnitudes of epistasis can favor the evolution of sexual reproduction have been clearly summarized (Barton 1995; Box 1 of Otto and Lenormand 2002). Synergistic epistasis has 2 effects on the evolution of genetic modifiers of sexual reproduction. First, synergistic epistasis can lead to lower genetic load in populations with more sex because selection acts more efficiently against genotypes carrying multiple mutations. This is a long-term advantage to sexual reproduction. Second, synergistic epistasis causes modifiers that increase sexual reproduction to experience lower fitness because they will be in genetic backgrounds with low average fitness. This causes a short-term disadvantage to modifiers that increase sexual reproduction. If synergistic epistasis is too large, the short-term disadvantage overwhelms the long-term advantage and sexual reproduction is not expected to increase in frequency (Barton 1995).

A second hypothesis concerning the effect of epistasis emphasizes the importance of dominance by dominance epistasis on the evolution of recombination in diploids with inbreeding (Roze and Lenormand 2005). Roze and Lenormand modeled a system in which all individuals undergo sexual reproduction and modifiers alter the amount of recombination that is exhibited among loci. They showed that with inbreeding the frequency of double homozygous genotypes is reduced as the rate of recombination between loci increases. If double homozygotes exhibit very low fitness, relative to their expected fitness, then this effect can favor the evolution of modifiers that increase recombination. Double homozygotes exhibit low expected fitness when dominance × dominance epistasis is large and negative (see Table 1). Roze (2009) has also shown that if the
Brand new mutation rate is not too large, negative dominance × dominance epistasis also favors increased recombination in structured populations.

Budding yeast, *Saccharomyces cerevisiae* is a facultatively sexual species, which is able to undergo sexual reproduction in response to certain environmental conditions. Although it can be maintained in the laboratory as an asexual haploid or diploid, in natural conditions it is always found as a diploid. The reason is because it is homothallic and undergoes a high rate of mating type switching, so a haploid cell line will quickly produce both mating types that then fuse to form a diploid. This ability to mate with itself is one reason to expect that yeast populations would show high levels of inbreeding. Furthermore, even when a haploid cell mates with a cell from a different strain, its partner may often be derived from the same ascus. All 4 products of a single meiosis may remain together in the ascus until appropriate germination and growth conditions occur. An exception is when the tetrad passes through an insect gut (Reuter et al. 2007). The biology of yeast thus implies that many, and perhaps most, cells in a population will be homozygous at most loci. Surprisingly, this expectation is not supported for yeast strains isolated from wineries (Mortimer 2000), suggesting that domesticated populations of *S. cerevisiae* may be quite different from nondomesticated populations.

A related, nondomesticated congener, *S. paradoxus*, does exhibit the predicted high homozygosity, with an estimated inbreeding coefficient of 0.99 (Johnson et al. 2004). When *S. cerevisiae* does undergo sexual reproduction, it exhibits very high levels of meiotic recombination between loci. In yeast 1 map unit represents ~2500 bp (Saccharomyces genome database, www.yeastgenome.org) which is a much higher recombination rate than observed in other eukaryotes (see Wilflert et al. 2007). Given yeast’s biology, one question is whether synergistic epistasis among mutations can explain the maintenance of sex in this species. Another question is whether strong, negative dominance × dominance epistasis can explain the high frequency of recombination. Several studies have addressed the first question, but none have addressed the second.

Previous work in yeast has primarily explored fitness interactions among loci to elucidate the pattern and magnitude of epistasis in haploids. These studies have examined growth rate and have uniformly found that epistasis is weak and the sign is, on average, close to zero, or slightly positive (Wloch et al. 2001; Jasnos and Korona 2007). The same result has also been seen in heterozygous diploids (Szafraniec et al. 2003). Both spontaneously arising

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mutations (Wloch et al. 2001; Szafraniec et al. 2003) and mutations obtained from the yeast deletion lines (Jasnos and Korona 2007) have been examined. These studies have been impressive in scope: The deletion line study examined more than 500 pairs of loci to calculate values of epistasis (Jasnos and Korona 2007). There are, however, some issues that were not addressed in previous studies. First, epistasis in homozygous diploids has not been examined in yeast. Second, the effect of the number of background mutations on 2-locus measures of epistasis has not been addressed. This may be important for the deletion study (Jasnos and Korona 2007) because each yeast deletion strain harbors 4 background mutations. Third, the pattern of epistasis across more than just pairs of loci has not been estimated. Fourth, only growth rate has been measured as a surrogate of fitness.

In this study, we estimated epistasis among deletion mutations at 6 biosynthetic loci, all of which occur in different biosynthetic pathways. We generated and scored fitness for all possible combinations of deleterious mutations at the 6 biosynthetic loci. We measured 4 components of fitness: 2 for haploid and 2 for homozygous diploid genotypes. These fitness components were haploid growth rate, haploid mating efficiency, diploid growth rate, and diploid sporulation efficiency. Our data allowed us to examine whether estimates of epistasis vary with the fitness component that is measured, whether patterns of multilocus epistasis differ from that observed between pairs of loci, and whether there is an effect of the number of background mutations on the sign or magnitude of epistasis. In addition, for every combination of 2 loci in a mutation-free background, we also generated all heterozygous genotypes so that we could partition diploid epistasis into additive × additive, additive × dominance, and dominance × dominance epistasis.

We found that, in agreement with previous studies, epistasis is small and shows no bias to synergistic values for haploid growth rate. This result also holds for haploid mating efficiency, sporulation efficiency, and diploid growth rate. The number of background mutations does have an effect on epistasis for mating efficiency and for haploid growth rate, but the effect is not in a consistent direction. Dominance by dominance epistasis estimates obtained by partitioning diploid epistasis for growth rates are both positive and negative. With the caveat that our results are based on only 6 biosynthetic loci, epistasis for fitness is not supported as an explanation for the maintenance of sexual reproduction or the high rate of meiotic recombination in yeast.
Materials and Methods
Generation of Experimental Lines

The 6 mutant loci we chose shared several characteristics: each represented a mutation in a different biosynthetic pathway, each was readily available as a deletion in the same genetic background, each could be easily genotyped in our laboratory using readily available dropout medium, and each was unlinked from all the others. We used 2 parental strains to generate all strains used in the experiments. The first is a yeast deletion strain (Euroscarf accession number Y00414) which is derived from BY4741 and has genotype: Mat α, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ade1::kanMX4. This strain was mated to BY4711 that has genotype: Mat α; trp1Δ. BY4711 and BY4741 are closely related strains; both were derived from a cross of strains FY3 with BY418, which were themselves recently derived from strain SC228 (Brachmann et al. 1998). Mating was confirmed by growth on minimal medium. The resulting diploid, which is heterozygous at the 6 biosynthetic loci and the mating locus but otherwise homozygous, was then sporulated (Burke et al. 2000). After sporulation, approximately 700 tetrads, representing 2800 spores, were dissected onto YPD complete medium (1% yeast extract, 2% peptone, 2% dextrose, and 2% agar). All spore colonies from each tetrad were then genotyped by replica plating onto appropriate selective media. Mating type was tested by attempted mating to 2 haploid tester strains.

Both Mat α and Mat a versions of each 6 locus genotype were identified and then confirmed with a second round of replica plating onto appropriate dropout medium. These 128 genotypes (64 of each mating type) were then used to generate 64 homozygous diploid genotypes by mating. Because the resulting homozygous diploids and their hemizygous haploid parents have the same phenotype with respect to growth on selective media, diploidy was confirmed by testing for lack of mating ability using haploid tester strains. All diploids were confirmed by retesting growth on selective media and repeating mating tests.

Finally, we also generated all possible heterozygous genotypes for pairs of loci in an otherwise mutation-free background. For example, with 2 loci there are 9 possible 2-locus diploids (Table 1). The 4 homozygous genotypes were generated as above, leaving only the 5 heterozygote-containing genotypes to be produced. We performed 6 matings to generate these genotypes so that we could score both the cis (coupling) and trans (repulsion) versions of the double heterozygote. Data obtained for the cis and trans double heterozygotes were pooled after confirming no significant difference between them. Mated diploids were selected on appropriate drop out media and diploidy was confirmed by failure to mate with the haploid tester strains.

All haploid and diploid experimental genotypes were then stored at −80 °C in 15% glycerol.

Fitness Components

We measured 2 components of fitness on Mat a haploid lines: growth rate in liquid medium and competitive mating efficiency. On diploid homozygotes, we measured growth rate and sporulation efficiency, and on diploid heterozygotes, we measured growth rate.

To measure growth rate in liquid medium, we followed the procedure outlined in Joseph and Hall (2004). In summary, each replicate was grown in 150 μl of YPD, starting at low density, in a microtiter plate well. A Bioscreen C spectrophotometer (Curve Growth Curve USA) maintained constant temperature at 30 °C with shaking and measured absorbance of 600 nm light every 10 minutes. Absorbance measures were log-transformed and then used to calculate a maximal growth rate using a sliding 120-min regression (see Joseph and Hall 2004). The maximal growth rate was measured on 10 replicates for haploid and homozygous diploids with one set of exceptions. For those strains that were used to partition diploid epistasis, 20 replicates of each genotype were measured. On each Bioscreen plate we also interdigitated the wild-type genotype. Relative Malthusian fitness of each replicate of a strain was calculated as its maximal growth rate divided by the maximal growth rate of the matched wild-type replicate. Previously we had observed that for some strains the position on a Bioscreen plate can affect the maximal growth rate (seen as a significant column and/or row effect in an analysis of variance [ANOVA]). Normalizing strains using a matched standard strain removed this position effect. So, by normalizing each of our replicates relative to the adjacent, wild-type strain, we experimentally controlled for any effects of position on the Bioscreen microtiter plate. The relative Malthusian fitness of a strain was then calculated as the average of the replicate measures.

Sporulation efficiency was measured for the 64 diploid strains by sporulating each strain using a protocol adapted from Codón et al. (1995) and then counting at least 400 cells per replicate, 3 replicates per strain. Lines were streaked from the freezer onto YPD and grown for 2 days. Three replicates, each from a separate colony of each line were grown overnight in liquid YPD at 25 °C. One milliliter of each overnight culture was then centrifuged and the pellet of cells resuspended in 2.5 ml of supplemented, presporulation medium (0.8% yeast extract, 0.2% peptone, 5% potassium acetate, 0.0005% adenine sulfate, 0.0005% uracil, 0.0005% histidine, 0.0005% methionine, 0.0005% tryptophan, 0.0005% leucine, and 2% dextrose) and grown overnight at 25 °C. The next day, 1.4 ml of each culture was centrifuged and the cell pellet resuspended in 2.5 ml of supplemented, sporulation medium (0.5% potassium acetate, 0.0005% adenine sulfate, 0.0005% uracil, 0.0005% histidine, 0.0005% methionine, 0.0005% tryptophan, 0.0025% leucine, and 2% dextrose). After 5 days at 25 °C, a 12–15 l sample from each culture was examined under ×40 magnification. At least 400 cells per replicate were counted and scored as tetrads (4 spores in the tetrad), aborted tetrads (2 or 3 spores in the tetrad), or nonsporulators. Within each group of lines measured at a particular time for sporulation efficiency, the wild-type diploid strain was also scored. Sporulation efficiency of each line was standardized relative to the wild-type strain that was sporulated at the same time.
Mating efficiency was measured for the 64 Mat a haploid genotypes by competing each experimental strain against a competitor of genotype Mat a; arg4::kanMX4; Nat R for access to a compatible Mat α, arg strain. The 3 strains were streaked from the freezer onto YPD and then grown for 2 days at 30 °C. Each was then grown overnight in liquid YD (1% yeast extract, 2% dextrose). The following morning, strains were mixed in a 1:10:10 ratio of experimental:competitor:compatible strain. In preliminary experiments, this ratio was found to give the largest difference between the wild-type strains and the 6-locus mutant strains. The ratio was obtained by adding 4 × 10⁷ cells of the experimental strain to 4 × 10⁸ cells of the competitor and compatible strains. The mix was then centrifuged and resuspended in 500 μl of YD. Aliquots of 50 μl were then placed onto 20-mm diameter disks of P2 cellulose filter paper (Fisher Scientific) and placed on a YD plate. Plates were left undisturbed for 3 h and then individual filter paper disks were placed in 1.8 ml of sterile water and vortexed vigorously. The solution was then diluted 100-fold and 100 μl plated on an arginine dropout plate (minimal medium + 0.0074% css-arg [MP Biomedicals]). This plate selects against the unmated competitor and unmated compatible strain, leaving the mated and unmated experimental strain. After 2 days, the number of colonies on a plate were counted and then replica plated to arginine dropout supplemented with nourseothricin (0.01%), on which only mated experimental cells will grow. The ratio of the number of colonies on the 2 plates is the mating efficiency for that replicate. Three replicates were made for each experimental strain. Within each group of haploid lines measured for mating efficiency at a particular time the wild-type strain was also measured. To control for differences in mating propensity of the competitor and compatible strains on different days, mating efficiency was standardized to the wild-type strain mated at the same time.

Malthusian and Darwinian Fitness

Our measure of fitness obtained from growth rate represents relative Malthusian fitness, whereas our measures for mating efficiency and sporulation efficiency represents relative Wrightian fitness. Standard population genetic models use relative Wrightian fitness, and we thus need to convert Malthusian fitness to Wrightian fitness. The relationship between absolute Wrightian fitness, \( m \), and absolute Malthusian fitness, \( w \), is

\[
  w = e^m
\]

(Crow and Kimura 1970). Importantly, \( m \) is absolute growth rate measured “per generation.” Equation 1 implies that the Wrightian fitness of a particular genotype, \( w_G \), relative to a standard genotype, \( w_S \), is

\[
  \frac{w_G}{w_S} = e^{(m_G - m_S)},
\]

where \( m_G \) and \( m_S \) are the per minute growth rates of the particular genotype and the standard genotype respectively, and \( g \) is the generation time in minutes. Using a standard curve of absorbance versus cell density, we calculated the average cell generation time (= \( g \)) for the wild-type genotype (our standard) to be \( \sim 74 \) min for both haploids and diploids (data not shown). To calculate Malthusian growth rate for a strain, we simply multiplied the average relative growth rate for that strain by the average growth rate of the wild type. Estimating the growth rate in this way retained our experimental correction for plate position (see above). We then used Equation 2 to calculate relative Wrightian fitness for each strain. For the remainder of the analyses, when we refer to fitness, we mean relative Wrightian fitness.

Epistasis in Haploids and Homozygous Diplolds

To examine whether there is evidence for synergistic or antagonistic epistasis across all loci, we plotted the logarithm of relative fitness versus the number of deleterious mutations present for haploids and homozygous diploids for each fitness measure. If mutations tend to interact multiplicatively, the relationship between log fitness and the number of mutations should be linear, with negative slope. If mutations exhibit epistasis, then the relationship between fitness and the number of mutations should have a significant quadratic component, with a negative coefficient indicating synergistic epistasis. We tested this by determining if the relationship between log fitness and the number of mutations was best fit by a linear regression or by a quadratic regression. JMP statistical software was used to perform these analyses (version 7, SAS Institute Inc.).

To examine the distribution of 2-locus epistasis values, we compared strains that varied for only a single pair of loci but were otherwise identical. These comparisons involved 4 genotypic fitness measures for each fitness component. Epistasis between 2 loci, \( \epsilon \), was calculated as

\[
  \epsilon = w_{BbC}w_{bc} - w_{Bc}w_{Bc}
\]

for haploids, and

\[
  \epsilon = w_{BBCC}w_{bbcc} - w_{BbCC}w_{bbCC}
\]

for homozygous diplods, where \( w_{BbC} \) or \( w_{BBCC} \) is the fitness of the nonmutant genotype, \( w_{bc} \) or \( w_{bbcc} \) is the fitness of the 2-locus mutant genotype, and \( w_{Bc} \) or \( w_{BbCC} \) and \( w_{bc} \) or \( w_{bbCC} \) are the fitnesses of the single mutant genotypes. Synergistic epistasis, \( \epsilon < 0 \), indicates that the 2 locus mutant has lower fitness than expected based on the products of the single locus fitnesses.

To examine the effects of the fitness component measured and the number of background mutations, we used ANOVA. We performed ANOVAs using PROC MIXED implemented in SAS (version 9, SAS Institute Inc.) because it allows for differences in variances across treatments (using the appropriate REPEAT statement).

Partitioning Diploid Epistasis

All 9 genotypic fitnesses for a pair of loci were required to partition diploid epistasis into additive by additive, additive
by dominance, and dominance by dominance epistasis. In Table 1, the fitnesses of each 2-locus genotype in terms of selection coefficients, dominance coefficients, and epistasis coefficients are shown. We used this table to estimate the various epistasis components with the following relationships.

\[ e_{\text{AxA}} = \mu_{\text{BBCC}} \mu_{\text{BbCc}} - \mu_{\text{BBCC}} \mu_{\text{BbCC}} \]  
\[ e_{\text{AxD}} = \mu_{\text{BBCC}} \mu_{\text{BbCc}} - \mu_{\text{BbCC}} \mu_{\text{BbCC}} - 2 e_{\text{AxA}} \]  
\[ e_{\text{AC}} = \mu_{\text{BBCC}} \mu_{\text{BbCc}} - \mu_{\text{BBCC}} \mu_{\text{BbCC}} - 2 e_{\text{AxA}} \]  
\[ e_{\text{DxD}} = \mu_{\text{BBCC}} \mu_{\text{BbCc}} - \mu_{\text{BbCC}} \mu_{\text{BbCC}} - 2 e_{\text{AcD}} + 4 e_{\text{AxA}} \]  
\[ -2 e_{\text{AxD}} - 2 e_{\text{AcD}} \]

where \( e_{\text{AxA}}, e_{\text{AxD}}, e_{\text{AcD}}, \) and \( e_{\text{DxD}} \) are the additive by additive, additive (locus B) by dominance (locus C), additive (locus C) by dominance (locus B), and dominance by dominance epistasis, respectively. Equations 5–8 were used to calculate point estimates for the epistasis measures for each of the 15 pairs of loci. To estimate the variance of these estimates, we generated sets of simulated data. For each genotype, we used a normal distribution with the same mean and standard error as our experimental samples. We randomly sampled values from the normal distributions to generate 1000 simulated data sets for each pair of loci, and for each we calculated the 4 epistasis values using Equations (3–6). We then calculated the means of the simulated epistasis values to confirm they were the same as we measured in our experiment, and the standard deviations, from which we could determine confidence intervals. All simulations were implemented in Mathematica (version 7.0, Wolfram Research).

Results

Epistasis in Haploids and Homozygous Diploids

In Figure 1, we plot the logarithm of fitness versus the number of mutations for all 64 haploid or homozygous diploid genotypes, for each of the 4 fitness components. For sporulation efficiency, in which we used the proportion of cells producing 4-spore tetrads, we plot the logarithm of 1 + fitness because some strains failed to give any 4-spore tetrads. Linear regressions of fitness on mutation number are significant for 3 of the 4 fitness components. We also fit linear regressions of the mean fitness within a background mutation category on mutation number and found that all were significant. In all cases, fitness declines with the number of mutations (Figure 1). Mating efficiency shows the weakest relationship between fitness and mutation number. For all regressions, adding a quadratic term does not significantly improve the fit to the data (\( F \) ratio test, \( P > 0.5 \) in all cases), indicating no support for nonmultiplicative fitness interactions among these loci. When we performed the quadratic regressions, we noted that the quadratic term had a (nonsignificant) positive coefficient in every case, which is consistent with antagonistic but not with synergistic epistasis.

The 2 locus epistasis estimates obtained from the fitness measures of the 64 genotypes are plotted in Figure 2 for each fitness component as a function of the number of common background mutations in the genotypes used to calculate epistasis. We first determined whether the mean of each distribution is significantly different from zero. We excluded the category with 4 background mutations that is represented by a singleton. After correcting for multiple comparisons (Benjamini and Hochberg 1995), only one of the groups of epistasis values (mating efficiency with zero background mutations) is significantly different from zero (\( t \)-test, \( P < 0.05 \)). This group exhibits antagonistic epistasis.

We then performed an ANOVA including the number of background mutations, the fitness component, and an interaction between the 2 in our model (Table 2A). We again omitted the 4-mutation category. We found that the number of background mutations and the interaction term had significant effects on epistasis (\( P = 0.04 \) and \( P < 0.0001 \), respectively), but fitness component was nonsignificant. Examination of Figure 2 suggested that mating efficiency might be causing the significant effects, so we reran the ANOVA with mating efficiency removed from the analysis (Table 2B). With this component removed, there is no significant effect of fitness component, number of background mutations, or their interaction on epistasis.

To gain more insight into the effects of background mutation seen in the ANOVA, we compared epistasis distributions across mutation classes within a fitness component. We tested whether the distribution of epistasis estimates differed based on the number of background mutations within a fitness category. We found that for diploid growth and sporulation efficiency, there is no effect of background mutations: none of the epistasis distributions for different numbers of background mutations differ from one another. For haploid growth, the distribution with 0 background mutations is significantly different from the distribution with 1 mutation (\( t \)-test, \( P < 0.05 \)), but none of the other comparisons are significant (Figure 2A). For mating efficiency, distributions for 0 and 2 mutations are significantly different (\( P < 0.05 \)) from the distributions for 1 and 3 mutations (Figure 2B). In all cases, significance was determined after correcting for multiple comparisons.

Partitioning Diploid Epistasis

The estimates for each component of epistasis obtained from the diploid growth data for each of the 15 pairs of loci are shown in Table 3. Also shown is the approximation of the standard deviation of these estimates obtained by simulation. Examination of this table reveals several patterns. First, additive by additive epistasis is close to zero. None of the 15 estimates of additive by additive epistasis are significantly different from zero. Very small additive by additive epistasis stems from the fact that the fitness estimates for single and double heterozygotes, \( \mu_{\text{BBCC}}, \mu_{\text{BbCC}}, \) and \( \mu_{\text{BbCC}} \) are all very close to the fitness of the nonmutant,
that is, the $h_i$ are small in Table 1, which results in a small value of $e_{AA}$ (Equation 3, see also Szafraniec et al 2003). None of the single or double heterozygotes are significantly different from the nonmutant fitness, after correcting for multiple comparisons (Kruskal–Wallis test). Second, additive by dominance epistasis, although positive on average, is again small in magnitude. Twenty-four of the 30 additive by dominance epistasis estimates are not significantly different from zero. Of the 6 that are different, 4 are positive and 2 are negative. This epistasis measure is variable in sign and small in magnitude. Third, dominance by dominance epistasis is more often significantly different from zero than other measures of epistasis. Six of the 15 dominance by dominance epistasis estimates are significantly different from zero. Fourth, dominance by dominance epistasis, although negative on average, is variable in both magnitude and sign. The range of values is $-0.0236$ to $+0.0282$ and of the 6 values that are significantly different from zero, 3 are negative and 3 are positive.

**Discussion**

Previous estimates of the distribution of epistasis in yeast have also found no consistent pattern of synergism (Wloch et al. 2001; Szafraniec et al. 2003; Jasnos and Korona 2007). We have extended previous results in 3 ways. First, we have shown that the same pattern holds for homozygous diploids. This is important given the expectation of high homozygote frequency in yeast (Introduction). Second, we have shown that the pattern holds for other components of fitness besides growth. Sporulation efficiency shows no evidence for significantly nonzero epistasis, either antagonistic or synergistic. Mating efficiency shows evidence for nonzero epistasis only when there are no background mutations, and in this one case epistasis is antagonistic. Third, we have demonstrated that the number of background mutations does not have a consistent effect on the value of epistasis measured. In some cases, the number of background mutations does effect the level of epistasis, especially for mating efficiency, but there is no monotonic
pattern to these effects: for mating efficiency, zero and 2 background mutations show higher epistasis than one and 3 mutations. Our finding that examining more than 2 loci does not alter conclusions regarding epistasis is in agreement with work by deVisser et al. (1997) for mycelium growth rate in *Aspergillus niger*. The absence of a pattern of consistent, synergistic epistasis does not support the deterministic mutational hypothesis (Kondrashov 1984, 1988) as a potential mechanism to explain the maintenance of sex in yeast, and perhaps other microbes (see Kouyos et al. 2007 for review).

Our results for dominance by dominance epistasis indicate that some pairs of loci do show significantly negative epistasis, but others have significantly positive values, and there is a range of intermediate values. To our knowledge, there is no theoretical work to address the effect of variation in the sign of dominance by dominance epistasis on the evolution of recombination with inbreeding. Theory in a haploid model has shown that variation in epistasis acts against the evolution of sexual reproduction (Otto and Feldman 1997). If the same holds true for the evolution of recombination with inbreeding in diploids, then our results would suggest that dominance by dominance epistasis does not show the pattern necessary to be a viable hypothesis for the high recombination rates observed in yeast. In the absence of the appropriate theory, with only a few loci tested, and with these loci representing an atypical class of mutations (large deletions), our failure to support hypothesis

**Figure 2.** Two locus epistasis versus the number of background mutations. Crosses indicate mean 2-locus epistasis for each category of number of background mutations. Panel A haploid growth, panel B mating efficiency, panel C diploid growth, and panel D sporulation efficiency. Diploid genotypes are homozygous. For panels A and B, means with different letters are significantly different at the 5% level, after correcting for multiple comparisons (see text). For panels C and D, none of the means were significantly different from one another.

**Table 2** (A) ANOVA of epistasis for haploid and homozygous diploid fitness measures (haploid growth rate, diploid growth rate, mating efficiency, and sporulation efficiency) and (B) ANOVA results with same model after removing mating efficiency data

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The model includes the fitness component being examined (component), the number of background mutations (mutations), excluding the 4 background-mutation category, and an interaction between the 2 (mutations × component). ANOVAs were implemented in SAS using PROC MIXED to allow for unequal variances across distributions.

$^a$ DF (num) is the degrees of freedom in the numerator of the F-test.

$^b$ DF (den) is the degrees of freedom in the denominator of the F-test.

does not alter conclusions regarding epistasis is in agreement with work by deVisser et al. (1997) for mycelium growth rate in *Aspergillus niger*. The absence of a pattern of consistent, synergistic epistasis does not support the deterministic mutational hypothesis (Kondrashov 1984, 1988) as a potential mechanism to explain the maintenance of sex in yeast, and perhaps other microbes (see Kouyos et al. 2007 for review).

Our results for dominance by dominance epistasis indicate that some pairs of loci do show significantly negative epistasis, but others have significantly positive values, and there is a range of intermediate values. To our knowledge, there is no theoretical work to address the effect of variation in the sign of dominance by dominance epistasis on the evolution of recombination with inbreeding. Theory in a haploid model has shown that variation in epistasis acts against the evolution of sexual reproduction (Otto and Feldman 1997). If the same holds true for the evolution of recombination with inbreeding in diploids, then our results would suggest that dominance by dominance epistasis does not show the pattern necessary to be a viable hypothesis for the high recombination rates observed in yeast. In the absence of the appropriate theory, with only a few loci tested, and with these loci representing an atypical class of mutations (large deletions), our failure to support hypothesis
Mean values of epistasis obtained from genotypic fitness estimates for all 15 pairs of 6 loci

<table>
<thead>
<tr>
<th>Loci</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Ade Met</td>
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<td>0.0018</td>
<td>0.0036</td>
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<td>0.0026</td>
<td>0.0044</td>
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<td>0.0010</td>
<td>0.0019</td>
<td>-0.0134</td>
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</tr>
<tr>
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<td>0.0054</td>
<td>0.0037</td>
<td>0.0065</td>
<td>0.0044</td>
<td>-0.0130</td>
<td>0.0069</td>
<td>0.0027</td>
<td>0.0020</td>
<td>0.0054</td>
<td>0.0037</td>
</tr>
<tr>
<td>Ade His</td>
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<td>0.0019</td>
<td>-0.0014</td>
<td>0.0040</td>
<td>-0.0058</td>
<td>0.0039</td>
<td>0.0041</td>
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<td>0.0019</td>
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<tr>
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<td>0.0040</td>
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<tr>
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<td>0.0027</td>
<td>0.0024</td>
<td>0.0042</td>
<td>0.0044</td>
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<tr>
<td>Met Trp</td>
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<td>0.0017</td>
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<td>0.0056</td>
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<td>0.0041</td>
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<td>-0.0005</td>
<td>0.0020</td>
<td>0.0023</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

Also shown are the standard deviations (SDs) for each value of epistasis, obtained by simulation. Values of epistasis whose confidence intervals (calculated as the mean ±2 SDs) do not overlap zero are shown in bold type. A × A, additive × additive epistasis; A × D, additive × dominance epistasis; D × A, dominance × additive epistasis; D × D, dominance × dominance epistasis. For A × D and D × A epistasis measures, the first letter refers to the first locus listed in left hand column. Thus for the first pair of loci (Ade Met), A × D is additive for adenine by dominance for methionine epistasis.

of Roze and Lenormand as an explanation for high recombination rates in yeast cannot be seen as a rejection of the hypothesis.

There is one possible complication that should be kept in mind when considering the absolute magnitude of the epistasis values for growth rates. These values are calculated using fitness measures that are estimated per cell generation (see above). However, the appropriate timescale when addressing hypotheses for the evolution of sex should perhaps be the average number of cell generations between episodes of sex. With that timescale, values of epistasis may be larger or smaller in magnitude. The reason is that the selection coefficients measured per sexual generation increase in magnitude as the number of mitotic generations between episodes of sex increases. If the number of mitotic cell generations is not too large, small differences in selection coefficients among genotypes will become larger which will increase the absolute magnitude of epistasis. However, if the number of mitotic generations between episodes of sexual reproduction is very large, selection coefficients will tend to unity, reducing the differences in fitness among genotypes and causing the magnitude of epistasis to decrease.

This study is one of the first that explicitly partitions diploid epistasis for fitness into additive by additive, additive by dominance, and dominance by dominance epistasis. We were able to do this by generating all possible 2-locus genotypes for our 15 pairs of loci, all with essentially identical genetic backgrounds. There is one important caveat to our study that is the pairs of loci we used to calculate epistasis are not independent. Only 6 different loci were utilized to partition epistasis, and so each locus is represented in 5 of the 15 pairs of loci. More work is clearly needed.

A few other studies have estimated additive by additive, additive by dominance, and dominance by dominance epistasis. Some studies involve examining covariances across relatives for a character as a function of inbreeding. These studies tend to find small estimates of components of epistasis (see Falconer and Mackay 1996 for discussion). The advent of large-scale sequencing allows for more direct approaches that are similar to ours. For example, Hua et al. (2003) examined the genetic basis for heterosis in rice by measuring 4 traits across a variety of hybrids generated by crossing recombinant inbred lines. Genotyping of 231 markers allowed identification of pairs of markers exhibiting epistasis, and because they had generated all possible 2-locus genotypes in their crosses, they were able to partition epistatic effects. They found that some pairs of loci showed dominance by dominance epistasis and, when present, it tended to be large and negative. Interestingly, the loci involved in epistasis were not the same as those with significant single locus effects. These types of analyses are important to evaluate hypotheses involving the various diploid epistasis measures and will hopefully become more common in the future.

Do the results obtained here and in previous studies lead us to reject models for the evolution of sex or recombination that require epistasis to be a particular sign or magnitude? One might reasonably conclude that such models should be viewed with reservation because several studies have found mixed evidence for epistasis of a particular sign. However, there is some indication that as genomic complexity increases, epistasis is more likely to be synergistic (e.g., productivity in Drosophila, Whitlock and Bourget 2000), perhaps as a result of mutational robustness (Sanjuan and Elena 2006). Furthermore, our understanding of genetic interactions at the molecular level, and how these
alter phenotype, suggests certain conditions under which synergistic or antagonistic epistasis should be common (Szathmary 1993). For example, under conditions favoring optimal pool size of a metabolite epistasis is likely to be synergistic. Selection for maximal flux, as might be common in microbes like yeast, is more likely to result in antagonistic epistasis. Models requiring synergistic epistasis to explain sex and recombination may thus be applicable only to multicellular species that experience selection for optimal metabolite pool sizes and have greater genomic complexity.

In this study, we have focused on estimating epistasis for fitness primarily with reference to specific models for the evolution of sex (Kondrashov 1984, 1988) and recombination (Roze and Lenormand 2005; Roze 2009). There has been a recent interest in sign epistasis and its importance for the evolution of sex and recombination. Sign epistasis occurs when the sign of the fitness effect of a mutation differs depending on genetic background (Weinreich et al. 2005). For example, a mutation that is deleterious in a wild-type background and beneficial in a particular mutant background exhibits sign epistasis. If sign epistasis is prevalent, it results in a rugged fitness landscape that can, in certain situations, select for sexual reproduction (Weinrich and Chao 2005; deVisser et al. 2009). Unfortunately, the conditions under which sex is favored can be difficult to predict for a given landscape (Misevic et al. 2009). Examination of Figure 2 indicates that sign epistasis is present in our data. However, a comprehensive analysis of sign epistasis, the resulting fitness landscape for these 6 loci, and whether the landscape would select for sexual reproduction is beyond the scope of the present study.

Despite the continued theoretical effort addressing the evolution of sex and recombination, there are many unanswered questions. It is becoming clear that a single genetic explanation to explain the evolution of sex and recombination in all sexual organisms is unlikely to be realized. Theories requiring synergistic patterns of epistasis do not seem to be applicable to microbes, which either show no epistasis or antagonistic epistasis. In yeast, the explanation for why sex is maintained and why meiotic recombination rates are so high, remains elusive.

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**References**


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