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Note added in proof: Analysis of sera for 94 days post-vaccination indicated that the anti-HBsAg titres increased, reaching values of 540 and 9,600 mIU ml^{-1} for rabbits 2 and 3, respectively.

Frequency of fixation of adaptive mutations is higher in evolving diploid than haploid yeast populations

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Adaptive mutations are shown to have a higher frequency of fixation in evolving diploid than in haploid populations of the yeast Saccharomyces cerevisiae, *providing direct evidence that it may be an evolutionary advantage to be diploid.*

A DOMINANT diploid phase is one of the major features of the life cycle of almost all eukaryotes, and can be considered to be circumstantial evidence for the adaptive significance of this phase. However, this subject has received scant attention from evolutionary biologists, especially when compared with the extensive literature on the adaptive significance of sex or recombination, a closely related, but independent issue (see refs 1, 2 and references therein). As early as 1929, Svedelius[3] argued that adaptive significance of diploidy was due to the ability of diploids to generate more variability than haploids. This argument and similar ones advanced by others[2,4–6] can be considered as belonging to the balance school[7] of population genetics, which asserts that genetic variation in natural populations is (1) ubiquitous and (2) somehow adaptive. A contrasting theory, which really owes its allegiance to the neoclassical school of population genetics, argues that the adaptive significance of the diploid phase lies in its ability to mask deleterious recessive mutations in either somatic[5,8,9] or germ-line cells[5,10–12].

There exist significant criticisms of both sets of theories. On the one hand, many of the postulated advantages of diploidy advanced by the balance school are unlikely to have been manifested as soon as an organism converted to diploidy and may only be consequences rather than causes of a dominant diploid phase. Furthermore, the adaptive significance of variation must involve either heterozygous advantage, considered by some[7,13,14] to be an uncommon phenomenon in natural populations, or mechanisms of group selection which are by no means universally accepted. On the other hand, the advantages for diploidy postulated by the neoclassical school will be on the order of the genomic mutation rate. These values are extremely small for populations of microorganisms (1×10^{-3}), although they may be appreciable for more complex organisms such as *Drosophila*[15]. Nevertheless, the greater accumulation of deleterious alleles in diploids will result in a higher mutational load as equilibrium is approached, resulting in an adaptive disadvantage for diploidy.

Here we investigate an alternative hypothesis for the adaptive significance of the diploid phase—namely that the rate of adaptation of diploid cells to new environments may be faster than that of haploid cells. The rationale for this hypothesis is simple; diploids can be considered to have undergone a duplication for every gene in the genome. As such, the rate of occurrence of adaptive mutations in diploids should be twice that of haploids, and so they should enjoy increased rates of adaptation. Similar hypotheses have been advanced to explain the success of organisms that have undergone gene duplications[16–20], and of polyploids[21]. Diploidy may be considered to be no more than a special case of polyploidization.

An important assumption implicit in this hypothesis is that most (>50%) adaptive mutations are expressed in the heterozygous state; *a priori* this assumption is not unreasonable. Advantageous mutations may be expected to improve or change gene function, and hence be dominant or semi-dominant whereas deleterious mutations are likely to cause the loss or attenuation of function and thus be recessive. The available data are not particularly revealing on this issue. Most mutations identified in *Drosophila* are recessive[22] whereas most of those identified in humans are dominant[23]. However, these results are difficult to interpret as few advantageous mutations have been studied; the mutations studied may be a biased sample of all mutations, and the results may vary from organism to organism.

Rates of adaptation to a new environment, in this case a carbon-limited chemostat environment, were measured in *Saccharomyces cerevisiae*. This organism is particularly suitable for such studies as it is a single-cell eukaryote with both stable haploid and diploid phases. Furthermore, it has been well characterized both physiologically and genetically.

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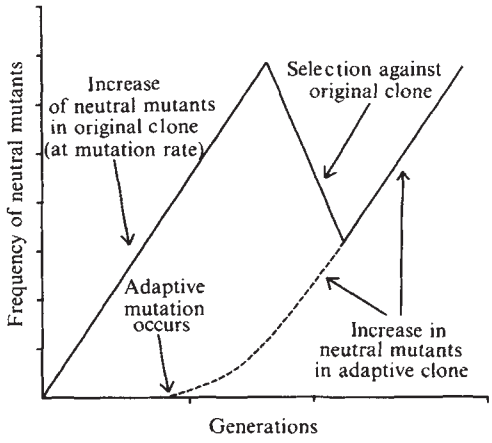

Fig. 1 Diagrammatic representation of the fluctuation in the frequency of neutral mutants in an asexual population showing the occurrence of, and selection for, an adaptive mutation.

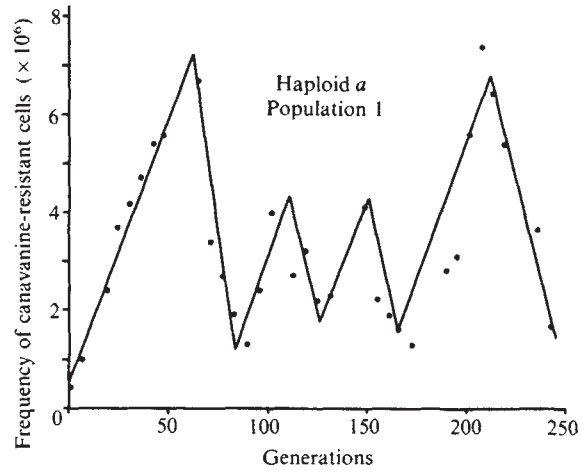

Fig. 2 Fluctuation in the frequency of canavanine resistance in one population of the haploid *a* strain, CP1AB-1A[28]. The line illustrates the fluctuations in the frequency of canavanine resistance expected assuming the occurrence and fixation of four adaptive mutations. The cells were grown in minimal medium +0.08% glucose[6] in continuous culture with a dilution rate of ~0.20 h^{-1}. Increasing the concentration of glucose in the medium above 0.08% resulted in a proportionate increase in the population size, demonstrating that glucose was the limiting substrate. Selection for mutants such as *abs* and *wal*[56], which cause cells to clump and sink to the bottom of continuous culture apparatuses, was reduced by pumping medium and cells from the bottom of the continuous culture apparatuses at ~67% of the dilution rate. Despite this, clumping cells were selected for after 100–300 generations. Each chemostat was sampled about every 24 h. Cells were sonicated for 15–30 s (Branson sonicator Model 185 with a microtip; power setting 3; equivalent to ~15 W) to break up clumps of cells, then plated onto minimal medium at appropriate dilutions to determine the number of viable cells per ml, and plated onto minimal medium plus canavanine[6] to determine the frequency of canavanine resistance. Canavanine resistance is recessive, determined by a single locus (*can1*)[57], and is neutral in our experimental conditions (ref. 6 and Table 1). An unsonicated aliquot was frozen in 15% glycerol at −70 °C for later analysis. Experiments were stopped when clumped cells could not be separated by sonication. The frequency of canavanine resistance was estimated as the number of mutant cells per ml divided by the number of cells per ml as determined from the viable counts. The population size for each sample was estimated as the number of cells per ml as determined by the viable count multiplied by the volume of the chemostat. The number of generations was determined from $t \times \ln 2/D$ where D is the dilution rate (h^{-1}) and t is the time (h) from the start of the the experiment[58]. The number of cell generations between samples was determined as the average of the population sizes for each sample weighted by the number of generations between samples. At the end of the experiment, the cells were tested for mating ability, sporulation, ploidy and for complementation with *gal2* and *gal3* tester strains[28] to verify that the cells had the same genotype as the strain used to inoculate the chemostat.

Table 1 Relative fitnesses of the adaptive clones isolated from the haploid *a* population (see Fig. 2)

Strain 1		Strain 2		Fitness of strain 2 relative to a fitness of 1 for strain 1 ±s.e.m. per generation
Isolated at generation	Putative no. of adaptive mutations	Isolated at generation	Putative no. of adaptive mutations	
0	0	0*	0	0.99 ± 0.01
0*	0	30	0	1.02 ± 0.01
0.5	0	48*	0	1.02 ± 0.01
25*	0	97	1	1.10 ± 0.01
30	0	97*	1	1.11 ± 0.01
97*	1	133	2	1.05 ± 0.01
133*	2	203	3	1.16 ± 0.02
203*	3	245	4	1.10 ± 0.01

For each experiment the strain which had been isolated after the smaller number of generations of growth in the chemostat is designated as strain 1. * Indicates that the strain was marked with canavanine resistance by plating ~1×10^8 cells on to minimal medium + 2 mM canavanine sulphate and picking the spontaneous mutant colonies that appeared. At least five colonies of each of the two strains to be compared, were picked, pooled and inoculated into 50–75 ml of minimal medium and grown overnight at 30 °C with shaking in a water bath. The cells were then inoculated into a chemostat in approximately equal frequency at approximately the cell densities observed in long-term experiments' 5×10^7 cells ml^{-1}. Medium flow commenced immediately after inoculation. Samples were taken every 12–24 h for 3–5 days. The samples were sonicated as described in Fig. 2 legend, plated onto minimal medium after appropriate dilution, incubated at 30 °C for 3–5 days and then replica-plated onto selective media to monitor changes in the frequency of the resistance marker. Approximately 500 colonies were counted for each sample. Fitnesses (±s.e.m.) were calculated as described previously[52], relative to a fitness value of unity for strain 1.

Detection of adaptive changes

It is of course necessary that all adaptive mutations be detected to obtain unbiased estimates of the rate of adaptation in haploid and diploid yeast populations. In a sexually reproducing population this would be unreasonably difficult as it would require (1) *a priori* knowledge of the phenotypes of all possible adaptive mutations and (2) that all adaptive mutations be unambiguously detectable. However, in an asexually reproducing population the spread of a selectively favoured gene throughout the population may be detected without a knowledge of the phenotype of the adaptive mutation by monitoring the frequency of an unrelated neutral mutation. In such populations recombination is virtually absent so all genes may be considered to be completely linked. In a large population (that is, 10^8–10^{10} organisms) in which the initial frequency of the neutral mutation is very close to zero, it will increase in frequency at the mutation rate such that after t generations, the frequency will be about μt (ignoring reverse mutation), where μ is the mutation rate. If at some point an adaptive mutation occurs in the population, the new adaptive clone will increase in frequency and both the old clone and the neutral mutants it has accumulated will be selected against. The probability that the adaptive mutation will occur in a cell carrying the neutral mutation will equal the frequency of the neutral mutant, $\sim\mu t$, which for small t, will be extremely small, of the order of 1×10^{-5} to 1×10^{-7}. As the

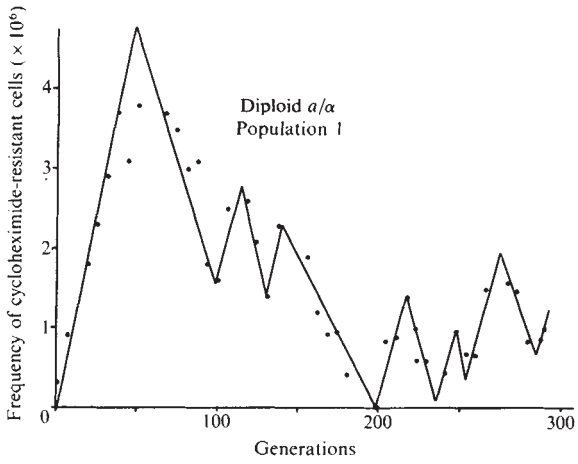

Fig. 3 Fluctuation in the frequency of cycloheximide resistance in one population of the diploid a/α strain, CP1AB-1AB[28]. The line illustrates fluctuations in the frequency of cycloheximide resistance expected assuming the occurrence and fixation of six adaptive mutations. The experiment was carried out using the procedures described in Fig. 2 legend with the following modifications. The frequency of dominant cycloheximide resistance, determined by a single locus (*CHY4*)[55,57], was determined by plating cells onto minimal medium + 1.8 μM cycloheximide. Dominant cycloheximide resistance is selected against in our experimental conditions (selective coefficient ≈ 0.10). The genotype of the cells at the end of the experiment was determined by sporulating the cells and assaying the spores as described in Fig. 2 legend.

new adaptive clone has existed for fewer generations than the old clone it will have accumulated fewer neutral mutants and consequently there will be a net decrease in the frequency of neutral mutants. As cells from the old clone become rare the frequency of neutral mutants will again rise as the new clone accumulates mutants at the mutation rate, until the next adaptive mutation appears and the cycle repeats. A diagram of this cycle is shown in Fig. 1. Slightly deleterious mutations may also be used to detect adaptive changes in populations. They will show the same cyclic fluctuations in frequency except that the rate of increase of a slightly deleterious mutant will be less than the mutation rate by an amount proportional to the selective coefficient. In general this will not compromise their utility in detecting adaptive change in populations, provided the selective coefficients are small[24]. These fluctuations are known in the microbiological literature as periodic selection[25]. If there is tight linkage between the adaptive mutation and the neutral mutation monitored, similar correlated responses in gene frequency change may also occur in sexual populations, and in such cases the phenomenon has been termed 'hitchhiking'[26]. Thus, by following the frequency of neutral or slightly deleterious mutants over time in an asexual population, the number and frequency of adaptive changes may be determined without a knowledge of the phenotype of the adaptive mutant itself. Furthermore, the possibility exists of comparing the rate of fixation of two populations using this approach[27].

Analysis of fluctuations in mutant frequencies

In the early experiments, fluctuations in the frequencies of three different mutant phenotypes were monitored in asexual haploid and diploid populations of *S. cerevisiae* grown in glucose-limited chemostats. The frequency of canavanine resistance, cycloheximide resistance and 5-fluorouracil resistance was monitored in the haploid populations and cycloheximide and 5-fluorouracil resistance in the diploid populations. Canavanine resistance is recessive and so could not be monitored in the diploid populations. All markers monitored in these populations showed the same pattern of fluctuations, confirming that the fluctuations observed were not unique properties of the particular resistance marker. Therefore, in further experiments only canavanine resistance was monitored in the haploid populations, and cycloheximide resistance alone was monitored in the diploid populations as these two markers proved to be the easiest to score.

Figures 2 and 3 show the change in the frequency of canavanine- and cycloheximide-resistant mutants versus generation time in two representative populations, a haploid a population and a diploid a/α population, respectively. These two representative populations were analysed in detail to verify that the fluctuations in canavanine resistance (haploid population) and cycloheximide resistance (diploid population) were caused by the fixation of adaptive mutations in the populations.

The putative adaptive changes were reconstructed by isolating cells at different generations, and placing them in direct competition with each other in the same glucose-limited chemostat environment, one of the strains being marked with canavanine resistance in the case of the haploids and 5-fluorouracil resistance in the case of the diploids. The results (Tables 1, 2) are presented in the form of relative fitnesses—the convention in population genetics. Two sets of controls were performed for both haploids and diploids. In the first set (row 1 of Tables 1 and 2), the selective effect of the drug resistance marker was measured in the strains CP1AB-1A (haploid) and CP1AB (diploid), which had not been grown previously in glucose-limited chemostats. The results show that canavanine resistance is selectively neutral in these conditions, but that the 5-fluorouracil marker is selected against. Accordingly, most of the reconstruction experiments with diploids were carried out in duplicate, marking strain 1 with 5-fluorouracil resistance in one experiment, and strain 2 with 5-fluorouracil resistance in the other. Relative fitnesses were then calculated correcting for the selective effect of 5-fluorouracil.

The second set of controls (rows 2 and 3 of Tables 1 and 2) compared cells isolated at different generations, but which were still considered to be members of the same clone, as determined by the dynamics of the drug resistance marker frequency. Thus, in Table 1, cells isolated at the beginning of the experiment, at generation 0.5, were grown in competition with cells isolated at generation 48. The constant increase in the frequency of canavanine resistance until approximately generation 60 indicates that no adaptive change in the population should have occurred during that time. The results shown in Table 1 confirm the selective equivalence of cells isolated at these two times. A similar set of results (Table 2) was obtained for diploids.

Tables 1 and 2 also show the results of competition experiments between cells isolated before and after each adaptive shift. In every case fluctuations in the frequency of canavanine or cycloheximide resistance signalled the fixation of an adaptive mutation. In the case of the diploid population the reciprocal experiments showed no evidence of epistatic interactions between the gene for 5-fluorouracil resistance and the adaptive mutations. Surprisingly, the average fitness increment between the adaptive clones for the haploid population (0.10) was not significantly different from that (0.09) for the diploid population, suggesting that the adaptive mutations fixed in the haploid and diploid populations are equally advantageous.

It is also possible to calculate the selective coefficients directly from the sequential rates of decrease of the frequency of the neutral mutants in the long-term continuous culture experiments shown in Figs 2 and 3. The reliability of these estimates is questionable as they are based on few data, and also assume incorrectly that all mutants (canavanine- or cycloheximide-resistant) are derived from the strain not possessing the most recent adaptive mutation. Nevertheless these estimates (not shown) agree closely with the estimates from the reconstruction experiments.

Finally, to verify that the sequential increases in the frequency of the neutral mutants were consistent with the rates of mutation to canavanine and cycloheximide resistance respectively, these mutation rates were estimated independently by fluctuation tests (Table 3). These rates show an excellent correspondence

Table 2 Relative fitnesses of the adaptive clones isolated from the diploid a/α population (see Fig. 3)

Strain 1		Strain 2			
Isolated at generation	Putative no. of adaptive mutations	Isolated at generation	Putative no. of adaptive mutations	Fitness of strain 2 relative to value of 1 for strain 1 (±s.e.m.) per generation	Relative fitness of strain 2 corrected for selection of fluorouracil resistance marker
0*	0	0	0	1.10±0.01	–
0.5*	0	32	0	1.10±0.01	1.00
0.5	0	44*	0	0.90±0.01	1.01
26*	0	107	1	1.41±0.03	1.25
26	0	107*	1	1.06±0.02	1.16
101*	1	139	2	1.18±0.04	1.06
101	1	139*	2	0.95±0.06	1.04
139	2	213*	3	1.01±0.03	1.10
213*	3	243	4	1.20±0.02	1.09
213	3	243*	4	1.06±0.03	1.16
243	4	268*	5	0.96±0.03	1.05
262*	5	305	6	1.13±0.04	1.03

Experiments were carried out as described in Table 1 legend with the following modifications. * Indicates that in each experiment, the strain was marked with 5-fluorouracil resistance. The selective medium was minimal medium +0.2 mM 5-fluorouracil. This marker proved easier to score than cycloheximide resistance on the replica plates. Cells were inoculated at a cell density of 3×10^7 cells ml^{-1}, the approximate equilibrium cell density for diploids. Relative fitnesses (±s.e.m.) were calculated as before (see Fig. 2 legend). Fitnesses of strain 2 were corrected for selection against the 5-fluorouracil resistance marker by assuming selective effects to be additive before normalizing the fitnesses relative to a fitness value of unity for strain 1.

with those estimated from the continuous culture experiments. In total, the independent estimates of the selective coefficients and mutation rates provide overwhelming support for the hypothesis that the fluctuations in the frequency of the resistant mutants, are a manifestation of the sequential occurrence and fixation of adaptive mutations.

Comparison of haploid and diploid populations

Rates of adaptation were measured for two haploid (a, α) and two diploid (a/α and a/a) strains which were isogeneic[28] except for the mating type locus; 64 putative adaptive mutations were observed in 2,612 generations of 11 different populations. The results are summarized in Fig. 4. The average frequency of adaptive mutations fixed was 5.68×10^{-12} per cell generation for all diploids, whereas the average number of adaptive mutations fixed per cell generation for all haploid populations was 3.55×10^{-12}. The Wilcoxon Mann–Whitney non-parametric test (for example, see ref. 29) showed a highly significant difference ($P=0.0002$) between the haploid and diploid populations. To test for an effect of the mating type locus, the same test was applied to the two sets (a and α) of haploid populations and the two sets (a/a and a/α) of diploid populations. As expected there was no significant difference in either case. Thus, the data show that diploids fix ~1.6 times as many adaptive mutations per cell as haploids. Furthermore, it can be seen that the rate of fixation of adaptive mutations does not decrease with time, as might be expected.

Frequency and nature of adaptive change

Fluctuations in the frequency of neutral mutants, signalling repeated adaptive genetic changes in *Escherichia coli*, were reported as early as 1950[25,30]. Our results demonstrate for the first time that similar changes may occur in populations of the single-celled eukaryote *S. cerevisiae*, and show that the rate of adaptive change in different populations may be compared. Two aspects of these results are noteworthy.

(1) The genetic changes in both haploid and diploid populations occur surprisingly frequently, higher than has been reported previously. The only study that allows a direct comparison of the rate of adaptive change is that of Novick and Szilard[30,31] concerning adaptation to a tryptophan-limited environment. The estimated frequency of fixation of adaptive mutations observed by these authors was $1.9–2.3\times10^{-12}$, values significantly lower than those observed here. Clearly this difference may be explained by the different genetic and physiological characteristics of the two species but may also be explained by the difference in environmental conditions used. Novick and Szilard identified genetic changes occurring during adaptation to a tryptophan-limited environment where the number of genetic events leading to increased adaptation may be limited.

The identification of rapid adaptive genetic changes reported here has important implications for industrial applications of *S. cerevisiae* and related yeasts. Although some evidence exists for the occurrence of significant genetic changes during long-term continuous fermentation of the brewery yeast *Saccharomyces carlsbergenesis*[32,33], no information has previously been available on their frequency. Genetic changes in the frequency reported here may seriously attenuate the economic properties of the organism. This problem may be particularly acute in industrial uses of *S. cerevisiae* as a host for artificial chimaeric plasmids (for example, see ref. 34), which themselves may be subject to frequent genetic changes[35] and high rates of segregational loss[36].

(2) Not only are the rates of adaptive change high in comparison with previous studies, but these rates of fixation of adaptive mutations did not decrease with the number of generations spent in the chemostat. Thus there is no evidence for the exhaustion of capacity for adaptive genetic change during the time period over which the populations were observed (up to 327 generations). The most obvious conclusion is that the number of loci and/or alleles for adaptive genetic change is extremely large. However, it is also possible that the epistatic or functional relationships between the adaptive mutations is sufficiently complex that adaptive mutations may cycle through the population over long time periods[31,37]. This 'circular evolution' may lead to an infinitely repeating succession of adaptive changes with a limited repertoire of adaptive mutations. We present elsewhere[38] evidence from estimates of selective differences between clones differing in more than one adaptive mutation which suggests that such a phenomenon may be occurring in our populations. It has also been suggested to occur in evolving populations of RNA viruses[39].

Implications for the evolution of diploidy

The fundamental result obtained from these experiments is that the rate of adaptive change in diploid populations of

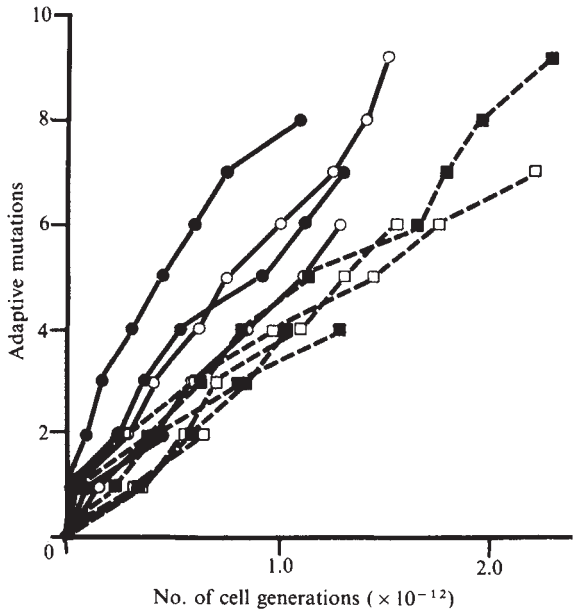

Fig. 4 Number of adaptive mutations fixed versus the number of cell generations elapsed for the 11 populations studied. Isogeneic strains used to inoculate the continuous cultures were: CP1AB-1A haploid, *a* (■); CP1AB-1B haploid, α (□); CP1AB-1AA diploid, *a*/*a* (●); CP1AB diploid, *a*/α (○). The isolation and complete genotypes of these strains have been described previously[28]. The mutation rates estimated for haploid *a* population 1 and diploid *a*/α population 1 are consistent with the data from the other nine populations and were used as the rate of increase in the resistant mutants in analysing the data from these populations. The number of adaptive mutations fixed in a population was determined by counting the number of decreases observed in the frequency of the cycloheximide- and canavanine-resistant mutants. Decreases in resistant mutant frequency were counted only when more than one sample showed a decrease and when it was consistent with our estimates of the mutation rates for such a decrease to have occurred. The time of occurrence of each adaptive mutation was assumed to be the time at which the first decrease in the frequency of the resistant mutant was observed. Although the actual time of occurrence of the adaptive mutations will be slightly earlier (see Fig. 1), this will not affect the estimates of the rates of adaptation because they depend on the elapsed time between adaptive mutations rather than their exact time of occurrence.

S. cerevisiae is 1.6 times higher than the rate in isogeneic haploid populations. To our knowledge, this represents the first direct experimental demonstration of an adaptive advantage for the diploid phase of the life cycle of a eukaryote. The deviation of this value from the theoretically expected value of two, which assumes that all adaptive mutations are dominant, suggests that a small proportion are not expressed in the heterozygous state. However, the equality of the fitness increments between the adaptive clones in the haploid and diploid populations suggests that, of those adaptive mutations fixed in the diploid population, all exhibit complete dominance. This assumes, of course, that the spectra of adaptive mutations fixed in the two populations are the same. Clearly it would be of interest to compare the characteristics of the adaptive mutations and to measure directly their level of dominance. However it must be remembered that our approach for the detection of adaptive change does not allow the identification of the loci of the adaptive mutations. Without this information it is impractical to separate the combinations of adaptive mutations necessary for the direct measurement of dominance and the determination of the phenotypes of the individual mutations.

A higher rate of adaptive mutation for diploid cells should be common to all eukaryotes having an asexual diploid stage in the life cycle. Thus, it is tempting to suggest this as a general

Table 3 Mutation rate estimates

Mutant	Mating type	Estimate from continuous culture experiment	Estimate from fluctuation test	Published estimates
Canavanine resistance	*a*	1.06×10^{-7}	$9.7\pm5.0\times10^{-8}$	1.5×10^{-7} *
Cycloheximide resistance	*a*/α	8.66×10^{-8}	$2.0\pm1.5\times10^{-7}$	

The fluctuation test was carried out as described previously[53]. Cells were grown in minimal medium and viable counts used to determine the total cell number. To estimate the mutation rate to cycloheximide resistance, cells were grown to a density of 5×10^5 cells ml^{-1} to minimize background growth which commonly occurs at higher cell densities. More cultures (90 instead of 15) were grown and larger volumes (1 ml instead of 0.2 ml) were plated using a soft agar plating technique to offset the smaller number of cells per culture. Mutation rates and 95% confidence intervals for these rates were calculated using either the P_0 or median method of Lea and Coulson[54]. Mutation rate estimates from the continuous culture experiments were the least-squares estimates of the slope of the initial increase in frequency of canavanine- or cycloheximide-resistant mutants versus the generation time. * Ref. 59.

mechanism for the evolution of diploidy. Although the rate of mutation may not be the limiting factor determining macroevolutionary change[2], this parameter may be critical in determining short-term adaptation to new environments for asexual organisms which cannot rely on recombination to generate variation in fitness. A similar argument has been used to explain the predominance of RNA viruses compared to DNA viruses[39], and at the microevolutionary level, to explain the selective advantage of the mutator alleles at the *mutT* and *mutH* loci in laboratory continuous cultures of *E. coli*[40–43]. In addition, on a theoretical level several workers[44–46] have argued that in asexual populations selection may favour genotypes which increase mutation rate.

Although we consider a higher rate of adaptive change in diploids to be an important mechanism in the evolution of diploidy in many organisms, it is unlikely to explain satisfactorily the predominance of the diploid phase in all organisms. For organisms having no asexual diploid stage in the life cycle, such an effect may explain the maintenance of a predominant diploid phase, but not its evolutionary origin. In addition, Darlington[47] has suggested that a predominant diploid phase evolved independently at least six times, and several independent origins have been proposed for the ascomycetes alone[48]. Indeed, it would be surprising if the mechanism of evolution of diploidy were the same in all cases. Clearly there is a link between multicellularity and a predominant diploid phase. Lewis and Wolpert[49] have suggested that this link may be causal and that diploids are more able to evolve the complex systems of gene regulation that are characteristic of multicellular organisms having complex somas. Protection from the deleterious effects of somatic mutation may also be more important in such organisms.

A further consideration is that the rate of fixation of adaptive mutations will also depend on population size. A higher frequency of adaptive mutation in diploids may be offset by the increased cost of producing a diploid cell. In nutrient-limited environments this effect may be significant. In our experiments the average cell density for diploids was 3.4×10^7 cells ml^{-1} during the early generations, whereas for haploids in the same environment the average cell density was 4.7×10^7 cells ml^{-1}. Thus the haploid population was larger by a factor of 1.4 and this increase in size may largely compensate for the reduced variation in fitness produced by haploids. In this respect it is interesting to note that marine yeasts, most of which are haploid, also live in a nutrient-limited environment[50]. On the other hand it is commonly agreed that in laboratory cultures of *S. cerevisiae* on rich media containing both haploid and diploid cells, the diploid cells will eventually outgrow the haploid cells[48,51] despite the fact that no physiological growth rate advantage for diploid cells has been detected[6]. Thus one factor which may determine

the evolution of diploidy is the type of environment in which the organism lives.

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Transmission of conformational change in insulin

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Crystal structures of insulin contain molecules that are similar but not identical in conformation. Packed helices move relative to each other, these shifts being accommodated by motions of side-chain atoms arising from small changes in torsion angles. Such low-energy conformational adjustments can accommodate shifts of no more than ~1.5 Å. This limits the extent to which conformational changes can be dissipated locally, causing their transmission over long distances.

MANY properties of proteins, including the regulation of their activity, depend on transmission of conformational changes between distant sites. Little is known about the detailed mechanisms of these processes, as few protein structures are known to sufficiently high accuracy in more than one conformational state. Highly refined structures of several forms of insulin[1–3] reveal a similar fold of the chains but extensive differences in conformational details. Here we extend the previous comparisons of these structures[4–6] to elucidate the mechanism of transmission of conformational changes in insulin.

We find that the backbones of helices tend to move as rigid bodies during conformational changes. Small relative displacements of packed helices (up to ~1.5 Å) are readily accommodated by small adjustments in torsional angles (up to ~15°) without the repacking of interfaces. This may be thought of as the limit of plastic deformation of helix interfaces. Larger displacements of packed helices are less easily accommodated, and occur very rarely. We suggest that it is the limited extent to which internal motions can be dissipated locally that is responsible for the transmission of conformational change over long distances.

Although individual proteins have individual mechanisms of function, these ideas should apply generally to conformational changes in proteins, including allosteric transitions.

Structure of pig insulin

The insulin monomer contains two chains, A and B, containing 21 and 30 residues, respectively, and linked by two disulphide bridges, A7–B7 and A20–B19. A third disulphide bridge lies within the A chain at A6–A11. Two monomers form a dimer, held together by hydrogen bonding between two strands of antiparallel β-sheet, and by van der Waals' contacts.

In the presence of Zn^{2+} the dimers assemble into hexamers. Their conformation is solvent-dependent[7,8]—crystallization at low ionic strength yields the 2Zn form, with two Zn^{2+} ions bound per hexamer; crystallization at high ionic strength yields the 4Zn form, which can bind four Zn^{2+} ions per hexamer. Both crystal forms are rhombohedral, with the crystallographic 3-fold axis relating the three dimers in the hexamer (Fig. 1*a*). The asymmetric unit of each crystal contains two monomers

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