consistent with an Acheulian industry. Artefacts were made from two local varieties of lava and apparently exotic quartz, quartzite and chert.

Lainyamok can be compared with Olorgesailie, the middle Pleistocene site north-east of Lake Magadi, as both sites sample broadly similar lake margin or lake flat habitats[15]. However, Olorgesailie and Lainyamok differ in that: (1) Olorgesailie is rich in giant geladas (*Theropithecus oswaldi*) but lacks *Papio*, the only monkey at Lainyamok; (2) only four carnivore specimens are known from Olorgesailie (C. Koch, personal communication), whereas 21 specimens of at least four carnivore genera are known from the much smaller Lainyamok collection; (3) large hand-axes and cutting or heavy-duty tools typify the Olorgesailie assemblage[15] whereas the known Lainyamok artefacts are small or light-duty implements.

It is not clear if the faunal contrasts are linked to the archaeological differences. Olorgesailie was apparently repeatedly occupied by many hominids engaged in specific, highly organized activities (for example, extensive tool manufacture[15] or systematic butchery of large animals[16]). Lainyamok's paucity of artefacts, single animal scatters and sole artefact cluster suggest it was transiently occupied by only a few hominids. A working hypothesis is that Lainyamok preserves small, short-term occupations where more opportunistic killing and butchering of carcasses of smaller species occurred. Such small-scale sites are common in the ethnographic record[17,18], but are not known from the Pleistocene.

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Relative fitness can decrease in evolving asexual populations of *S. cerevisiae*

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It is generally accepted from the darwinian theory of evolution that a progressive increase in population adaptation will occur in populations containing genetic variation in fitness, until a stable equilibrium is reached and/or the additive genetic variation is exhausted. However, the theoretical literature of population genetics documents exceptions where mean population fitness may decrease in response to evolutionary changes in gene frequency, due to varying selective coefficients[1–4], sexual selection[5,6] or to epistatic interactions between loci[7]. Until now, no examples of such exceptions have been documented from fitness estimates in either natural or experimental populations. We present here direct evidence that, as a result of epistatic interactions between adaptive mutations, mean population fitness can decrease in asexual evolving populations of the yeast *Saccharomyces cerevisiae*.

Populations of haploid and diploid *S. cerevisiae* were grown in glucose-limited continuous cultures (chemostats) for up to 300 generations. Population sizes were large—approximately 4.9×10^9 for the haploid population and 4.5×10^9 for the diploid population. In these conditions reproduction is exclusively asexual by budding, and evolutionary changes occur by the replacement of one adaptive clone by another. As recombination is virtually absent in these populations, two or more adaptive mutations must be incorporated into the population in a strict sequential fashion.

Adaptive changes were identified by monitoring the frequencies of independent neutral or weakly selected mutations in the populations. Being asexual, these evolving populations may be regarded as consisting of a series of clones over time, each new clone having a selective advantage over the immediately preceding clone. In each clone the initial frequencies of the independent neutral or weakly selected mutations will be zero, and their frequencies will increase over time at the mutation rate or slightly less, depending on the intensity of selection against the independent marker. The frequencies of the independent mutations will necessarily be lower in the newly emergent adaptive clone than in the preexisting clone, as the former has had less time to accumulate mutations. Therefore, during an adaptive change the population frequencies of the independent mutations will exhibit transient decreases. Repeated replacements of adaptive clones will result in repeated fluctuations in the frequency of the independent markers. These fluctuations, known as periodic selection in the microbiological literature, allow the unambiguous detection of all adaptive changes without prior knowledge of the phenotype of the adaptive mutation itself[8].

The frequencies of mutants resistant to canavine, cycloheximide and 5-fluorouracil were monitored in the haploid population, and mutants resistant to cycloheximide and 5-fluorouracil were monitored in the diploid population. (Canavanine resistance is recessive and so could not be monitored in the diploid population.) Samples were taken every three to six generations (12–24 h) and aliquots of the cell suspension were stored at −70 °C in 15% glycerol for future analysis. All markers monitored showed the same pattern of fluctuations in each population. From these data, 4 adaptive changes were identified in 245 generations in the haploid population and 6 adaptive changes were identified in 305 generations in the diploid population. The dynamics of the fluctuations of canavanine resistance for the haploid population and cycloheximide resistance for the diploid population are shown in Figs 2 and 3 of ref. 8.

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To determine the epistatic interactions between the adaptive mutations, cells isolated after successive adaptive shifts, containing different numbers of adaptive mutations, were placed in pairwise competition in the same glucose-limited chemostat environment. The fitness of each adaptive clone was estimated in this way relative to each other adaptive clone. For each experiment one of the two strains was marked with an appropriate resistance marker (canavanine for diploids and 5-fluorouracil for haploids) to allow the two strains to be distinguished. Competition experiments between strains carrying canavanine or 5-fluorouracil resistance and the same strains without the marker, showed canavanine resistance to be neutral and 5-fluorouracil to be selected against[8] (selective coefficient = 0.09). Therefore, all experiments using the 5-fluorouracil resistance marker were corrected for selection against this marker. To correct for possible interactions between the adaptive mutations and canavanine or 5-fluorouracil resistance, many of the competition experiments were performed twice, with one strain marked with the resistance marker in the first experiment and the other strain marked in the second experiment. No interactions between the resistance markers and the adaptive mutations were observed.

The results for the haploid and diploid populations are shown in Tables 1 and 2. It is important to realize that, since the populations are asexual, each adaptive mutation becomes part of the genetic background in which further adaptive mutations are selected. Therefore, a strain carrying a new adaptive mutation also carries all the adaptive mutations previously fixed in the population. Tables 1*a* and 2*a* show the results of competition experiments between clones differing in only one adaptive mutation. These experiments therefore reconstruct each putative adaptive shift. As expected, the relative fitnesses of the later isolated clones were always higher than those for the clones isolated immediately preceding the adaptive shift. Thus, these results confirm the occurrence of the adaptive shifts in the population signalled by the fluctuations in the frequency of canavanine resistance (haploids) or cycloheximide resistance (diploids).

Tables 1*b* and 2*b* show the results of competition experiments between clones differing by more than one adaptive mutation. These data clearly reveal significant epistasis between the adaptive mutations. In all cases the fitness differentials between adaptive clones differing in more than one adaptive mutation

Table 1 Relative fitnesses of the adaptive clones isolated from the haploid *a* population

	Strain 1		Strain 2		Fitness of strain 2 relative to 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	
a	25*	0	97	1	1.10 ± 0.01
	30	0	97*	1	1.11 ± 0.01
	97*	1	120	2	1.05 ± 0.01
	133*	2	203	3	1.16 ± 0.02
	203*	3	245	4	1.10 ± 0.02
b	30*	0	133	2	1.08 ± 0.01
	30	0	133*	2	1.18 ± 0.03
	30	0	203*	3	0.85 ± 0.03
	30	0	203*	3	0.84 ± 0.04
	30	0	245*	4	1.16 ± 0.04
	30*	0	245	4	1.12 ± 0.01
	97*	1	203	3	1.08 ± 0.01
	102*	1	245	4	1.08 ± 0.01
	133*	2	245	4	1.09 ± 0.02

The adaptive clones were isolated from long-term glucose-limited continuous cultures inoculated with the haploid *a* strain CP1AB-1A[22]. Experiments were carried out as described previously[8]. 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.

* Strain marked with canavanine resistance. Canavanine resistance has been shown to be neutral in our conditions[8]. Fitnesses (±s.e.m.) were calculated as described previously[23] relative to a fitness value of unity for strain 1.

Table 2 Relative fitnesses of the adaptive clones isolated from the diploid a/α population

	Strain 1		Strain 2		Fitness of strain 2 relative to strain 1 ± s.e.m. per generation	Relative fitness of strain 2 corrected for selection of fluorouracil resistance marker
	Isolated at generation	Putative no. of adaptive mutations	Isolated at generation	Putative no. of adaptive mutations		
a	26*	0	101	1	1.41 ± 0.03	1.25
	26	0	101*	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	294	6	1.14 ± 0.04	1.03
b	26	0	139*	2	0.99 ± 0.01	1.08
	26*	0	213	3	1.28 ± 0.02	1.15
	26	0	243*	4	0.88 ± 0.02	0.97
	20	0	262*	5	0.98 ± 0.08	1.07
	26*	0	301	6	0.84 ± 0.05	0.75
	26	0	305*	6	0.71 ± 0.06	0.80
	101*	1	262	5	1.47 ± 0.10	1.30
	101	1	262*	5	1.04 ± 0.05	1.14
	101	1	305*	6	0.95 ± 0.04	1.04
	139*	2	243	4	1.06 ± 0.10	0.97
	139	2	262*	5	0.81 ± 0.03	0.90
	213*	3	268	5	1.16 ± 0.02	1.05
	213	3	268*	5	0.94 ± 0.01	1.03

The adaptive clones were isolated from long-term glucose-limited continuous cultures inoculated with the diploid a/α strain CP1AB[22]. Experiments were carried out as described previously[8]. 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.

* Strain marked with 5-fluorouracil resistance. 5-Fluorouracil is selected against in our conditions (selective coefficient = 0.09). Relative fitnesses (±s.e.m.) were calculated as before[23]. 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.

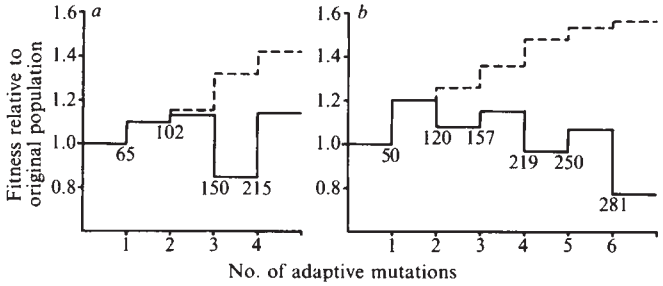

Fig. 1 Change in population fitness relative to a fitness value of unity for the original population of the haploid *a* strain CP1AB-1A (*a*) and of the diploid *a*/*α* strain CP1AB-1AB (*b*). ----, Expected rate of increase of population fitness due to substitution of four (*a*) or six (*b*) adaptive mutations, assuming fitnesses are additive. ——, Observed rate of change of population fitness due to substitution of four (*a*) or six (*b*) adaptive mutations. Standard errors are given in Tables 1 and 2. Fitness values for each adaptive clone are estimated from reconstruction experiments with the original clone used to initiate the experiment. The numbers shown against each change in population fitness are the times of occurrence (generations) for each adaptive mutation, estimated as described previously[8].

are smaller than those expected from the sum of the fitness differentials between clones differing in only one adaptive mutation. For example, in Table 1 the fitness differential between the first adaptive clone carrying 1 adaptive mutation and the clone isolated at generation 245 carrying 4 adaptive mutations is only 0.08. The predicted differential assuming no epistasis, calculated from Table 1*a*, is $0.05+0.16+0.10=0.31$. Of particular interest, however, is the nature of certain of the epistatic interactions. In one case in the haploid population, and in several cases in the diploid population, the relative fitnesses of adaptive clones carrying multiple adaptive mutations were lower than the fitnesses of clones isolated earlier in the experiment, even though each clone carrying a new adaptive mutation has an increased fitness relative to the immediately preceding clone. Thus, in both the haploid and diploid populations, the fixation of several adaptive mutations can actually decrease the fitness of a strain relative to the fitness of the clones at the beginning of the experiment. To emphasize this point, Fig. 1 shows the changes in relative fitness of the haploid (Fig. 1*a*) and diploid (Fig. 1*b*) populations with respect to the fitness of the original strain as each adaptive mutation becomes fixed in the population. The dashed line in the figure indicates the expected rate of increase of population fitness assuming the adaptive mutations are additive. The expected rate of increase of population fitness, assuming fitnesses are mutiplicative, is slightly higher.

This apparent paradox is resolved when it is considered that fitness is a relative measure and is defined by the numbers of descendants left by a given genotype relative to others in the population[9]. The mean fitness of a genotype may depend on many variables each of which may affect the intrinsic rate of natural increase of the population (*r*-selection) and/or the ability of a genotype to alter the numbers of offspring left by its neighbours (*K*-selection)[4,9]. It is clear that in our experimental system the variable responsible for each adaptive shift may be different. In this context it is not unreasonable that a combination of adaptive mutants may result in a maladaptive clone when compared in competition with a clone isolated several steps earlier. If the adaptive shifts only resulted in a change in the intrinsic rate of natural increase, such decreases in relative fitness should not occur. However, growth in chemostats is always resource-limited and the rate of growth of the population is fixed by the dilution rate[10]. Therefore, it is unlikely that the majority of adaptive clones involve change in the intrinsic rate of natural increase.

The suggestion that extensive interactions occur between loci affecting fitness is not new. Such interactions have been observed for loci determining drug resistance in both *Escherichia coli*[11] and the bacteriophage T_4 (ref. 12). Furthermore, ample indirect evidence exists from observations of multilocus gametic associations in sexual populations[13], from the well-known and widespread lack of concordance between predicted and observed responses to selection for components of fitness in animal and plant breeding, and from the results of artificial selection experiments[14,15]. However, the present results present the first direct evidence for and measurements of epistatic interactions between multiple adaptive mutations in evolving populations. Furthermore, the results represent the first observation that population fitness may decrease in response to evolutionary changes. Such a phenomenon has been postulated theoretically by several workers[1–4,6,7].

Our capacity to observe such decreases is clearly dependent on the asexual nature of our populations, in which adaptive mutations occur and are fixed in the population strictly sequentially. In such populations only certain combinations of adaptive mutations will necessarily occur. In sexual populations, recombination potentially will allow the generation of all combinations of adaptive mutations and also will allow the incorporation of mutations in parallel. This may be sufficient to obscure any significant fitness decreases. However, the importance of recombination in sexual populations will be minimized if population sizes are small[16] and if adaptive changes occur sufficiently rapidly that each adaptive mutation is fixed before the occurrence of the next[17]. The results described here and previously[8] suggest that such rates of evolutionary change are not unreasonable. Therefore, sexual populations may also exhibit fitness decreases during evolutionary change.

The rates of occurrence of the adaptive mutations in these and other similar populations[8] illustrate a further unexpected characteristic of these changes. Figure 1 shows the estimated times of occurrence of each adaptive mutation. The rate of occurrence of adaptive mutations being fixed in the populations did not decrease over time as would be expected if the possible range of adaptive changes for this environment were becoming exhausted. This result may not be so surprising given that the relative fitnesses of the populations are not constantly increasing. Mutations selected for during one period of adaptation may be selected against during subsequent periods of adaptation in a different genetic background. It is possible that certain categories of adaptive mutations are continually being fixed and then lost from the population as their fitnesses become modulated by the genetic background. Thus, for these populations evolution may be 'circular', an infinitely repeating succession of adaptive changes involving a limited repertoire of adaptive mutations[18,19]. Such a phenomenon has previously been suggested to occur in evolving populations of RNA viruses[20]. The concept that there is no 'adaptive peak'[21] corresponding to a single genotype or genetic composition of the population has profound implications for our understanding of the maintenance of genetic variation under directional selection.

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Single neurones can initiate synchronized population discharge in the hippocampus

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The synchronized firing of neuronal populations is frequently observed in the mammalian central nervous system. The generation of motor activities such as locomotion[1] and respiration[2] requires the simultaneous activation of many neurones and synchronous firing also underlies the cortical α rhythm[3] and the hippocampal θ rhythm[4]. However the influence that single neurones may have on such neuronal population discharges is not clear. We have examined this question using small isolated segments of the CA3 region of the guinea pig hippocampus. We report here that in the presence of picrotoxin, a γ-aminobutyric acid (GABA) antagonist, these segments spontaneously generate synchronized rhythmic bursts comparable with the interictal epileptiform discharges observed in the hippocampus and neocortex in the presence of penicillin[5–7]. The activation of some individual neurones by intracellular current injection can partially entrain and reset the rhythm. The probability that a synchronized burst will follow stimulation of a single cell increases with time after a spontaneous synchronized discharge, suggesting that each population discharge is followed by a period of relative population refractoriness. A delay of 40–200 ms elapses between the activation of a single neurone and the synchronized discharge. We suggest that during this time activity elicited in one neurone spreads to other neurones through multisynaptic excitatory pathways and leads eventually to the participation of the whole population in a synchronous burst.

Transverse slices (400 μm thick) of guinea pig hippocampus were prepared with a vibratome (Oxford). Two further cuts made orthogonally to the cell-body layer in the CA3 field and meeting at the hippocampal fissure resulted in a segment of neural tissue measuring 400–600 μm along the stratum pyramidale. Consideration of soma diameter (20–40 μm) and packing density (two to three cells deep) in the CA3 stratum pyramidale[8] suggests that segments contained approximately 1,000 pyramidal cells. Segments were transferred to a recording chamber and maintained as described previously[9]. Intracellular and extracellular field potential recordings were made from the stratum pyramidale with 2 M K-acetate-filled microelectrodes of resistances 80–120 MΩ and 40–60 MΩ respectively. Synchronized discharge was induced by exposure to picrotoxin (10^{-4} M) which blocked spontaneous and evoked inhibitory synaptic potentials.

The active and passive properties of more than 70 neurones recorded in this study were similar to those described for pyramidal cells from the same area of the intact hippocampal slice[10,11]. In the presence of picrotoxin, spontaneous synchronized bursts of period 7.3 ± 2.9 s (mean ± s.d.) were observed in 27 CA3 segments measuring 400–600 μm along the stratum pyramidale whereas such activity was never seen in smaller segments. Rhythmic population bursts, when all neurones discharged synchronously with the field potential burst, were blocked by low-Ca^{2+} (0.5 mM) high-Mg^{2+} (8 mM) solution in six segments. This behaviour therefore seems to require a minimum neuronal substrate for its expression and depends on local synaptic mechanisms within the CA3 region. With the size of neuronal population reduced to the minimum which supports synchronized discharge we tested the capacity of single members of the population to influence population activity. In other neuronal systems this question has been investigated by applying repetitive intracellular stimulation at frequencies differing from that of the population rhythm[12]. We found that 10 out of 36 neurones tested could influence the rhythm of synchronized activity (Fig. 1). Repetitive intracellular stimulation partially entrained the population discharge when applied at frequencies 30–60% faster than the mean spontaneous frequency (at higher frequencies synchronized events followed some multiple of the stimulus period as in Fig. 3). The frequency of population bursts increased by 23 ± 11% of control ($n = 10$) and they often followed an intracellular stimulus with latency up to 200 ms (Fig. 1*c*). A similar delay may be observed *in vivo* between afferent stimulation and the occurrence of an interictal discharge[5–7]. The degree of entrainment, expressed as the proportion of synchronized bursts which occurred within 200 ms of intracellular stimulation, varied between 17 and 60% whereas the proportion of bursts expected to occur within this time period by chance was 3–7%.

The influence of a single neurone on population activity was characterized more systematically ($n = 11$) by making single intracellular current injections at different times following a spontaneous synchronized burst (Fig. 2). Even at times much shorter than the mean period of spontaneous bursting the activation of some neurones could elicit a synchronized burst and reset the population rhythm such that the time to the next spontaneous burst was comparable with the mean period of the population rhythm. However the proportion of trials in which a constant intracellular current injection led to a population burst increased with time following a spontaneous burst (Fig. 2*c*). Failure to elicit a population burst was not due to failure to activate the neurone but instead appears to reflect a period of relative population refractoriness[5,7,13], which follows each synchronized burst and which outlasts the intracellular afterhyperpolarization (Fig. 2*b*). The latency with which a synchronized discharge followed activation of this neurone varied between 40 and 120 ms (Fig. 2*d*). Synaptic interactions have often been implicated in the synchronization of neuronal activity[5,14,15]. If synaptic transmission is involved it must be primarily chemically mediated and excitatory since the synchronized activity discussed here was blocked in low-Ca^{2+} high-Mg^{2+} medium and observed in the absence of GABA-dependent inhibition. Anatomical[16,17] and physiological[18] evidence suggests that pyramidal cells in the CA3 region of the hippocampus possess recurrent excitatory collaterals. However the time elapsing between activation of a single neurone and a following population discharge greatly exceeds that needed for transmission across a single synapse and the short intercellular distances in a segment preclude long axonal impulse conduction times. Therefore, as has been suggested in a theoretical model[19], a synchronized discharge may depend on activity spreading from the activated cell, through local recurrent pathways, to other cells in several steps, with an increasing number of cells discharging at each step until the whole population becomes synchronously active.

Paired intracellular recordings ($n = 10$) were made to examine directly the involvement of synaptic mechanisms in the initiation of a synchronized burst. A multisynaptic connection existed between the two cells shown in Fig. 3. Moreover, activation of the presynaptic neurone could elicit a simultaneous long-latency discharge in both neurones, whereas activation of the postsynaptic neurone had no effect. On stimulating intracellularly at a faster frequency than simultaneous bursts could be evoked, it was apparent that the period of population refractoriness was associated with a reduced postsynaptic response to activation of the presynaptic cell. Here presynaptic current injections of constant amplitude and duration were made at 1-s intervals

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