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Evolution of digital organisms at high mutation rates leads to survival of the flattest

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Darwinian evolution favours genotypes with high replication rates, a process called 'survival of the fittest'. However, knowing the replication rate of each individual genotype may not suffice to predict the eventual survivor, even in an asexual population. According to quasi-species theory, selection favours the cloud of genotypes, interconnected by mutation, whose average replication rate is highest[1–5]. Here we confirm this prediction using digital organisms that self-replicate, mutate and evolve[6–9]. Forty pairs of populations were derived from 40 different ancestors in identical selective environments, except that one of each pair experienced a 4-fold higher mutation rate. In 12 cases, the dominant genotype that evolved at the lower mutation rate achieved a replication rate >1.5-fold faster than its counterpart. We allowed each of these disparate pairs to compete across a range of mutation rates. In each case, as mutation rate was increased, the outcome of competition switched to favour the genotype with the lower replication rate. These genotypes, although they occupied lower fitness peaks, were located in flatter regions of the fitness surface and were therefore more robust with respect to mutations.

Mutation and natural selection are the two most basic processes of evolution, yet the study of their interplay remains a challenging area for theoretical and empirical research. Recent studies have examined the effect of mutation rate on the speed of adaptive evolution[10,11] and the role of selection in determining the mutation rate itself[12–14]. Quasi-species models predict a particularly subtle interaction: mutation acts as a selective agent to shape the entire genome so that it is robust with respect to mutation[1–5]. (See refs 15–18 for related predictions expressed in other terms.) In particular, selection in an asexual population should maximize the overall replication rate of a cloud of genotypes connected by mutation, rather than fix any one genotype that has the highest replication rate. Thus, a fast-replicating organism that occupies a high and narrow peak in the fitness landscape—where most nearby mutants are unfit—can be displaced by an organism that occupies a lower but flatter peak. Thus, 'survival of the flattest' may be as important as 'survival of the fittest' at high mutation rates. This prediction has proved difficult to test experimentally, but a recent study[19] with an RNA virus reported that two populations, derived from a common ancestor, have mutational neighbourhoods with different distributions of fitness effects.

Direct evidence for the displacement of a fast replicator by a more robust, slower one must come from experiments in which such organisms are squarely pitted against each other. The systematic (repeatable) winner of such a competition is, in effect, the fitter one, although the loser may have the higher replication rate. For example, imagine that a particular mutation yields a more robust genotype, but at the cost of a slightly lower replication rate. It is an empirical question whether the advantage of the mutational robustness is sufficient to offset its disadvantage in terms of replication rate. Quasi-species theory predicts that, under appropriate conditions (high mutation pressure), such a mutation can be fixed in an evolving population, despite its lower replication rate. This prediction does not depend on the details of the organism chosen for experiments, but only on mutation rate, replication speed, and robustness to mutations. Microorganisms, such as bacteria and viruses, are often used to test evolutionary theories, and competition experiments are typically performed to quantify fitness in the course of these tests. However, it would be difficult to disentangle the contributions of replication rate and robustness, because competitions measure the combined effect of both processes. Here, we use a more convenient system for disentangling these effects: digital organisms that live in, and adapt to, a virtual world created for them inside a computer.

Digital organisms are self-replicating computer programs that compete with one another for CPU (central processing unit) cycles, which are their limiting resource. Digital organisms have genomes (series of instructions) and phenotypes that are obtained by the execution of their genomic programs. The evolution of these programs is not simulated in the conventional (numerical) sense. Instead, they physically inhabit a reserved space in the computer's memory (an 'artificial Petri dish'), and they must copy their own genomes. Moreover, their evolution does not proceed towards a target specified in advance; instead it proceeds in an open-ended manner to produce phenotypes that are more successful in a particular environment. Digital organisms acquire resources (CPU cycles) by performing certain logical functions, much as biochemical organisms catalyse exothermic reactions to obtain energy. They lend themselves to evolutionary experiments because their environment can be readily manipulated to examine the importance of various selective pressures. The only environmental

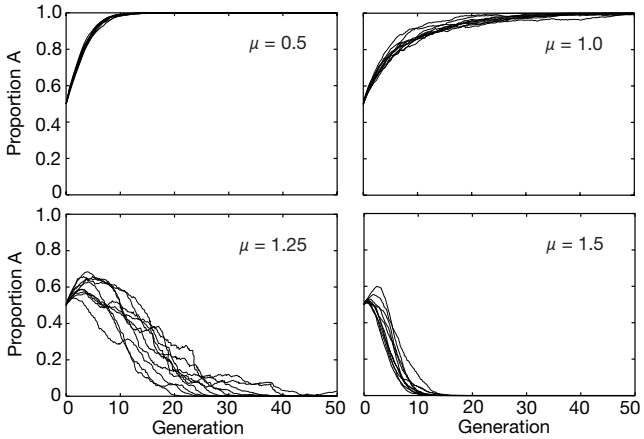

Figure 1 Competitions for one pair of organisms at four different mutation rates. Organism A replicates 1.96 times faster than B. μ, Genomic mutation rate.

factor varied here is mutation rate (such as might be achieved by varying temperature or radiation with biochemical organisms). Digital organisms replicate by copying their genomes one instruction at a time, just as biochemical organisms copy DNA (or RNA) base by base. This process yields overall copy fidelity $F \equiv e^{-\mu}$, where $\mu = RL$ is the mutation rate per genome, R is the error rate per instruction copied, and L is the length of the genomic sequence.

Populations of digital organisms were used to test directly the prediction from quasi-species theory that natural selection can favour genotypes with slower replication, provided they occupy flatter peaks surrounded by mutants that are also reasonably fit. We evolved 40 pairs of digital organisms; one of each pair adapted to a low (A) mutation rate and the other to a high (B) rate. Evolution at high mutation rate creates a selective force that favours robustness[20,21] and thus increases the likelihood of obtaining genotypes for competition experiments that are informative in the present context. Among all 40 pairs, we found 12 in which A achieved a more than 1.5-fold advantage in replication relative to B. For these 12 pairs, A and B were then mixed in equal proportions and allowed to compete across a range of mutation rates. After 50 generations, we measured the percentage of organisms derived from A in the mixed population.

For all 12 pairs, A excluded B at low mutation rates, whereas B prevailed at high mutation rates. Figure 1 shows the actual dynamics of competition for one pair. Figure 2 shows, for this same pair, that the competitive reversal reflects a shift toward less fit genotypes, which is more pronounced for A than B. These data therefore demonstrate that A occupied a higher but narrower fitness peak, whereas B was on a lower but broader peak. We note that for all pairs and all mutation rates, A could increase to its carrying capacity in the absence of its competitor. Thus, the reason for the extinction of A was not simply an unbearably high mutation rate.

We then sought an effective measure of the breadth of any fitness peak. Such a measure would allow us to define the critical mutation rate, μ_{crit}, at which A and B perform equally well; and thereby predict, for any given mutation rate, whether A or B would prevail in competition. The digital organisms in our study are asexual, that is, there is no recombination between individuals associated with their replication. We initially tried an approach that relies on an approximation from population genetics theory, which says that the equilibrium genetic load (reduction in mean fitness) from deleterious mutations in an asexual population is equal to the genomic rate of deleterious mutation[12–24]. For each organism in the 12 pairs, we examined every possible one-step mutation[8] to determine the exact proportion of deleterious mutations, which could be scaled by the mutation rate to predict μ_{crit}. To our surprise, this approach was highly unsatisfactory for predicting the outcome of competition or the critical mutation rate at which the competitive reversal occurs (data not shown). The weakness of this approach lies in the implicit assumption that competing populations remain tightly centred on the original genotypes. This assumption is clearly false, as seen in Fig. 2 by the large proportion of genotypes that differ by two or more steps from the original type.

We thus characterized each organism's mutational neighbourhood in a different way. Specifically, we measured the actual growth rate of the population spawned from a single organism across a range of mutation rates. The decay in growth rate with increasing mutation rate was well described by:

$$w(\mu) = w_0 \exp(-a\mu - b\mu^2), \quad (1)$$

where μ is the genomic mutation rate, w_0 is the intrinsic replication rate of the organism, and a and b are parameters estimated by fitting the data. (A similar function can be derived from quasi-species theory, see Supplementary Information.) Because the function $w(\mu)$ describes the realized growth rate of a population at a given mutation rate, the outcome of a competition between A and B should be determined by whose realized growth rate $w(\mu)$ is greater at a certain mutation rate, not by whose intrinsic replication rate w_0 is larger. The predicted critical mutation rate, μ_{crit}, is then simply the value where the two organisms' realized growth rates cross. (We note that the function $w(\mu)$ used here is fundamentally different from the decay function in ref. 8. Our function describes the decay of the realized growth rate of a population with increasing mutation rate, whereas the earlier function reflects the decay of intrinsic

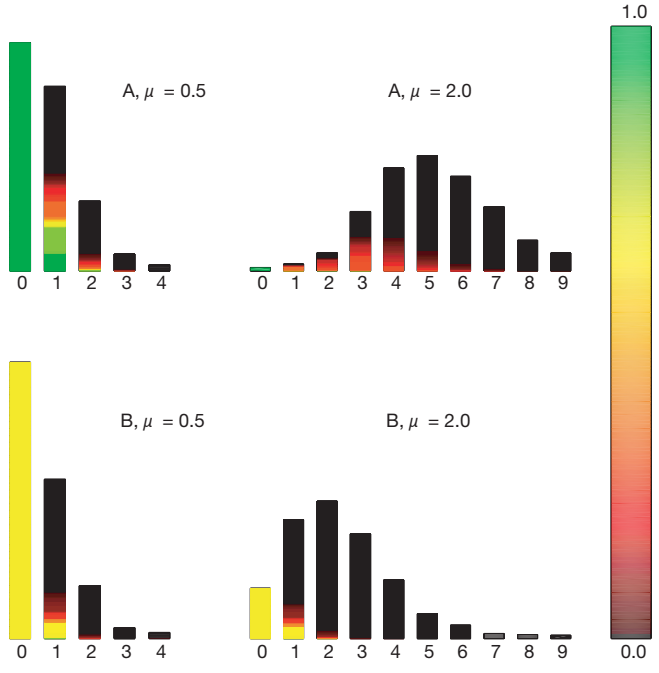

Figure 2 Genotype distributions after 15 generations from populations seeded uniformly with either A or B. Numbers below the bars are Hamming distances from the seeding genotype; bar heights are relative frequencies of genotypes at those distances. Colour coding indicates the intrinsic replication rates of all the genotypes. The colour scale was normalized to the intrinsic replication rate of A. The pair of organisms in this graph is the same pair for which the dynamics of competition were shown in Fig. 1. The right tails of the distributions are truncated for the purposes of illustration only.

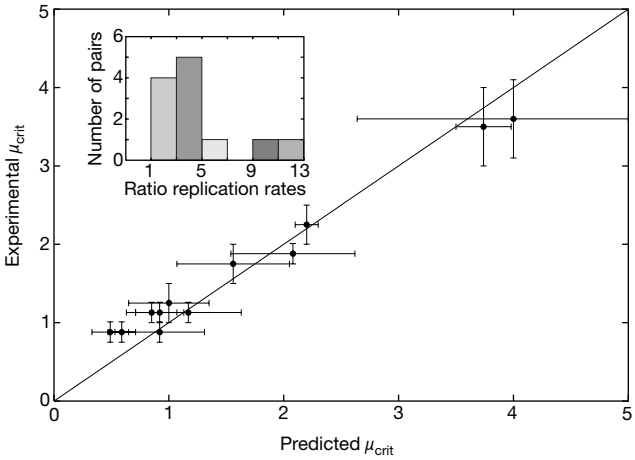

Figure 3 Critical mutation rates obtained from competition experiments versus predicted estimates based on each organism's intrinsic replication rate, w_0, and mutational robustness parameters, a and b. Points along the diagonal line would imply perfect agreement between experiment and prediction; the actual correlation coefficient is 0.991 ($P < 0.0001$). The inset shows the distribution of intrinsic replication rate ratios ($w_{0,A}/w_{0,B}$) for the 12 pairs.

replication rate as a function of genetic distance averaged over all mutants.) To test the utility of our new measure of peak breadth, we obtained the function $w(\mu)$ for all 24 organisms; for each corresponding pair we then calculated the predicted μ_{crit} value. Figure 3 shows the experimental μ_{crit} values (obtained by interpolation from the competitions) plotted against the predicted values. In all 12 cases, the experimental and predicted values agree well, and the overall correlation coefficient is 0.991 ($P < 0.0001$).

Thus we have demonstrated that faster replicating organisms can easily be out-competed at high mutation rates by organisms that replicate more slowly, if the latter obtain sufficient support from their mutational neighbourhood. Even a 12-fold difference in replication rate could be overcome by greater mutational robustness of the slower replicator (inset to Fig. 3). We emphasize that this robustness was not caused by any difference in replication fidelity, but rather by differences in 'canalization' with respect to mutational perturbation[17]. We also showed a widespread trade-off between intrinsic replication rate and mutational robustness, which arose during divergence from a common ancestor in environments that differed only in the imposed mutation rate. These findings demonstrate the importance of the mutational cloud, as described by the quasi-species model. The mutation rates where we saw these effects were on the order of one per genome per generation. Such mutation rates are not unusually high; they occur in RNA viruses[25,26] and many DNA-based eukaryotes[26–28].

One difference between the digital organisms studied here and biochemical organisms is that the latter can control, to some degree, their own mutation rates through DNA editing and repair. Thus, mutation rate may depend on the particular genotype as well as the environment. Nonetheless, evolution and competition experiments with bacteria or viruses could be performed at different mutation rates by varying the concentration of some mutagenic agent. We expect that more robust organisms would prevail over faster replicating, but more brittle, organisms at high mutation rates. □

Methods

All experiments were performed using the Avida platform, version 1.4, which can be obtained, along with the configuration files necessary to reproduce our experiments, from http://dllab.caltech.edu/pubs/nature01/. The particular organisms used here were taken from a pool generated in another study[8]. The organisms had genome lengths between 54 and 314 instructions, and they typically performed between 20 and 30 one-, two-, or three-input logical operations. In all experiments reported here, we disabled changes in genome length, which ensured that the genome-wide mutation rate did not change during evolution of the pairs from a common ancestor. We also prevented the organisms from evolving new computational functions by rewarding only logical operations that their common ancestor already possessed.

Adaptation

For the adaptation phase, we used 40 previously diverged ancestors to seed 40 pairs of populations at low and high mutation rates of 0.5 and 2.0 per genome per generation, respectively. Apart from the mutation rate, the selective environment was identical for each pair derived from the same ancestor. After 1,000 generations, we extracted the most abundant genotype from each population, giving us 40 pairs of organisms in which one member, designated A, was adapted to the lower mutation rate and the other, designated B, was adapted to the higher mutation rate.

Among the 40 pairs, we found one pair where both dominant organisms were nonviable (this can occur, for example, if another abundant genotype has a genome for which a lethal miscopy occurs with a high probability). In three cases, B evolved a higher replication rate than A. Because we were interested in situations where B could out-compete A despite having a lower replication rate, we ignored these cases. Among the 36 remaining pairs where A had a higher replication rate than B, we found 24 pairs with replication-rate ratios between 1.0 and 1.5, and 12 pairs with ratios above 1.5. We used the latter group for competition experiments, as these should both provide a clearer signal of the phenomenon of interest and provide a more stringent hurdle to be overcome by differences in mutational robustness.

Competition

The competition experiments lasted 50 generations. We seeded the mixed population with 50% each of A and B. We marked A with an inherited label, so that we could measure the percentage of descendants of each type in the mixed population. Population size was fixed at 3,600. For each pair of A and B, we ran competitions at genomic mutation rates of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0, with tenfold replication. We also ran additional competition at intermediate rates to locate the critical mutation rate more closely, and we tested up to a mutation rate of 4.0 in two cases where A prevailed at mutation rates of 3.0 and below.

To obtain an independent measure of the mutational robustness of each organism, we seeded a population with that single genotype and measured mean fitness (number of organisms born per unit time) after 15 generations, at genomic mutation rates ranging from 0 to 3.0 in steps of 0.5. The mean fitness at vanishing mutation rate corresponds to the intrinsic replication rate w_0 of that genotype. The robustness parameters, a and b, were determined from a nonlinear fit of equation (1) to the data.

Critical mutation rate

We determined the critical mutation rate from the competition experiments as follows. For all 12 pairs, the population consisted almost entirely of the descendants of only a single competitor (that is, one of the two had disappeared) after 50 generations, except at mutation rates near the critical point, where more time was needed for extinction. The critical mutation rate is the midpoint between the highest rate where A prevailed and the lowest rate where B prevailed. The error is one-half the corresponding interval.

The independently predicted critical mutation rate is given by the smallest positive root to the quadratic equation $(a_A - a_B)\mu + (b_A - b_B)\mu^2 = \ln(w_{0,A}/w_{0,B})$, where subscripts indicate organism A or B, a and b are robustness parameters from equation (1), and w_0 is the intrinsic replication rate. The corresponding error is obtained by propagating the errors of the robustness parameters from the fit of equation (1).

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