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# Macroevolution simulated with autonomously replicating computer programs

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The process of adaptation occurs on two timescales. In the short term, natural selection merely sorts the variation already present in a population, whereas in the longer term genotypes quite different from any that were initially present evolve through the cumulation of new mutations. The first process is described by the mathematical theory of population genetics. However, this theory begins by defining a fixed set of genotypes and cannot provide a satisfactory analysis of the second process because it does not permit any genuinely new type to arise. The evolutionary outcome of selection acting on novel variation arising over long periods is therefore difficult to predict. The classical problem of this kind is whether 'replaying the tape of life' would invariably lead to the familiar organisms of the modern biota<sup>1,2</sup>. Here we study the long-term behaviour of populations of autonomously replicating computer programs and find that the same type, introduced into the same simple environment, evolves on any given occasion along a unique trajectory towards one of many well-adapted end points.

When replicate lines of microbes are cultured in the same conditions, they usually evolve higher rates of growth in parallel. In principle, this need involve no more than the successive substitution of the same series of mutations and thus the emergence of the same multilocus genotype<sup>2,3</sup>. The tempo of adaptation can be analysed through the theory of periodic selection<sup>4,5</sup>; the lack of substantial genetic variance of fitness among replicate lines suggests that this simple description might often be adequate<sup>6,7</sup>. In a few cases where the relevant loci are known this has been confirmed directly<sup>8-10</sup>, but in other experiments replicate lines have diverged in fitness<sup>11</sup>, or the pattern of correlated responses has shown that they have diverged genetically, despite their similar direct responses<sup>12,13</sup>. In some cases, the physiological and genetic responses occurring in replicate lines have been identified, showing how the same selection pressure can lead to quantitatively and qualitatively different outcomes<sup>14,15</sup>. In experiments with large multicellular organisms such as Drosophila, differences among replicate lines can be caused by initial differences among the founding populations, or by inbreeding or drift during the course of the experiment<sup>16,17</sup>. In microbial experiments with large populations of asexual organisms derived from isogenic founders, these explanations are ruled out, raising the possibility that long-term evolutionary change is often highly contingent.

We studied an auto-adaptive genetic system called Tierra<sup>18,19</sup>, comprising a virtual microcosm inhabited by self-replicating computer programs ('creatures'). The genome of each creature is a sequence of instructions that directs the flow of control. Each instruction performs some elementary operation, such as incrementing a register or moving the instruction pointer to another location in the genome. When an appropriate sequence of instructions is correctly executed, the sequence is copied elsewhere in computer memory; changing the sequence of instructions usually changes the rate of replication. Selection between the variants produced by mutation is caused by competition for CPU time: variants that replicate more rapidly tend to increase in frequency. The evolving population of programs thus resembles an evolving population of bacteria or viruses, and such auto-adaptive genetic systems are now coming into use to supplement conventional analytical methods in evolutionary theory<sup>20</sup>. We have shown previously that the microevolutionary dynamics of Tierran populations are adequately described by conventional population genetics theory<sup>21</sup>.

We began each experiment with a single creature that was allowed to proliferate indefinitely, with an initial carrying capacity of 500 individuals and a mutation rate of 0.1 per genome per replication.



**Figure 1** Variety of evolutionary end points. Genotypes are classified according to the presence and position of key instructions such as *div* (divide), *mal* (allocate memory), *IncC* (increment register C), *PushX* (put a value in register X onto a stack), *ifz* (if register equals zero then execute next instruction, else advance two instructions) and *ret* (return). DD types are density-dependent and cannot replicate as single copies in pure culture; DI types are density-independent. The diversity of size-22 DI types is arranged according to the position of instructions relative to *div* and *mal*. a is *adrb* or *adro*; b is *subAAC* or *movBA*; c is *subCAB* or *pushB*. The numbers in parentheses give the number of observed outcomes of a given kind. **a**, Small-population experiments; **b**, large-population experiments.

The environment was the simplest possible, in which individuals competed for CPU time alone and other biotic interactions were not permitted. The process was continued until no further genetic change could be discerned. In each case the population at the end point was fixed for a single genotype (with a certain amount of neutral variation). We collected these genotypes for 109 independent experiments.

Selection for more rapid replication was effective in all cases, causing a reduction in the number of instructions executed by a creature in the course of replication. As expected, this was accompanied by a correlated decrease in genome size. The ancestral genome of 80 instructions was eventually replaced by much shorter sequences of fewer than 30 instructions, reflecting a decrease in the number of instructions executed per replication ( $I_E$ ) from 827  $I_E$  in the ancestor to as few as 135  $I_E$  in its remote descendants. The direct phenotypic response to selection was thus mostly, although not completely, in parallel (the mean among small-population lines was 177.4  $I_E$ , s.d. 25.8  $I_E$ ).

In contrast, the genotypic basis of the response was highly divergent, with several structurally different genomes emerging as possible end points of evolution (Fig. 1a). The two main groups of genotypes comprised either 27 or 22 instructions and were qualitatively different in structure. Genomes of size 27 invariably had the div (divide by copying the parental sequence into the memory assigned) instruction as close as possible to the end of the sequence, whereas in size-22 genomes div was in the front half of the sequence, often very near the beginning. The size-27 genomes were rather uniform, with most of them belonging to a single type, whereas the size-22 genomes were highly diverse. They often differed with regard to the presence or position of important instructions that direct the flow of control; they also differed with regard to the genetic organization of the region around the key instructions div and mal (allocate a block of memory into which the offspring sequence can be copied). Furthermore, both size-27 and size-22 genomes had density-dependent and density-independent versions. The densityindependent versions are normal self-replicating algorithms, but the density-dependent versions are unable to replicate as single copies and require the presence of a similar sequence nearby. Finally, several other end points were also observed, including bizarre genotypes such as one bearing two copies of ret (return from the copy sequence and reinitiate the sequence of events leading to replication). The microscopic details of these genotypes are in themselves of little interest, but the manner in which epistatic and social interactions affected evolutionary outcomes suggests the existence of general principles that might apply to all systems of self-replicators.

The time taken to reach the end point also differed between replicate experiments. Even for the rather uniform size-27 densitydependent genotypes it varied by a factor of two, without including



Figure 2 Change in sequence length through time of experiments for which a size-27 density-dependent sequence was the end point.

one aberrant case in which the population remained almost static for 1,000 million instructions (Fig. 2). Moreover, the nature of the intermediate genotypes was different in every experiment. This wide range of trajectories indicates that the genetic background in which mutations arise affects the route to the end point and the time taken to reach it, an observation consistent with the assertion that mutational order is important in causing divergence between populations<sup>22</sup>.

The trajectory itself was studied in more detail through 'go-back' experiments, in which an intermediate genotype was recovered and used to initiate a new evolutionary process. At the beginning of any experiment the founding genotype is 'totipotent' and can evolve towards any end point. As time goes by, the sequence of changes that its descendants have accumulated tends to restrict the range of end points that it can attain, until eventually, near the end of the process, it is nearly certain to reach a particular end point. In the simple Tierra system, the crucial fate-determining step is the placement of *div* either at the rear or near the front of the genome, which usually directs subsequent evolution towards either size-22 or size-27 genotypes. This step depends on the epistatic interaction between an *ifz-ret* pair situated in the copy procedure, near the middle of the genome, with div. (The ifz instruction can cause the instruction pointer to move forward two places, rather than a single place.) The *ifz-ret* combination is normally strongly deleterious, but increases replication rate in conjunction with an anterior *div* (see Methods). When this combination arises, whether early or late in the history of a lineage, evolution usually leads to one of the short sequences that retain an anterior div; otherwise, it tends towards one of the longer sequences with a posterior *div* (see Methods).

Long-term evolutionary dynamics depend on the flux of beneficial mutations, which is determined by population size N and mutation rate per site u (refs 23, 24). If Nu > 1 then most mutations, beneficial or not, occur at once, the most fit are substituted simultaneously, and replicate populations evolve in parallel; if  $Nu \ll 1$  then beneficial mutations are substituted successively and replicate populations diverge because each experiences a unique sequence of beneficial mutations. In our experiments the genome size is on average about 50, so that  $Nu \approx 500 \times 0.1/$  $50 \approx 1$ . Moreover, beneficial mutations are less frequent: the frequency of mutations of small effect that increase fitness is about 0.35u, and that of mutations of large effect less than 0.02u(ref. 21). The extensive divergence that we observed might have been attributable to a restricted supply of mutations caused by small population size. We therefore conducted a further series of 20 experiments with N = 10,000 size-80 creatures and thus  $Nu \approx 20$ . The predominance of size-27 end points was more marked and they were, as before, rather uniform in structure. Nevertheless, a number of smaller types of size 21-24 evolved, together with a highly aberrant size-39 creature that incorporated a sequence (involving call in conjunction with an anterior div and lacking the conventional 'head' sequence seen in all other types) never before seen in our experiments (Fig. 1b). Thus, evolution continues to be highly contingent even at relatively high values of Nu, because of the strongly epistatic nature of fitness. In an infinite population, evolution would in principle be completely predictable and populations would not diverge permanently, because in an infinite population not only would any combination of mutations occur but each would be infinitely abundant. However, in real populations only a very small fraction of possible combinations of mutations occur in any generation, and moderate degrees of epistasis ensure that replicate populations often diverge towards different end points.

Our experiments indicate that contingency might be a general feature of evolution in systems with unbounded, nearly neutral variation. It is possible to construct complex environments in which, for example, programs are rewarded for their ability to manipulate integers<sup>25,26</sup>. In such conditions it might not be very

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surprising to learn that many effective solutions exist and that populations consequently diverge in both phenotype and genotype. However, the situation represented in these experiments is the simplest possible: directional selection acting on beneficial mutations that reduce genome size, leading to strong phenotypic convergence and the eventual fixation of a single genotype. The situation is strikingly similar to the evolution of the virus QB in vitro, where it evolves strongly reduced genome size and increased replication rate<sup>27</sup>. Nevertheless, we have demonstrated not only that the genetic basis of evolutionary change varies between replicate realizations but also that the evolutionary fate of evolving lineages becomes steadily more closely determined through time and is strongly influenced by key innovations involving irreversible epistatic interactions between loci. Thus, even in this simplest of situations, the outcome of evolution can be any of several genotypes, and it cannot be predicted which will emerge in any particular realization.

#### Methods

All experiments were conducted using Tierra v.5.0, and run on computers based on Intel Pentium II and Pentium III running the Microsoft Windows 98 and Windows 2000 operating systems. The end points of the small-population and large-population experiments can be downloaded from McGill University's website (http://www.mcgill.ca/ biology/faculty/bell).

#### Basic experiments and 'go-back' experiments

A single copy of the Tierran founder genotype, 80aaa, was used to seed a series of 109 small-population and 20 large-population replicates conducted with the methodology of Yedid and Bell<sup>21</sup>. Evolution was considered to have ceased when the only replication-competent genotypes being produced were nearly neutral variants and no further change in size or fitness seemed possible. Genotypes from two experiments that resulted in the two major products of evolution (27 density-independent (DI) and 22 DI types) were selected for a series of 'go-back' experiments. We extracted a dominant genotype from points near the end, middle and beginning of each experiment, and inoculated the system with that genotype alone, using the same culture conditions as for the original experiment. Ten replicates were run for each genotype. This protocol was repeated for the 20 large-population experiments, except that the starting carrying capacity was 10,000 size-80 creatures.

#### Test of fitness effects of div/ifz-ret combination

Two genotypes from an experiment that resulted in a size 22 DI end point, one of size 56 lacking the *div/ifz-ret* fingerprint and a second of size 54 containing the fingerprint, were selected to investigate whether or not occurrence of the fingerprint by itself conferred enhanced fitness. Dummy instructions were added to a non-critical section of the genome of the size-54 genotype to slow its rate of replication, resulting in a size-57 genotype equal in fitness to the size-56 type. The two genotypes were competed against one another, saving all progeny genotypes so as to trace unequivocally the lineage of subsequent dominant types; the added instructions provided a marker for identifying progeny of the size-54/57 genotype. One set of experiments was inoculated with a single individual of each type at low density; a second set was inoculated with high and equal numbers of each. In the great majority (>90%) of the tests conducted, dominant genotypes derived from the size-54/57 type ultimately prevailed, even in cases where progeny of the size-56 type initially had a short-term advantage.

#### Determination of end point by div/ifz-ret combination

A size-63 genotype from an experiment that resulted in a size-27 DI end point was modified to contain the *div/lfz-ret* fingerprint, and used to inoculate the system as in the 'go-back' experiments. It was found in all attempts that the resulting end points were smaller than size 27 (mostly of size 22) and retained the *div/lfz-ret* fingerprint. A second set of experiments was conducted in which the same size-63 genotype was modified with only the anterior *div* near the creature's head, to examine whether the presence of this instruction was a necessary preadaptation for genotypes smaller than size 27 to occur. Wherever it was retained, a genotype smaller than size 27 was the eventual end point, although the anterior *div* was lost through mutation roughly half the time, resulting in size-27 types with the posterior *div-ret* combination.

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# Long-term dendritic spine stability in the adult cortex

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The structural dynamics of synapses probably has a crucial role in the development and plasticity of the nervous system. In the mammalian brain, the vast majority of excitatory axo-dendritic synapses occur on dendritic specializations called 'spines'. However, little is known about their long-term changes in the intact developing or adult animal. To address this question we developed a transcranial two-photon imaging technique to follow identified spines of layer-5 pyramidal neurons in the primary visual cortex of living transgenic mice expressing yellow fluorescent protein. Here we show that filopodia-like dendritic protrusions, extending and retracting over hours, are abundant in young animals but virtually absent from the adult. In young mice, within the 'critical period' for visual cortex development,  $\sim$ 73% of spines remain stable over a one-month interval; most changes are associated with spine elimination. In contrast, in adult mice, the overwhelming majority of spines ( $\sim$ 96%) remain