Microevolution in an Electronic Microcosm

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ABSTRACT: The evolution of microbial populations in simple environments such as chemostats is still not fully understood. The classical interpretation of adaptation involves a process of successive substitution whereby a new dominant genotype arises by mutation from the genotype previously dominant and spreads more or less rapidly through the population until it is nearly fixed. The population is, thus, nearly uniform most of the time. Some observations suggest that the process may be more complicated, but it remains formidably difficult to assemble the phylogeny of an evolving culture in sufficient detail to be sure. We report experiments with an electronic microcosm inhabited by self-replicating computer programs whose phylogeny can be rendered completely transparent. The physiology of these programs is different in many respects from that of organic creatures, but their population biology has many features in common, including a very extensive, if not unbounded, range of variation. Experimental populations evolved through point mutations (many of which were quasineutral when they were viable) and through rearrangements that led to a change in genome size and often had large effects on fitness. As a general rule, smaller genomes execute fewer instructions in order to replicate, the rate of replication increases as the number of instructions executed declines, and the rate of replication in pure culture is a good predictor of success in mixture. When cultured with CPU (central processing unit) time as the sole limiting resource, smaller genomes, therefore, evolve as a correlated response to natural selection for faster replication. The genetic basis of adaptation was highly contingent and always differed in replicate experiments. The pattern of evolution depends on mutation rate. At low mutation rates of 0.01 per genome per generation or less, we observed classic periodic selection, with each dominant genotype descending from the previous dominant and rising to a frequency of 0.8 or more. At higher mutation rates of about 0.1 per genome per generation, the most abundant genotypes rarely exceeded a frequency of about 0.4, and rare genotypes present in a few copies comprised a large part of the population. New dominant genotypes did not usually descend directly from previous dominants but,

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instead, from one of the many rare or moderately abundant genotypes. We suggest that the conventional chemostat paradigm may hold only as a special case at very low mutation rates and that the dynamics and diversity of evolving populations, even in the simplest conditions, may be more complex than is usually recognized. Artificial genetic autoadaptive systems are likely to be useful in constructing theory for situations that lie beyond the boundary of conventional population genetics.

Keywords: periodic selection, successive substitution, genetic algorithm, autoadaptive genetic system, experimental evolution, Tierra.

Microbial populations in chemostats are the most thoroughly understood of all ecological systems. It is only for chemostats, indeed, that we have a complete theory of the growth and regulation of single species (Tempest 1970) and mixtures of species (Tilman 1982). Both chemostats and serial passage experiments have also been used in evolutionary biology to investigate the process of successive substitution that underlies adaptation (e.g., Bennett et al. 1990; Dykhuizen and Dean 1990; Lenski and Travisano 1994). In this case, a complete theory has not yet been developed because of the continual appearance of novel variation through an unpredictable sequence of mutations. The paradigm that emerged early in the history of chemostat research (Novick and Szilard 1950, 1951; Atwood et al. 1951a, 1951b), however, has continued to dominate the interpretation of evolving chemostat populations down to the present day. It involves a process of "periodic selection" (Atwood et al. 1951b). Beneficial mutations that increase fitness in the particular conditions of growth provided by the chemostat occasionally arise and increase in numbers sufficiently to be driven to fixation by a nearly deterministic process of selection. After a longer or shorter interval the process is repeated, when a new beneficial mutation becomes established in the lineage in which the previous mutation was fixed (fig. 1A; Crow and Kimura 1965; Paquin and Adams 1983a). The interval between successive substitutions will depend on the population size, N, the rate of beneficial mutation, $u_{\rm b}$, and on the mean selection coefficient associated with beneficial mutations, $s_{\rm b}$. Thus, when $Nu_{\rm b}s_{\rm b}$ is large, new mutations will sweep through the population rather frequently, and the time during which substitution is occurring will be relatively long, compared with

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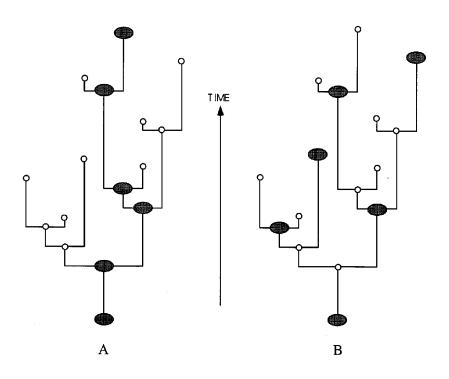


Figure 1: Genealogies associated with (A) classical periodic selection and (B) indirect descent of dominants; nodes are populations of genotypes

the interval between successive substitutions. This is likely to be true early in the history of the chemostat, when u_b will be large (because few of the available mutations have yet been fixed) and s_b will also be large (because mutations of large effect are likely to be fixed first). As time goes on, the supply of beneficial mutations will dwindle, their average effect on fitness will fall, and the interval between substitutions will increase. Long-term cultures will, therefore, show a nonlinear, decelerating increase in fitness (Lenski and Travisano 1994). Regardless of these quantitative changes, however, the underlying process remains the same: the successive substitution of beneficial mutations, each occurring in a descendant of the previous dominant mutant, with the population remaining essentially uniform in the intervals between substitutions.

Some experiments have yielded results that seem inconsistent with the paradigm (see Dykhuizen 1990). For example, Adams et al. (1985) found that the turnover in chemostat populations of yeast (about one substitution every 40 generations) seemed too rapid to be consistent with the assumption that the populations were nearly uniform in the intervals between substitutions. Moreover, these intervals did not increase over time in the expected way. Adams and Oeller (1986) pointed out that chemostat populations are often so large that almost all possible single mutants will be present at any given time. A population might then contain hundreds of beneficial mutations of different effect, changing in frequency at different rates. This diversity might be maintained indefinitely if a new genotype spreading through the population modified culture conditions so as to alter the relative fitness of others (Bell 1997). The chemostat would then be, as Adams and Oeller (1986) suggest, a reservoir of adaptive mutations at different frequencies, and a new dominant genotype spreading through the population might descend from any of these rather than from the genotype previously dominant. This interpretation is radically different from the paradigm: in place of a regular and rather repeatable succession of dominants, each descended from the last, it posits a churning, inchoate mixture in which few genotypes ever approach fixation, and those that do rise to high frequency might descend from obscure ancestors (fig. 1B).

In order to distinguish between these two theories, we must be able to evaluate the diversity of the evolving chemostat population and inspect its phylogeny in order to determine the pattern of succession. It is not yet practicable to do this satisfactorily because current technology does not allow us to characterize the genotypes of thousands of cells per generation, still less to determine with certainty their ancestor-descendant relationships, except in the simplest systems, such as phages and ribozymes (Wichman et al. 1999; Lehman et al. 2000). Moreover, conventional population genetics provides no well-defined prediction because the range of variation available for selection in conventional models and simulations is defined at the outset, and it is impossible for any novel type to arise. Population genetics, as currently understood, therefore, cannot chart the evolution of an open system with indefinite variability. This is an important limitation on evolutionary theory because, if we cannot determine the mechanism underlying short-term adaptation in the simplest and best understood of ecological systems, it may be premature to speculate about processes that occur on larger spatial and temporal scales in natural communities. To circumvent these practical and theoretical limitations, we have turned to a system where these difficulties can be overcome and which may be regarded either as the simulacrum of a chemostat or as an extension of population genetics to open systems.

Tierra

Tierra is an electronic microcosm inhabited by selfreplicating computer programs (Ray 1991, 1994*a*, 1994*b*, 1998). These programs, the Tierran creatures, consist of short sequences of instructions written in code similar to assembly language. The way in which the instructions are constructed and implemented is analogous in many ways to the transmission and expression of genes, although there are important differences between the two systems, as we shall explain below (see table 1 for a brief summary of the system's most important elements). An artificial system of self-replicators can furnish a useful analogue to populations of microbes only if it will evolve and, moreover, only if its evolution is governed by similar principles. Most existing

Table 1: Basic elements of the model system

computer machine languages are too brittle to be useful: any change in an instruction is almost certain to be lethal, so most conventional computer programs will almost certainly be broken by random change. Even self-replicating programs written in such languages are, therefore, confined to a very small range of genotypes, beyond which they cannot evolve. Natural genetic systems based on nucleic acids, on the other hand, are extremely flexible and, because most random changes are quasi-neutral, can give rise to an essentially infinite variety of genotypes through cumulative evolutionary change. The Tierran system lies between these extremes (table 2). Tierra's original instruction set, instruction set 0 (used in this study), is of the same order of magnitude as the genetic code, using a set of 32 instructions of 5 bits each, which are analogous to nucleotide triplets (or, more accurately, to amino acids). Mutations in any of the five bits will change the instruction, which may change the way the program is implemented. The instruction set is "contained" so that mutation will always result in another instruction of the set and not in undefined bit patterns. The Tierran instruction set is more brittle than the organic genetic code because each instruction is implemented directly rather than being meaningful only in the context of a larger unit such as a gene or a protein molecule. Single-instruction changes are, therefore, more likely to be severely deleterious than single-base changes and less likely to be beneficial (Lenski et al. 1999). Nevertheless, the effect of a single-instruction change will depend, in some degree, on the overall structure of the program, so quasi-neutral changes are, in practice, not unusual and beneficial changes are observed quite often. It has not been established whether the set of genotypes that can evolve in Tierra is infinite, but it is

Term	Definition
Soup	The computer memory space in which Tierran creatures reside
Creature	A Tierran virtual organism, which consists of a virtual CPU and an associated genome of instructions
Genome	The list of Tierran instructions directing replication of a given creature
Instruction	A specific computer operation that, when executed, modifies the local state of a CPU; Tierran instructions operate only on a given creature's virtual CPU (their effects are contained within Tierra) and are considered the "atomic" unit of a Tierran genome
Instruction set	The full ensemble of instructions that defines the assembly language used to program a computer through manipulation of a CPU; it varies with CPU architecture (in both software models and real instantiations)
Virtual CPU	A software emulation of a computer's central processing unit; in Tierra, each creature has its own virtual CPU, which serves as a collection of state variables describing the local state of an executing genome and containing the numeric values manipulated by the instructions of the genome
Bit flip	Conversion of a bit to its complementary value $(0\rightarrow 1 \text{ or vice versa})$
Background mutation	Random bit flips effected continuously by the Tierran operating system on both occupied and free soup space
Copy mutation	Bit flip effected randomly on a codon position but only during copying of an instruction from one location in memory to another
Flaw	Error in execution of an instruction that alters its default behavior

Genetic/evolutionary property	Organic/biological	Digital model (Tierra)
Genetic code ^a	4[A, T/U, G, C]/3	2[0, 1]/5
Code degeneracy	Present	Absent
Mutational mechanisms	Base substitution, cross-linking, mobile genetic elements, errors in germ-line replication	Bit conversion, errors during process of replication (flaws)
Recombination	Consequence of sexual fusion and crossing over or, more rarely, of transformation	Consequence of transformation, if this is permitted
Extent of variation	Indefinitely large number of meaningful genomes	Extremely large (perhaps indefinite) number of possible meaningful genomes
Limiting resource(s)	Many kinds (chemical, spatial, energetic)	Master CPU time
Genotype frequency	Frequency of individuals bearing a particular allele or combination of alleles	Frequency of a specified sequence
Stochastic changes in		
frequency	Predominate in small populations under weak selection	Predominate in small populations under weak selection
Inheritance	Usually vertical transmission; horizontal trans- mission possible but rare in most organisms	Usually vertical transmission, capacity for hori- zontal transmission is usually user enabled
Effect of mutation	Most point mutations quasi-neutral or mildly deleterious	Most point mutations lethal (Lenski et al. 1999)
Variance of fitness	Most standing genetic variation quasi-neutral	Most standing genetic variation quasi-neutral (this article)
Differential reproductive		
success	Differences in rate of production of offspring	Differences in rate of copying of genome

Table 2: Parallels and contrasts between genetic and evolutionary properties of the organic/biological world versus the Tierran computer model

^a Values listed according to "number base [elements]/codon size."

certainly extremely large. It seems reasonable to explore the system as a means of investigating issues in microevolutionary dynamics that lie beyond the reach of conventional population genetics. The macroevolutionary dynamics of Tierran communities have been described by Ray (1991).

The creatures are injected into culture medium (referred to as the "soup") consisting of computer memory, where they replicate and compete. The limiting resource in a Tierran culture is "energy," which is represented by central processing unit (CPU) time. This is freely available to all creatures, although some may require more than others in order to replicate and are to this extent less efficient. While the culture memory is a limiting factor on the population, the organisms do not really adapt to it in a meaningful way. The creatures will evolve to make optimal use of the available CPU time, and the ability to perform specific computations beyond self-replication may (at the user's discretion) be rewarded with extra CPU time (Adami 1994, 1995; Adami and Brown 1994).

The creatures are placed in a "slicer queue," whereby each cell is allotted a certain amount of CPU time in a manner that may be designated by the user. Every cell has a virtual (software representation) CPU associated with it, whose state is modified by the instructions in the creature's genome. When a given creature is marked active by Tierra, the "hardware" CPU will be executing the Tierran instructions pointed to by the instruction pointer associated with that particular cell. Because the size and number of creatures may vary, generation time in Tierra is not fixed (generation time also scales with soup size), and the most appropriate measure of time is the total number of instructions executed, divided by half the soup size (since at any time about half the soup consists of nonexecuting "embryonic" code), termed an "update" (denoted here by "U"). Each creature first examines itself to determine its size, requests that much memory from the operating system, and copies its own code into that space, giving birth to a daughter. If the parent breeds true, the daughter will be identical to its parent and will function in the same manner. The daughter may differ from its parent if either sustains a mutation (since no protection from mutation is provided during gestation) or if the parent is innately incapable of breeding true. Three kinds of mutation are allowed. The first, background mutation, is caused by random bit flips that occur "continuously" and that affect living, embryonic (incompletely gestated), and dead code anywhere in the soup. The second, copy mutations, occur only during the transfer of instructions from parent to daughter: one of the five bits of the instruction currently being copied is randomly selected and flipped. And the third, flaws, are arithmetic errors during the execution of the code that may affect the sequence and phenotype of the daughter but need not be heritable. They are usually manifested as point insertions, deletions, or duplications of whole instructions, thus constituting a kind of slippage or frameshift. All three kinds of mutation may affect the speed and fidelity with which the daughter creature is implemented. A growing population quickly fills up the memory space, and it is then necessary to remove creatures in order for replication to continue. This is usually done by constructing a "reaper queue" in which the rank of a creature is determined by the creature's age and the number of "errors" (defined as instructions that fail to execute correctly and set the creature's error flag, which constitutes a sort of "senescence" for that cell) it has made during execution. The oldest and/or most error-prone cells advance to the head of the queue. When there is not enough memory available to fulfill a request, the creature at the top of the queue is killed by de-allocating its memory, and the space is given to the creature that has requested it. In our experiments, however, we set the reaper to kill at random so that older creatures and cells with high mutational loads are not doubly penalized; in a real chemostat, cells of any age and fitness have a nominally equal chance of being washed out. In this way, the variation created by mutation is exposed to selection through competition for CPU time, and the population evolves.

One of the main reasons for turning to an electronic microcosm is that the phylogeny of the Tierran creatures can be made completely transparent. When a new genotype appears, a record is created that stores its parent, its time of birth, and its name. The name of a creature consists of its length, in instructions, and a sequential three-letter code. Thus, the ancestral creature for this instruction set is 80 instructions in length, and its name is 80aaa. The first descendant of the same length but different genotype would be called 80aab, the first descendant of length 79 instructions would be called 79aaa, and so forth. In most realizations of Tierra, the birth records are not sufficient to construct a completely accurate phylogeny for two reasons. First, names are saved only if a genotype exceeds a certain frequency, and if unsaved can be reused; consequently, creatures may bear the names of lost ancestral genotypes, complicating genealogical reconstructions. We overcame this by brute force, simply saving all possible genotypes so that every genotype is assigned a unique name. And second, creatures can usually read and execute the code of other creatures, leading to the evolution of parasites that use the copy loops of neighbors to replicate their own code (Ray 1991). When this happens, the physiological parent named on the record is not the genetic parent. We have prevented this by invoking the memory protection option available in recent versions of Tierra so that parasites cannot evolve and so that the parent recorded is, in all cases, the genetic parent. With these two modifications, a completely transparent phylogeny can be obtained. We can then use Tierra to obtain an extremely detailed account of the first few hundred generations of adaptation to a novel environment. It has been said that one of the great advantages of bacterial chemostats is the ability to store an evolving population at intervals so that one can go back in time to resample the population at will; in Tierran systems, the complete fossil record is available, with every individual labeled.

Material and Methods

Tierra System

We used Tierra version 5.0 (Ray 1998), with the original Tierran instruction set, Instruction Set 0 (Ray 1991, 1994*a*). The complete setup files are available on request. The minimum creature size was set to 12 instructions (far below anything likely to be viable), and the memory protections were adjusted to prevent creatures from reading, writing, and executing the code of other living creatures. This prevents the occurrence of parasites, although, in principle, it permits the uptake of "dead" code in unallocated space. It is unlikely, in practice, that creatures could be transformed by dead code because disturbance (which causes large-scale extinctions) were turned off and there was little free space in the soup at any given time.

Operating Environment

All experiments and trials were run on an Hewlett-Packard Vectra VL Pentium 200 running under Microsoft Windows 95, at the McGill University Biology Department. The birth/death files were analyzed with two accessory software tools, the PaleoBeagle Explorer and Evolvability (T. Ray, personal communication). Because of the number of genotypes preserved, the run_info data necessary for completion of this analysis were generated at binoc.tamu.edu, a Unix machine at the Department of Biochemistry and Biophysics at Texas A&M University, running under Evans and Sunderland Operating System (ES/OS) version 2.3, through the courtesy of R. Swanson.

Population Parameters

A soup of 80,000 Tierran instructions in size was inoculated with a single copy of 80*aaa*, the founder of instruction set 0, near the center of the soup. The seed value for Tierra's random number generator was set by the system clock independently for different experiments. Dominant genotypes were determined from firsthand observation of the run and by reviewing Tierra's log file (a condensed record of events printed at intervals of 1 MI, where MI denotes millions of instructions executed, the basic measure of time in Tierra). All genotypes and birth/death information were saved so that with a complete fossil record a full genealogy of the dominant genotypes could be constructed.

Experiments were run at low and high mutation rates: two were run at very low mutation (0.001 per genome per generation), two at moderately low mutation (0.005 per genome per generation), and five at high mutation (0.1 per genome per generation). Experiments were ended when the performance of Tierra became severely degraded by genome storage, which occurs sooner when mutation rates are high. Consequently, the high-mutation experiments ran for about 300 Tierran generations (ranging between 3,500 and 3,875 U), the moderately low-mutation experiments for 1,000-2,000 generations (11,300 and 29,375 U, respectively), and the very low-mutation experiments for about 10,000 generations (127,000 and 114,500 U, respectively). We used relatively small populations (about 500 creatures) because of the need to save every genotype in order to obtain completely known phylogenies. In particular, it is impracticable to run large populations with high mutation rates because the large number of variants that appear quickly degrades system performance. We, therefore, ran three large-population experiments, with a memory size of 1,600,000 instructions (about 10,000 size 80 creatures), at low mutation rate to verify that the patterns observed in the previous experiment were not effects of small-population number. These experiments ran for 897, 1,320, and 1,776 generations (10,628, 15,713, and 20,000 U, respectively) with all three types of mutation at 0.001 per genome per generation.

Verification of Mutation Rate

We measured mutation rates directly and found that they did not correspond to input parameter values. Empty memory space was seeded with 250 copies of the ancestral genotype 80*aaa*, which was then allowed to replicate once to yield a total population of 500. The parental individuals are exposed to background mutation; the offspring, to background and copy mutations. Knowing the number of mutant genotypes appearing after one generation, the background and copy rates can be estimated. For an input value of 0.05 for both rates, a series of five trials yielded estimates of the background rate of 0.0974 and of the copy rate of 0.0971. It appears that the actual mutation rate is, thus, about twice the specified rate so that in these experiments the actual total rate of mutation per genome per generation was 0.002, 0.01, and 0.2 at the three levels studied.

Nature and Distribution of Fitness

Relative fitnesses and selection coefficients were determined for two random samples of creatures generated during one of the high-mutation experiments. One was a sample of 50 creatures from a point early in the experiment (at around 375 U), and the second was a sample of 50 from a point near the experiment's end (around 3,500 U). Fitness was assessed first by measuring the rate of increase of a population of each genotype in mutation-free pure culture, which is defined as the number of creatures produced per 1,000 instructions executed. The estimate for each genotype was the mean of five replicate trials with different starting positions and random seeds. Relative fitnesses were estimated using 80*aaa* as the reference genotype according to the method of Lenski et al. (1991), and coefficients of selection for each genotype were calculated from these. We then estimated selection coefficients in mixed cultures by following the rate of change in frequency of the test genotype through time in competition with 80*aaa*.

Competition Trials

Genotypes appearing in the course of one of the highmutation experiments were used in a series of competition trials to investigate whether simple pairwise interactions were sufficient to explain the events that we observed. All mutation was turned off during these trials. Five kinds of trial were conducted.

Trial 1: Pairwise Combinations of Dominant Genotypes. One copy of each genotype was inoculated into a paused (i.e., nonexecuting) system that is then restarted. The object of this experiment was to identify any intransitive interactions involving the displacement of genotypes evolving later by those evolving earlier.

Trial 2: Mixture of All Dominant Genotypes. In one set of 10 trials, a paused system was inoculated simultaneously with a single copy of each of the dominant genotypes. The run was allowed to progress until one of the genotypes became fixed. A second set of 10 trials was performed in which the inoculum consisted of equal numbers of all the dominant genotypes so as to begin each trial with a full soup (38 copies of each genotype). The object of this trial was to establish whether consistent success in pairwise encounters indicated a global maximization of fitness.

Trial 3: Invasion Trials. One dominant genotype was inoculated and allowed to expand until it had filled the soup. A single copy of a dominant genotype that had evolved later was then inoculated. Five trials of each invasion attempt were run. This trial showed whether genotypes that were superior when competing at high frequency were able to invade from low frequency. A second set of trials was conducted in a similar manner using genotypes of known fitness from the samples used to characterize the distribution of fitness.

Trial 4: Interference by Another Genotype. The object of this experiment was to determine whether the outcome of competition between two genotypes with high fitness was affected by the presence of a large number of individuals of another genotype of lower fitness. A paused soup was inoculated with a few copies of two genotypes of known fitness and competitive ability, 80aao and 80asv, drawn from the sample of 50 genotypes taken from early in the experiment (see above). It was established in advance that 80aao was slightly superior to 80 asv. The soup was then inoculated with 300 copies of 81 aae, a genotype from the same sample that replicates less rapidly than either tester, and allowed to resume execution. Two replicate trials with 10, 20, and 30 copies of each were run, each with a different starting position for each block of genotypes and different random seed. A fourth trial was set up with equal numbers of all three genotypes.

Trial 5: Context-Dependent Selection. The object of these experiments was to determine whether the outcome of competition between two genotypes of high fitness was affected by the specific composition of the remainder of the population. Two genotypes (67 adm and 67 ast) from late in the same high-fitness experiment used previously were used; it was determined beforehand that 67 adm was superior. Three samples of five genotypes each were chosen randomly from the same population and used to create differing genetic backgrounds by inoculating each of them along with 67 adm and 67 ast into a paused soup in the quantity in which it existed in the experiment at the time of sampling. Experiments were conducted with each background genotype, with all combinations of genotypes from a sample and with all samples combined. Finally, a second set of samples was drawn from the same population in the same way, and the trial repeated using all 30 genotypes. The chemostat equations, and their relations to biological fitness, have been described by Dykhuizen (1990).

Results

Mutational Variation

Genotypes sampled from an evolving population vary in fitness, whether extracted soon after inoculation (F = 14,786, df = x, y; P < .0001) or much later (F = 4,734, df = x, y; P < .0001; fig. 2). When they were examined in more detail, they were found to fall into four categories.

Category 1: Stable Replicators, Which Breed True in Pure Culture. Most genotypes were of this kind (37/50 in both early and late samples). Significant variance of fitness among genotypes can be demonstrated within this category, mainly because replication error is very small, but

differences in fitness are slight, and these genotypes collectively constitute a cloud of quasi-neutral variants whose extent will depend on the genomic mutation rate. It is this category that makes gradual directional evolution possible.

Category 2: Inviable Genotypes Incapable of Replication. This category is a substantial minority in both early and late samples (nine and eight individuals, respectively). They are the outcome of deleterious mutation and are eventually removed from the population.

Category 3: Mutators. A few individuals (one in the early sample, three in the late sample) were very low-fidelity replicators that usually produced mutant progeny.

Category 4: Specific Mutators. These are unusual, rare genotypes (one in the early sample, two in the late sample) that consistently produce the same genotype in offspring, but this genotype is not the parental genotype. They play an important part in evolutionary change, as explained below in "Evolutionary Mechanisms."

Nature of Fitness

Rate of Replication in Pure Culture in Relation to Success in Mixture. Selection coefficients were estimated both from the relative rates of increase of two genotypes grown in pure culture and from their change in frequency when grown as a mixture. These two are very closely related in the genotypes we have tested (fig. 3), so the response to selection can be predicted from knowledge of behavior in pure culture. The response is somewhat less than the pureculture fitnesses would suggest, however, probably because it is measured at high density whereas pure-culture rate of replication is measured at low density.

Number of Instructions Executed in Relation to Rate of Replication. There is a very strong negative relationship between the rate of increase and gestation time, the number of instructions required for a genome to complete replication (I_E ; fig. 4).

Genome Size in Relation to Number of Instructions Executed. At any given time, gestation time is correlated with genome size (fig. 5), although the correlation is not perfect; genomes of the same size may have different values of gestation time. Moreover, although rapidly replicating genomes are usually small, not all small genomes replicate rapidly; many replicate slowly or not at all. The relationship between gestation time and genome size changes through time as more efficient genotypes evolve. This causes a progressive reduction in the elevation of the regression, although the slope remains more or less the same.

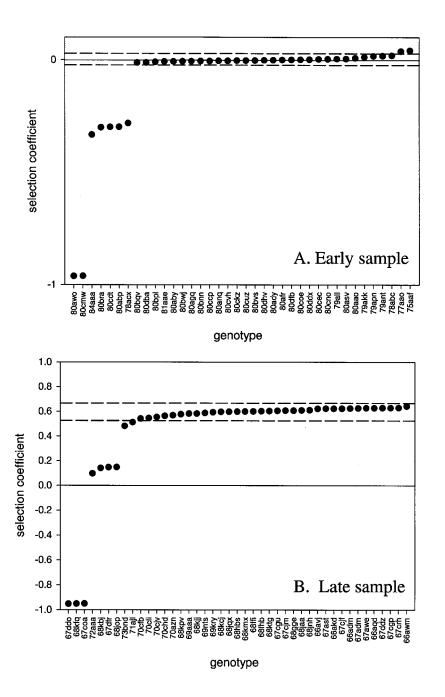


Figure 2: Variation in fitness. Genotypes were sampled from a high-mutation population early in the experiment (ca. 375 updates; *A*) or later (ca. 7,500 updates; *B*). Selection coefficients refer to fitness in pure culture relative to the ancestor 80*aaa*, which is indicated by the solid line at 0. The dashed lines mark two standard deviations on either side of the mean for the quasi-neutral genotypes only.

Fidelity of Replication. A genotype is especially vulnerable to flaws if it incorporates many arithmetic instructions, such as those incrementing registers or moving information between registers. The copy region is particularly rich in instructions of this kind. The design of a creature may affect

its success in this way, through variation in fidelity even among creatures with the same genome size and $I_{\rm E}$.

Physiology of Fitness. We have identified a range of specific pathologies affecting replication, other than complete ste-

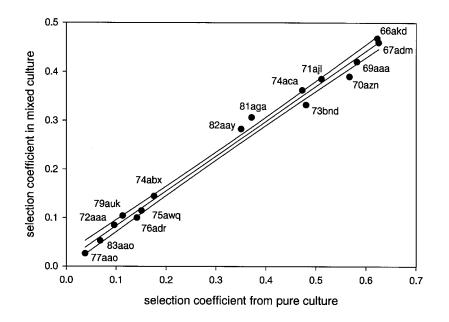


Figure 3: Fitness in pure culture and in mixture. Selection coefficients calculated relative to 80*aaa* as rates of increase of genotypes in pure culture and calculated from change in frequency of genotypes grown as mixtures with 80*aaa*. The regression is y = 0.717x + 0.012, with $r^2 = 0.99$. Curved lines are 95% confidence intervals.

rility. Most concern genotypes that produce their first daughter in a seemingly normal fashion but then experience difficulties in replicating subsequently. "Multipliers" fail to locate their head or tail correctly because they lack complementary template sequences and give rise to daughters of steadily increasing size. "Wasters" fall through the copy loop on their second attempt and are able to replicate only if they fall right through to the tail and can reinitiate selfidentification. "Loopers" encounter a jump-back instruction that returns them to the template sequence they have just left and so get stuck in an endless loop. "Nonreturners" are deleted for "ret" (return from copy procedure) and are unable to produce a second daughter because they send the instruction pointer to the nearest available patch of free memory. There are doubtless many other categories of defect.

Outcome of Selection

Pairwise Competition between Dominants. The experiment from which the genotypes used in the pairwise competition trial were drawn showed a general trend toward smaller genomes, as did all the others. The pattern of events underlying the trend was as follows: the small variant 72*aaa* appeared abruptly at 1,025 U, but the larger creature 79*auk* arose shortly afterward, and the two, having displaced the ancestor 80*aaa*, continued to fluctuate at high frequency

until both were eliminated by the rise of 76*adr* after 1,400 U. There followed a long period during which the population was dominated by a single very abundant genotype: 76*adr* was displaced by 74*aca* at 1,675 U, which was in turn displaced by 69*aaa* at 2,025 U, which retained its dominance until 2,850 U. The final phase of the experiment was more complicated. Three genotypes (68*baw*, 67*adm*, and 69*fdx*) were abundant from 2,850 U to 130 MI 3,250 U, with sometimes one and sometimes another having the highest frequency. They were then replaced by another set of genotypes of about the same size (67*ast*, 69*kvp*, 66*akd*, 68*gge*, and 68*hbs*), which fluctuate in frequency until the end of the experiment at 3,575 U.

This history can be compared with the outcome of the pairwise competition trials (table 3). The founder 80*aaa* is displaced by all subsequent dominants. Genotypes 72*aaa* and 79*auk* coexist at high frequency throughout the 3,150 U of the trial. A simple rule holds for the genotypes dominant between 1,400 and 3,225 U: the genotype evolving later displaces the genotype evolving earlier, with no evidence of intransitivity (cf. Paquin and Adams 1983*b*). Among the genotypes that were abundant after 3,325 U, temporal priority was no longer decisive. It was genome size, instead, that determined the outcome of competition, the smaller genotype being superior. The smallest dominant used in the pairwise competition trials, 66*akd*, never achieved overall numerical superiority but was, nevertheless,

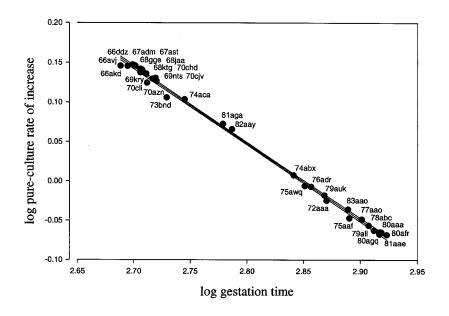


Figure 4: Rate of increase and number of instructions executed (*gestation time*). The regression is $\log y = 2.755 - 0.967 \log x$, with $r^2 = 0.99$. Curved lines are 95% confidence intervals.

almost invariably successful in pairwise competition. Over a broader range of genotypes, dominants with smaller genomes are usually superior, but fitness is not completely correlated with genome size and we have encountered exceptions to the rule.

Mixture of All Dominants. The single-copy inoculum (low density) trials ran for between 2,075 and 10,000 U (average $4,694 \pm 2,449$), while the block inoculum (high density) trials ran for between 2,109 and 8,457 U (average 4,741 \pm 2,038). In all 20 trials, either 66*akd* or 67*adm* (the two fittest genotypes obtained) were the eventual victors: 66akd became fixed in six of the 10 low-density trials, while it was fixed in nine out of 10 high-density trials. We attribute this result to two principal factors. First, the difference in relative fitness between the two types is only about 2%. Genotype 66*akd* has a gestation time of 492 instructions, versus 502 instructions for 67 adm, even though we determined pure-culture selection coffeicients of 0.623 for 66akd and 0.625 for 67 adm. Second, the probability of stochastic loss of any of the quasi-neutral dominant types (all with relative fitnesses within 5% of each other) is greater at low density since there is a lower chance of any of them attaining the frequency necessary for invasion in a population whose mean fitness nearly approximates their own.

Invasion Trials. The results of the invasion trials, where a single individual of the invading genotype is inoculated into a large population of the resident genotype, were not

completely consistent with the outcome of pairwise competition when both genotypes were inoculated at low frequency (table 4). Three generalizations seemed to hold. In the case of the first few dominants (80aaa, 79auk, 72aaa, and 76adr), the later-evolved type invades a population of the earlier-evolved type, except that 76adr was unable to invade 80aaa or 79auk. Second, these first few dominants were always invaded by subsequent genotypes. Finally, later dominants, from 74aca onward, were not invasible under the conditions of the trial. The only exception was a single successful invasion (in five attempts) of 74aca by 67ast. We attribute this pattern to the trend toward smaller selection coefficients through time, increasing the probability of stochastic loss of a unique beneficial mutation. We examined this interpretation by recording the invasion success in 100 trials of each of four genotypes slightly superior to 80*aaa*. They were fixed in four cases (s = 0.0085), three cases (s = 0.0165), seven cases (s = 0.0378), and 22 cases (s = 0.1416), respectively. With weak selection, the expected probability of fixation of a unique beneficial mutation is 2s, so the expected number of invasions in all three trials together was 40.56, which is fairly close to the observed number of 36. The results of the invasion trials, thus, seem to reflect the dominance of selection in the earlier stages and demographic stochasticity at later periods when the intensity of selection has declined.

Interference Experiments. When introduced at low frequency, both testers tend to be eliminated stochastically

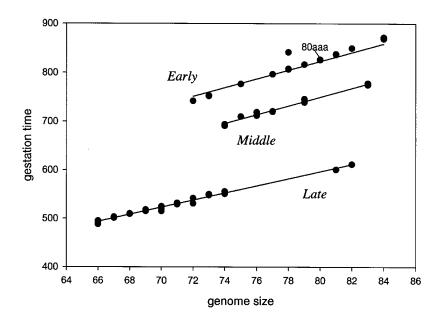


Figure 5: Genome size and speed of replication. Samples were drawn from a high-mutation population at three periods: *early* (1–775 updates), y = 104.4 + 8.98x; *middle* (776–2,650 updates), y = 26.7 + 9.04x; and *late* (2,651–7,500 updates), y = 8.0 + 7.36x.

within a few dozen generations. Whenever they persist, however, the type with greater fitness (80*aao*) invariably eliminates the type with lower fitness (80*asv*), regardless of the abundance of the background genotype (81*aae*). These experiments yield no evidence, therefore, that the presence of variants of lower fitness can alter the outcome of competition between two superior genotypes.

Context-Dependent Experiments. The superior genotype (67*adm*) of the experimental pair eventually became fixed in all trials, sometimes after considerable fluctuations in frequency. There was, therefore, no indication that the eventual outcome of selection is influenced by the composition of the population. The details of substitution might be more labile; although the inferior genotype (67*ast*) of the experimental pair usually increased in frequency as the random background genotypes declined, it was occasionally eliminated by one of the background genotypes before the eventual fixation of 67*adm*.

Evolutionary Dynamics

Tierra displays vigorous microevolutionary dynamics, with a large number of viable genotypes appearing through mutation and persisting for varying lengths of time. Furthermore, there was a marked process of successive substitution, with novel genotypes spreading and displacing those previously dominant before being displaced themselves. Evolution was highly contingent, with different sequences of dominant genotypes in each experiment; we were unable, indeed, to find any case in which a dominant genotype appeared in more than one experiment.

Directional Trend in Genome Size. Contingency at a genotypic level was accompanied by phenotypic convergence. There was a consistent tendency in all experiments for reduced genome size, indicating strong natural selection for rate of replication. Final sizes in the five high-mutation experiments after 300 generations of culture were 67, 69, 64, 68, and 68 instructions (mean 67, 1.7 SD), representing an average loss of nearly 20% of the ancestral genome. With lower mutation rate, the response to selection was less, as expected: genome size decreased about 10% to 73 instructions over 1,000 generations in one experiment and 15% to 68 instructions over 2,000 generations in the other. At very low mutation rate, genome size decreased by only a few percent over comparable periods of time, but the total response to selection after about 10,000 Tierran generations was similar to that observed in the other experiments. The large-population experiments featured final sizes of 63, 66, and 62 (mean 63.67, 2.08 SD) after selection times ranging between about 10,628 and 20,000 U. Size reduction was not an invariable rule: on many occasions a longer sequence displaced a shorter sequence, although it was never much longer. Although this was observed within populations, however, within lineages there is almost always a monotonic decline in genome size. Optimization proceeded almost exclusively through reduction in genome size and the accom-

Earlier	Later															
genotype	genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	80 <i>aaa</i>		2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	79auk			=	4	5	6	7	8	9	10	11	12	13	14	15
3	72 <i>aaa</i>				4	5	6	7	8	9	10	11	12	13	14	15
4	76adr					5	6	7	8	9	10	11	12	13	14	15
5	74 <i>aca</i>						6	7	8	9	10	11	12	13	14	15
6	69 <i>aaa</i>							7	8	9	10	11	12	(13)	14	15
7	68baw								8	9	10	(11)	7	7	(14)	15
8	67 <i>adm</i>									8	10	11	8	8	8	15
9	67 <i>ast</i>										9	9	9	9	9	15
10	68 <i>gge</i>											(10)	10	10	(10)	15
11	68 <i>hbs</i>												11	11	(14)	15
12	69 <i>kvp</i>													13	14	15
13	69 <i>fdx</i>														14	15
14	68 <i>fhb</i>															15
15	66 <i>akd</i>															

Table 3: Outcome of pairwise competition experiments

Note: The genotype named was the winner. When in parentheses, (X), genotype X was considerably more abundant and was still increasing in frequency at the end of the experiment; "=" is a case in which the outcome was a stable polymorphism.

panying shortening of gestation time; we did not observe the phenomenon of "unrolling the loop" in any of the experiments (see, e.g., Cho and Ray 1995). (This mechanism allows creatures to gain considerable efficiency by copying more than one instruction during a single iteration of the copy loop.) We do not interpret the occasional appearance of a larger genotype in a dominant lineage, or the occasional displacement of a smaller dominant type by a larger one, to be evidence of "code bloat" (see, e.g., Soule and Foster 1998). This has been postulated to be a mechanism that might protect the creatures from the destructive effects of processes such as mutation and crossing over; a creature that accumulates nonexecuted junk code has a decreased probability of vital code being affected by these processes. In an autoadaptive genetic system populated by self-replicators (particularly under the conditions used here), fitness is highly related to the rate of replication. This need not be the case in standard evolutionary computation approaches, where fitness is defined by an extrinsic function that evaluates the competence of a particular program at performing a specific task and where replication is effected by the supervisory software (Fogel 2000). Thus, any considerable increase in size will almost always have a detrimental effect on fitness (under the conditions used here). A concrete example can be given for a genotype such as 82aay from the experiment used for the competition trials. This creature descends from a size 74 ancestor at around 1,875 U of the experiment (while 74aca was dominant) and has indeed acquired a section of extra code that is copied but not actually executed. However, the resulting increase in gestation time (612 instructions vs. 556 for 74aca) and reduced pure-culture rate of increase (1.164 creatures per thousands of instructions executed vs. 1.27 creatures per thousands of instructions executed for 74*aca*) ensured that this genotype was unsuccessful compared to shorter, more rapidly replicating types that arose during the experiment.

Periodic Selection in Relation to Mutation Rate. At very low mutation rate, dominant genotypes attained very high frequencies and were often fixed. As a dominant genotype was being replaced by its successor, there was necessarily a period of transient polymorphism and an increase in diversity. It sometimes happened that during this transition period a third genotype superior to both its predecessors arose, in which case a more lengthy period of polymorphism would ensue. The basic pattern, however, was one of simple periodic selection. At intermediate mutation rate, the frequencies attained by the dominant genotypes were generally <0.95, and the population included a large number of rather rare genotypes and maintained a greater level of diversity. At high mutation rate, dominant genotypes seldom attained very high frequencies. The occasional genotype maintained frequencies of 0.8-0.9 for short periods of time, but this was very unusual: most dominant genotypes never exceeded a frequency of 0.4 or thereabouts. At the same time, there were always very many rare genotypes so that the frequency distribution of abundance was J-shaped, albeit with a clear mode (see Adami et al. 1995 and Bell 2000 for descriptions of models that can give rise to such a distribution). The mode of the distribution (at low abundance) was very dynamic, its composition changing rapidly over time as the result of mutation pressure and stochastic loss.

The distribution of abundance among genotypes can be summarized as a measure of genotypic diversity, such as the

Resident	Invading															
genotype	genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	80 <i>aaa</i>		+	+	0	+	+	+	+	+	+	+	+	+	+	+
2	79auk	0		0	0	+	+	+	+	+	+	+	+	+	+	+
3	72 <i>aaa</i>	0	=		=	+	+	+	+	+	+	+	+	+	+	+
4	76adr	0	0	0		+	+	+	+	+	+	+	+	+	+	+
5	74 <i>aca</i>	0	0	0	0		0	0	0	+	0	0	0	0	0	0
6	69 <i>aaa</i>	0	0	0	0	0		0	0	0	0	0	0	0	0	0
7	68 <i>baw</i>	0	0	0	0	0	0		0	0	0	0	0	0	0	0
8	67 <i>adm</i>	0	0	0	0	0	0	0		0	0	0	0	0	0	0
9	67 <i>ast</i>	0	0	0	0	0	0	0	0		0	0	0	0	0	0
10	68 <i>gge</i>	0	0	0	0	0	0	0	0	0		0	0	0	0	0
11	68 <i>hbs</i>	0	0	0	0	0	0	0	0	0	0		0	0	0	0
12	69 <i>kvp</i>	0	0	0	0	0	0	0	0	0	0	0		0	0	0
13	69 <i>fdx</i>	0	0	0	0	0	0	0	0	0	0	0	0		0	0
14	68 <i>fhb</i>	0	0	0	0	0	0	0	0	0	0	0	0	0		0
15	66akd	Ο	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table 4: Outcome of invasion experiments

Note: The "+" indicates that the invasion was successful, the invader eliminating the resident; "O" indicates that it was unsuccessful. In two cases there appeared to be long-term coexistence with both types at appreciable frequencies.

variance of population frequencies. We have used the wellknown Simpson's Index (SI), which takes high values (close to 1) when diversity is least and low values (close to 0) when diversity is greatest. Periodic selection should show a perennially low level of diversity, with the population dominated by a single genotype, punctuated by spikes of high diversity marking the passage of a beneficial mutation. Broadly speaking, this pattern is shown at very low mutation rate, where the fixation of successive dominants is marked by peaks of high SI separated by troughs of low SI (fig. 6A). The peaks are rather abrupt, however, because most dominants began to be displaced soon after having been fixed. As the mutation rate increases this pattern becomes more obscure (fig. 6B), and at high mutation rate the populations maintained a more or less constant level of high diversity (fig. 6C). In high-mutation-rate populations, most individuals belong either to very rare or to very abundant genotypes, with a deficiency of individuals belonging to genotypes of intermediate abundance.

Temporal Pattern of Substitution. The temporal pattern of substitution can be seen most clearly in low-mutation experiments, where there is a fairly simple sequence of dominants. At very low mutation rate the durations of successive dominants following the ancestor 80*aaa* were 6,075, 6,300, 17,350, 5,600, 7,375, 5,950, 1,525, 2,575, 10,400, 8,575, 10,200, 15,320, and 4,225 U in one experiment and 13,350, 11,575, 2,025, 500, 2,500, 4,550, 425, 31,575, 225, 16,000, and 12925 U in the other. At intermediate mutation rate, the corresponding durations were 1,350, 300, 2,975, 1,025, 1,525, and 1,250 U in one experiment and 4,675, 2,800, 1,625, 14,400, and 5,800 U in the other. At high mutation

rate dominants overlap extensively, and it is difficult to give a simple description of the duration of successive dominants, but in the least complicated case, the durations of successive dominants following the ancestor 80*aaa* were 175, 475, 350, 350, 375, 25, and 700 U. The last figure is a minimum since the run was terminated while the genotype was still dominant. In no case does there seem to be any tendency for the interval between substitutions to lengthen over time. The pattern in the other high-mutation experiments, although more complex, was consistent with this conclusion.

Evolution in Large Populations. The large-soup experiments displayed results similar to the other low-mutation-rate experiments, in spite of the larger soup size. The sequence of succession in these experiments was as follows (length of dominance [time given in update]):

First, 80 <i>aaa</i> (1,979)—78 <i>abd</i> (2,877)—75 <i>aeg</i> (2,805)—
70 <i>acf</i> (1,116)—63 <i>aaa</i> (1,850(end)).
Second, 80 <i>aaa</i> (3,249)—76 <i>abc</i> (1,935)—
72 <i>aab</i> (990)—71 <i>acu</i> (4,680)—69 <i>aay</i> /70 <i>acn</i> /71 <i>acu</i>
polymorphism(125)—69 <i>aay</i> (1,585)—69 <i>aay</i> /
66 <i>aaj</i> polymorphism(39)—66 <i>aaj</i> (3,107(end)).
And third, 80aaa(1,161)-78aau(1,344)-
77 <i>aen</i> (2,344)—74 <i>ach</i> /77 <i>aen</i> polymorphism(59)—
74 <i>ach</i> (4,375)—69 <i>aac</i> (5,160)—69 <i>aac</i> /65 <i>aab</i>
polymorphism(28)—65 <i>aab</i> (2,880)—65 <i>aab</i> /62 <i>aad</i>
polymorphism(20)—62 <i>aad</i> (2,615(end)).

Again, there is evidently no consistent tendency for the interval between successive substitutions to increase through time.

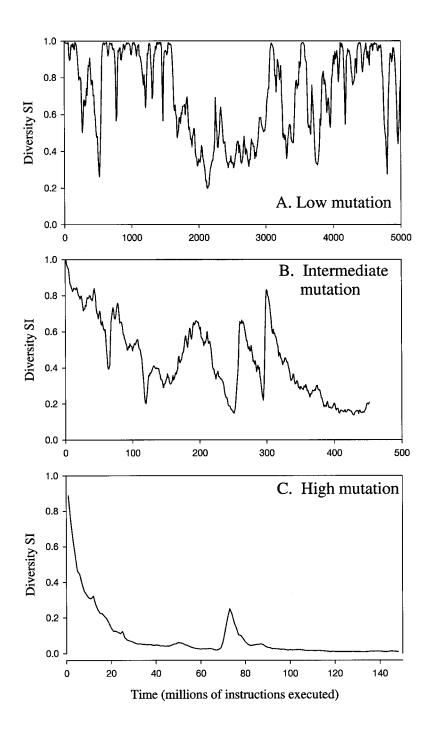


Figure 6: Plots of diversity as Simpson's Index (SI) through time at low (A), intermediate (B), and high (C) mutation rates

Genealogy of Dominants. At very low mutation rate each new dominant genotype descends directly from the previous dominant, as the classical account of successive substitution anticipates (fig. 7). (The only exception to this rule is the

bridging genotype involved in the mechanism of size change, which is explained in the section below.) At intermediate mutation rate the same process can be observed, but dominants sometimes descended from genotypes that

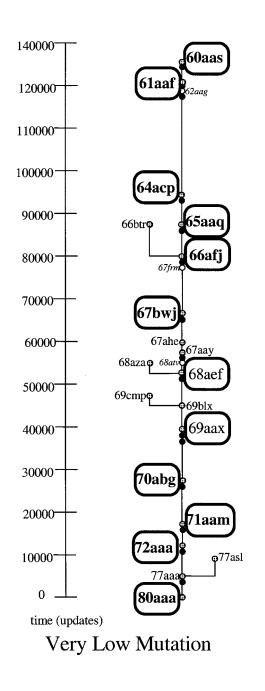


Figure 7: Genealogies of dominants. Lines connect successive descendants. Symbols indicate nature and frequency of genotype. Filled circles are specific mutators in all plots. Mutation = 0.002 per genome per generation. Stippled circles indicate dominant genotypes. Large ovals with thick borders indicate a maximum frequency > 0.8; name in bold type indicates fixation of that genotype.

were only moderately abundant (fig. 8). At high mutation rate the simple classical picture breaks down completely, and dominant genotypes do not, in general, descend directly from previously dominant genotypes (fig. 9). Rather, dominants descend from previous dominants through a string of intermediate, nondominant genotypes. In the great majority of cases, indeed, dominants emerge from the fog of rare genotypes that collectively constitute a large fraction of the population.

We considered two interpretations of this phenomenon. The first was that new dominants arose from "hibernating" genotypes that arose early in the experiment, persisted for

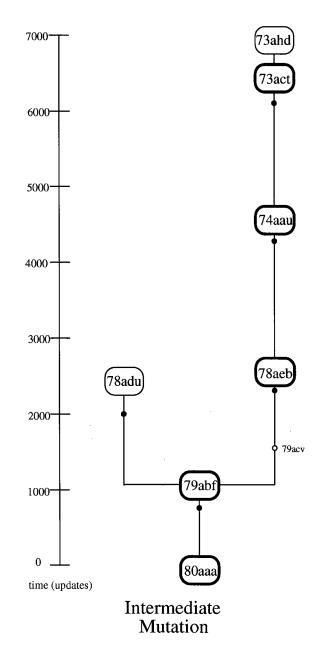


Figure 8: Mutation = 0.01 per genome per generation. Filled circles represent specific mutators. Open circle indicates a maximum frequency < 0.05; large ovals with thin border, frequency = 0.4-0.8; and large ovals with thick border, frequency > 0.8.

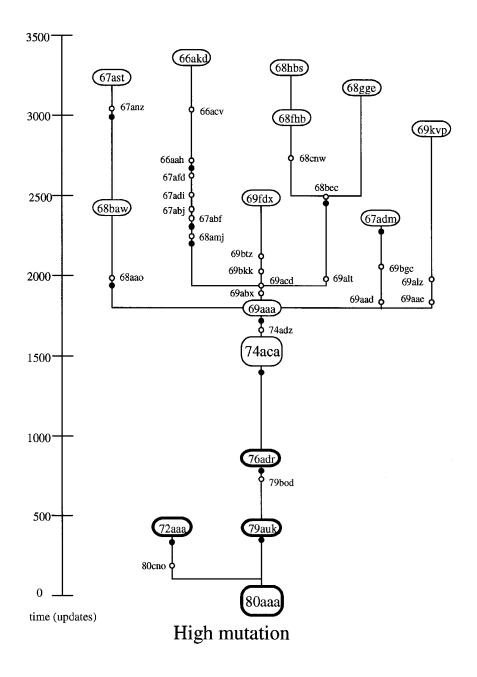


Figure 9: Mutation = 0.2 per genome per generation. Filled circles represent specific mutators. Open circles indicate a maximum frequency < 0.05; small ovals with thin border, frequency = 0.05-0.01; small ovals with thick border, frequency = 0.1-0.4; large ovals with thin border, frequency > 0.4-0.8; and large ovals with thick border, frequency > 0.8.

long periods as rare types, and much later succeeded in spreading through the population. The second was that new dominants descended from rare types that had appeared for the first time only recently. We did not observe in any case an early-evolving rare genotype persisting as such for many generations and then suddenly breaking through and becoming dominant at a much later point in time. There is, instead, a clear temporal succession taking place, whereby later-evolved genotypes succeed earlier-evolved ones.

The genealogies from the large-population experiments were largely consistent with those from the low-mutation experiments with small populations, although they displayed a greater degree of clonal interference (Gerrish and Lenski 1998). In each of the three experiments, there is at

	Initial genotype	<u>80aaa</u>	<u>80cno</u>	<u>80dwy</u>	<u>72aaa</u>	Final genotype
10	no operation, value 1	nop1	nop1	nop1	nop1	no operation, value 1
I 1	no operation, value 1	nop1	nop1	nop1	adrb	address back
I 2	no operation, value 1	nop1	nop1	nop1	nop0	no operation, value 0
I 3	no operation, value 1	nop1	nop1	nop1	mal	allocate memory to daughter
I 4	set value in register to zero	zero	zero	zero	nop0	no operation, value 0
I 5	flip first-order bit of register	not0	not0	not0	nop0	no operation, value 0
I 6	shift left	shl	shl	shl		
Ι7	shift left	shl	shl	shl		
I 8	move contents of one register to another	movDC	nop1	nop1		
I 9	address back	adrb	adrb	adrb		
I 10	no operation, value 0	nop0	nop0	nop0		
I 11	no operation, value 0	nop0	nop0	mal		
I 12	no operation, value 0	nop0	nop0	nop0		
I 13	no operation, value 0	nop0	nop0	nop0		
	phenotype:	wild- type	quasi- neutral	specific mutator	superior smaller	

mutational series

Figure 10: Mechanism of genome size change

least one case where a dominant mutant arises from a nondominant ancestor, which is itself derived from the previously dominant type. The nondominant intermediate experiences a second beneficial mutation that then sweeps to dominance, eliminating both the parental and grandparental types. One of the these experiments even featured a "leapfrog" event of the type described by Gerrish and Lenski (1998), where a first beneficial mutant of size 70 is displaced by a second, fitter size 69 mutant derived from the same size 71 ancestor. However, we still did not observe the multiplicity of coextensive quasi-neutral types seen in the highmutation experiments, and in general, both new dominant types and nondominant intermediates arise "directly" from the previously dominant type, displaying a pattern of sequential substitution.

Evolutionary Mechanisms

We identified two qualitatively different kinds of genetic mechanisms underlying evolutionary change, "Gradual Selection among Quasi-Neutral Variants" and "Change in Genome Size through Specific Mutators," which are discussed below.

Gradual Selection among Quasi-Neutral Variants. Nonlethal point mutations can increase efficiency without changing

genome size, usually by reducing gestation time. Their effect is always slight, generating selection coefficients of 0.01 or less, and drives a slow and limited process of adaptation.

Change in Genome Size through Specific Mutators. Evolution in Tierra, as we have set it up, occurs largely by the successive substitution of progressively smaller genotypes. We have identified the genetic mechanism that usually underlies size reduction as an indirect consequence of the template matching used by Tierran creatures to calculate their size and to localize their various subroutines. It can be explained most clearly with a concrete example, which we take from one of the high-mutation experiments (summarized in fig. 10).

In order to determine its size, a creature first locates its beginning and end using a combination of the *adrb* (address back: put the value of the template represented by the immediately following instructions into a register for template matching and then search backward for the complementary template) and *adrf* (address forward) instructions. Each of these is followed by a series of *nopX* (no operation, value X = 0 or 1) instructions that form the actual template. Tierra works by seeking the complement of the current template; for example, the series *adrb-nop0-nop0-nop0-nop0-nop0* will search backward and attempt to find a series of four *nop1s*, storing the address of the first *nop1* in the CPU's

AX register. The series adrf-nop0-nop0-nop1 looks forward for nop1-nop1-nop0, which forms the creature's tail and stores the address of the last *nop0* in the AX register. Changes may arise when one of the template markers is changed by mutation. For example, genotype 80cno from the experiment used for the competition trials is a stable replicator, differing from 80aaa only by the replacement of a movDC (copy contents of CX register into DX register) instruction in position 8 to a nop1. This change does not appear to have any deleterious effect on the algorithm as a whole. In 80dwy, a descendant of 80cno, a nop0 in position 11 is changed to mal. The adrb in position 9 now reads the template as being only a single instruction, the nop0 directly succeeding it. Searching backward, it finds the first corresponding nop1 in position 8 and stores that as its beginning-positions 0-7 are not searched. This causes the creature to believe that its "head" actually begins at position 8 and not at position 0. When the creature finds its end through the *adrf* instruction at position 16 and then adds 1 to arrive at a measure of its size, it will calculate its length (through the subCAB instruction) as being only 72 instructions in length and not 80. This change is reflected in the progeny of 80dwy: 72aaa does indeed begin with the sequence nop1-adrb-nop0, corresponding to positions 8-9-10 in 80*dwy*. The genotype 80*dwy* is, thus, a directed mutator, consistently giving rise to a particular mutant genotype unlike its own. In this case, the mutant is not only viable but is actually fitter than its parent and acts as an intermediate step whereby size change is affected. "Bridging" genotypes such as 80*dwy* usually exist only as new single-copy mutants that give rise to and are eventually eliminated by a truebreeding descendant. In evaluating the ancestry of successive dominant types, therefore, it is important not to regard these bridging types as being equivalent to normal genotypes present at low frequency.

Other mechanisms whereby size can change have been identified, but they are much rarer and less completely understood. Almost all evolutionary change in the system that we set up was based on slightly beneficial variants produced by point mutation and the process of size change described above.

Discussion

Tierra as Simulacrum

The genetics, physiology, and ecology of Tierra differ in many respects from their counterparts in the organic world. In the first place, we anticipate the genetic system of its inhabitants to be more brittle than organic systems based on nucleic acids, for reasons we have pointed out. This should imply that a greater proportion of mutations will be severely deleterious, and a smaller proportion

quasi-neutral. In itself, however, this should lead to populations fixed for dominant genotypes, with very few rare variants, undergoing classical periodic selection at long intervals. What we observed, instead, when mutation rate was moderate or high was a large dynamic pool of rare genotypes, most of which were viable and had similar values of fitness. The system seems likely, therefore, to be rather well connected, in the sense that it will often be possible to pass from an inferior to a substantially different superior genotype through a sequence of quasi-neutral variants in which each step involves a single mutation only. This fulfils a basic requirement for evolution through natural selection. Robustness of this sort has previously been demonstrated at a phenotypic level when evolutionary methods are used to generate specific hardware configurations that are tolerant to various types of computational and environmental insults (see, e.g., Thompson 1996; Masner et al. 1999), and it may be a general feature of any evolved system. Robustness is also observed in autoadaptive systems when self-replicating programs are allowed to evolve in more complex environments than those used in this study (Lenski et al. 1999; Adami et al. 2000).

Second, the process of replication is quite different in Tierra. Genes made of nucleic acid are transcribed and translated in a linear fashion, generating and utilizing protein phenotypes. Under the conditions used here, there is no distinction corresponding to that between transcription and translation, or genotype and phenotype, although such distinctions emerge in environments conducive to learning tasks, where similar phenotypes (the task[s] performed) may have very different sequences underlying them (see, e.g., Adami 1998). The program directs its own replication without requiring the analogue of a replicase or any other protein support or catalyst, though the CPU is, of course, required to execute the instructions. The nearest organic analogue might be a truly self-replicating ribozyme. Even in this case, however, the Tierran mechanism of replication is quite different. It is based on execution loops, for which there seems to be no good analogy in the organic world. One property of such a system, for example, is that the instruction pointer can jump backward and forward within (or even outside) a program, resulting in a nonlinear flow of control (Ray 1991). This may have unexpected physiological consequences, the most remarkable being the genetic basis of size change described above.

Finally, although interactions among Tierran organisms may be affected by proximity, Tierra does not possess mappable space in any useful sense. It might be thought of as linear, in the sense that individuals are queued for replication, but this is likely to be misleading. Offspring may be born anywhere in the soup, regardless of the location of their parent. The instruction pointer does not necessarily pass in an orderly fashion from one point to the next but may be directed, instead, to an unexpected location in the process of executing a sequence of instructions. Because all points in memory are linked through the CPU, indeed, the meaningful distance between points is the time required to go from CPU to memory, which is the same for any pair of points. The ecology of Tierra is likely to have features for which no analogue exists in the organic world.

Evolution in Tierra

The exotic ecology, physiology, and genetics of Tierra obviously suggest that its evolutionary processes may also differ from those of organic systems. This is not necessarily the case. The principles that govern evolutionary divergence and biological diversity are different in kind from those involved in physiology and transmission genetics. They concern the behavior of open systems of self-replicators with indefinite variation and selection. These principles may apply to any such replicators. It would be absurd to use the Tierran organisms as models of individual animals and plants because the principles of physiology and genetics rest ultimately on molecular interactions that do not occur in computers. It is by no means absurd to use Tierran populations as models of populations of animals and plants because replication, variation, and selection can occur just as well inside computers as outside, and their consequences are not constrained in any fundamental way by the physical garb that individuals assume. There may well be some rules governing the evolution of chemostat populations that are attributable to the properties of DNA-protein systems or of some other aspect of microbial biology. There will be other rules, however, arguably the most important ones, that will characterize the evolution of any open system of selfreplicators, and it is these that experiments with autoadaptive systems can address. These systems have already been used to address different problems in evolutionary biology and genetics, such as the "age-area" abundance distribution problem of Willis (Adami et al. 1995) and the role played by epistasis in genome complexity and robustness (Lenski et al. 1999).

Tierran populations, indeed, seem to behave in a rather simple fashion that can readily be understood in terms of conventional theory. They possess heritable variation in replication rate that is directly associated with the outcome of selection in mixed cultures. The physiological basis of this variation—gestation time—is easy to understand, and because it is correlated with genome size, it leads to an indirect response that fuels a directional evolutionary trend. The process is obstructed by sampling error, especially in the establishment of new beneficial mutations. Moreover, the great range of available viable mutational changes causes the genetic basis of the indirect phenotypic response to vary among replicate selection lines. These observations indicate that Tierra is a tractable system that can be used to mirror the behavior of populations of organisms despite the exotic nature of its individuals.

The Microevolutionary Process

There is a clear general tendency for the evolution of smaller genomes, presumably through selection for more rapid replication. The parallel with the evolution of smaller genomes in viral populations maintained by serial transfer (reviewed by Spiegelman 1971; Orgel 1979; Biebricher 1983) is most striking. The Tierra results are perhaps the more surprising, in that viral genotypes are propagated using a replicase supplied by the experimenter, and can, therefore, shed a great deal of unnecessary RNA, including that encoding the replicase. Somewhat similar events can occur in Tierra when parasites capable of reading and executing the copy loops of other individuals evolve. This was prevented in our experiments, however, and all the successful short variants that appeared were autonomous self-replicators.

Selection does not favor small genome size itself but, rather, rate of replication, with which genome size is correlated. The distinction can be appreciated when the correlation breaks down. In the high-mutation experiment illustrated in figure 9, for example, 79*auk* was able to coexist for 350 U with 72*aaa*, despite being 10% larger. This is because rate of replication depends not on genome size itself but, rather, on the gestation time (I_E): a shorter genome may execute more instructions if it encodes redundant operations, such as reading a self-identification loop twice or making an unnecessary memory allocation without any corresponding attempt to replicate. In this case, 72*aaa* requires 742 I_E to replicate, whereas 79*auk*, though larger, requires only 739 I_E .

Phenotypic convergence was accompanied by marked genotypic contingency, with the identity and sequence of genotypes being different in all the lines that we observed. A similar pattern has been reported for chemostat cultures of the DNA bacteriophage fX174 (Wichman et al. 1999).

Microevolutionary Pattern

The pattern of events that we observed depended on the genomic mutation rate. Our simulations cover most of the range of reasonable values for the genomic mutation rate (reviewed by Lynch et al. 1999). Drake et al. (1998) suggest that mutation rates among microbes are only about 0.003 per genome when the estimate is confined to coding DNA, near the lower end (0.005 and 0.001) of the range we used. Most reports seem to establish a lower bound of between 0.4 and 1.0 per genome per generation for multicellular

eukaryotes (Mukai 1964; Mukai et al. 1972; Charlesworth et al. 1990; Johnston and Schoen 1995; Deng and Lynch 1997; Drake et al. 1998). This is broadly consistent with a rate of 0.003 per germ-line replication and lies slightly above the upper end (0.2) of the range we investigated.

At the lowest mutation rate, microevolution in Tierra approaches the classical picture of periodic selection, with the dominant genotype at any given time attaining very high frequency, having descended directly from the preceding dominant type. Furthermore, selection seems to operate quite straightforwardly by favoring genotypes with high rates of replication in pure culture. If the genomic mutation rate in haploid microbes is indeed much less than one per replication, then our results broadly support a simple interpretation of evolutionary dynamics in chemostats.

As mutation rate increases, this simple interpretation begins to break down, and it seems appropriate to ask whether a more complex interpretation, involving dominant genotypes often descending from rare ancestors and themselves attaining only moderate frequency, might sometimes be required to understand microbial evolution. The evidence for classical periodic selection remains strong but is, nevertheless, indirect. It is based primarily on three observations. The first is the fluctuation in frequency of rare quasi-neutral markers, which are interpreted to mark the passage of successive beneficial mutations. Although these fluctuations undoubtedly occur and must often bear the conventional interpretation, they cannot always be interpreted straightforwardly and sometimes seem to occur too rapidly to be associated with successive complete substitution (Adams and Oeller 1986). Rare or moderately abundant Tierran genotypes fluctuate rapidly in frequency, and any particular genotype would be likely to decline abruptly during the passage of an exceptionally successful mutation, as shown in figure 4C by the precipitous decline of diversity at around 1,750 U caused by the passage of 74aca. Second, there is a steplike increase in mean fitness, or any correlated phenotype, caused by the occasional passage of beneficial mutations. This is a phenotypic rather than a genotypic observation, and the pattern is not always clear even in the best experiments (e.g., Lenski and Travisano 1994; fig. 5). At a phenotypic level, indeed, the same phenomenon would often occur in Tierran populations-an abrupt shift from one genome size to another. Finally, chemostat populations remain uniform to all appearances between substitutions. Again, this is a phenotypic observation that would often apply to Tierran populations, where the bulk of the population is often of the same size or narrow range of sizes. It might be argued that the genotypic diversity that we observe merely reflects quasi-neutral variation around what is essentially a single type present at very high frequency. This is not always the case. Where several genotypes are fairly abundant, they are not very closely related and often belong to distinct, widely separated lineages. These moderately abundant genotypes are highly stable replicators: no new genotypes appeared, for example, when mutation was switched off during the pairwise competition trials, which comprised in excess of 37,500 U. Without denying that simple periodic selection may often be the rule, it may be worth investigating the possibility that microbial populations sometimes exhibit the same underlying diversity and patterns of succession that are seen in Tierra at moderate or high mutation rate.

At high genomic mutation rates of 0.2 per replication, the classical process breaks down completely, with the production of large numbers of quasi-neutral variants, many of which may be reasonably abundant at any given time. At the highest mutation rates that we have investigated, the population becomes a swarm of genotypes resembling the molecular quasi-species (Eigen et al. 1988). (The parallel may not be precise because quasi-species theory assumes the presence of a master sequence, and it is not always clear in our high-mutation experiments whether any such sequence exists, in populations where genome size is variable and no single genotype has very high frequency.) At this point, Tierra resembles a high-mutation system such as populations of RNA viruses. It will also resemble a population of multicellular organisms, given a genomic mutation rate close to one per generation. The main difference is that the Tierran genome is very small so that the fraction of instructions that mutate each generation is much greater than in a fly or a worm. The simple replacement of one dominant type by another occurred mostly in the early stages of evolution and was usually succeeded by a period in which several genotypes fluctuate in frequency without any one obtaining a decisive advantage, until the whole set is replaced by another set behaving in a like fashion. These periods of complex dynamics were sometimes interrupted by the ascendancy of a single genotype. There was no clear difference between the longevities of dominant lineages early and late in succession and, therefore, no clear evidence for a depletion of mutational variation or a weakening of selection in the short term of a few hundred generations. The replicate experiments differed chiefly in the time of onset of complex dynamics and the occurrence of subsequent dominants. The evolutionary process was highly contingent in detail, with each replicate following a unique pathway, even though the lines evolved phenotypically in the same direction, at least with respect to genome size.

Use of Autoadaptive Genetic Systems in Evolutionary Biology

Although the physiology of Tierran organisms is bizarre, their evolutionary dynamics seem likely to resemble those of microbes in most essential respects. Within the rather severe restrictions that we have imposed on the system, the microevolutionary patterns of short-term change within populations are consistent with conventional expectation and can be interpreted in a straightforward manner. Used in this way, the system can be viewed as a halfway house between conventional algebraic theory, which generates analytical predictions at the expense of making restrictive assumptions, and populations of microbes, where the details of evolutionary mechanics are often inaccessible. Tierra cannot be completely understood, in the sense that an algebraic argument can be understood, but it can be unambiguously interpreted because it permits complete knowledge of the outcome of experimental manipulations. It could find a role, therefore, in verifying predictions made by conventional theory in the context of an independent system of evolvable self-replicators. Its usefulness in these relatively simple situations, however, gives us confidence that it can be extended to tackle problems that lie beyond the scope of conventional theory. To give a simple example, it is not clear, even within the confined circumstances of the system that we used, whether there is a definite end point to the trend to smaller genome size. The lack of any appreciable diminution in the interval between dominants suggests that there remains an abundance of fresh mutational variation to fuel a continued decline, but it is not known whether this process will eventually come to a halt or result in some cyclical or fluctuating alternation of small genomes. If it does eventually come to a halt, it is not known whether there is a unique end point or a number of possible final states. These issues lie beyond the reach of conventional theory but are readily studied in autoadaptive systems like Tierra. However, these kinds of studies also hold considerable interest outside the purely biological realm; we will need to consider how robotic "lifeforms" (and the software that directs them) may evolve in the future, given that the feasibility of using evolutionary methods to design simple, self-replicating robots has now been demonstrated in principle (Lipson and Pollack 2000).

Carroll (2000) has argued strenuously that the evolution of the major features of organic design often involves qualitative changes in the organization of the genome. The example he gives is the origin, duplication, and divergence of the Hox genes underlying the origin and diversification of metazoan body plans. He criticizes contemporary population genetics theory for failing to come to grips either with the emergence of new patterns of gene action or with the ensuing dynamics of explosive diversification succeeded by stasis. It is difficult to see, however, that conventional theory is capable, even in principle, of dealing with evolutionary novelty. Self-replicating programs, on the other hand, provide open systems in which the emergence and fate of new modes of genome organization can be studied. We have investigated only a highly constrained version of Tierra in order to understand the mechanisms of microevolutionary change and to show that the patterns of change are consistent with established general principles so that we can plausibly use the system to accomplish a modest extension of conventional theory. Released from these constraints, however, it is known that qualitatively different kinds of replicator can evolve (Ray 1991, 1994b), although the macroevolutionary processes involved have yet to be investigated systematically. The expanded evolutionary synthesis that Carroll claims to be necessitated by recent advances in paleontology and developmental biology may require the expanded theoretical horizon provided by autoadaptive genetic systems. The major contribution that such systems can make to evolutionary biology is that they provide us with an open system in which adaptation is the consequence of new and unforeseen variants arising during the course of our experiments. This may enable us for the first time to construct rigorous theories of long-term evolutionary change.

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