Evolution of Stable Ecosystems in Populations of Digital Organisms

Tim F. Cooper and Charles Ofria

Center for Microbial Ecology
Michigan State University, East Lansing, MI 48824

Abstract

Competition for resources has long been believed to be fundamental to the evolution of diversity. However, the difficulty of working with natural ecosystems has meant that this theory has rarely been tested. Here, we use the Avida experimental platform to demonstrate the evolution of ecosystems composed of digital organisms. We show that stable ecosystems are formed during evolution in an environment where organisms must compete for limiting resources. Stable coexistence was not observed in environments where resource levels were not limiting, suggesting that competition for resources was responsible for coexistence. To test this, we restarted populations evolved in the resource-limited environment but increased resource levels to be non-limiting. In this environment, ecosystems previously supporting multiple genotypes could maintain only a single genotype. These results demonstrate the utility of the Avida platform for addressing ecological questions and demonstrate its potential in addressing questions involving ecosystem-level processes.

Introduction

One of the most striking features of life is its diversity. However, the selective pressures which have led to this diversity have not yet been comprehensively identified (Morin 2000; Schluter 2000; Tilman 2000). In part, the lack of progress has been due to the inherent difficulty of performing precise, replicated and controlled experiments on whole ecosystems (Morin 2000). In an effort to ameliorate these limitations, some researchers have turned to laboratory microcosms (reviewed in Travisano & Rainey 2000). However, even in these model systems, ecosystems can evolve to be much more complex than is experimentally tractable and identification of the causes of diversity can still be difficult (Notley-McRobb & Ferenci 1999; 1999; Rainey & Travisano 1998).

Competition between organisms for limited resources has long been suspected to play a key role in the structuring of communities (Tilman 2000; Schluter 1996; Tilman 1982). Though proof of this idea has been deceptively difficult to come by (Schluter 1996; 2000), the reason for its enduring appeal is obvious: if organisms deplete resources as they use them, the benefit for their continued use will diminish and organisms able to use underutilized resources will be favored. Over time density-dependent selection created by this process can lead to adaptive radiation and even speciation as organisms diverge to into distinct lineages (Schluter 2001).

In this study, we use digital organisms (self-replicating and evolving computer programs) to examine the diversity arising in populations during adaptation to an environment containing nine distinct resources. (We use the term population to refer collectively to all organisms in the environment.) We compare the result of adaptation when resources are in infinite supply to when resources can be depleted. Because there are no limiting resources in the former environment, only one niche is present; the organism most efficiently using the most valuable resource combination will always have a selective advantage and be able to outcompete all other organisms (Kassen, *et. al.* 2000; Hardin 1960; Tilman 1982). In contrast, in the depletable resource environment, all resources can be limiting, potentially creating as many niches as resources and thus allowing as many species as resources to coexist (Bell 1997; Huisman 1999).

We show that stable ecosystems evolved in populations grown in environments where organisms competed for depletable resources. Diversity was maintained for many thousands of generations when the mutation rate was set to zero but rapidly disappeared when we changed the environment to make resource levels infinite.

The Experimental System

The Avida platform is a computer software system in which digital organisms evolve by means of random mutation and natural selection (as opposed to artificial selection, as in genetic algorithms) (Ofria, Brown, & Adami 1998)[1]. The genetic code of the digital organisms in Avida is a Turing complete programming language. To replicate, a digital organism performs a self-analysis that determines information about its genome,

---

[1] The Avida software is available from http://nemus.dllab.caltech.edu/avida/

it then allocates enough extra memory to hold its offspring, and copies its genome line by line into that extra space before finally dividing off the offspring as an independent organism. This replication process is subject to two types of errors: copy mutations, where a random instruction is written in place of the ancestral one and insertion/deletion mutations, where a random instruction is added or removed from the genome.

In Avida, the success of an organism depends upon its growth rate. This is determined by an organism's replication efficiency and by its interactions with the environment. An Avida environment determines the mutation rates the organisms are subjected to, as well as the collection of resources that they can use to increase the rate at which they execute the instructions contained in their genomes. To "metabolize" a resource and garner a benefit from it, an organism must perform a mathematical computation specified by the experimenter. Resources are globally available to all organisms. In previous versions of Avida, resource levels were infinite, such that the number of organisms using that resource did not affect its level or the benefit gained for its use. In this study, we add the capability of feedback between the use of a resource and its level, such that the use of a resource by one organism lowers the availability of that resource to all others. A low concentration of a resource will reduce the benefit that can be gained by performing its associated computation. This model of resource competition is analogous to that occurring during competition in bacterial chemostats.

In Avida the experimenter determines the environment that populations will evolve in, but does not specify which organisms are favored. Instead, the outcome of the evolutionary process is determined by population genetic processes, that is, natural selection and genetic drift. For this reason, Avida populations are genuinely evolving systems, rather than being "simulations" of evolution. Avida allows perfect knowledge of the genetic, phenotypic and ecological state of the system at all times and ensures that no measurement influences the course of evolution. These attributes have enabled Avida to be used to address fundamental evolutionary questions in ways not possible with traditional systems (Lenski, *et. al.* 1999; Adami, *et. al.* 2000; Wilke, *et. al.* 2001).

Materials and Methods

The experiments reported here were performed using version 2.0beta of the Avida software. Unless otherwise noted, each replicate evolving population was started with identical initial conditions but with a different random number seed. This seed causes runs to differ at points where a random choice is made, such as the occurrence of mutations or the location of an offspring in the population.

The genome of the ancestral organism in each run was 100 instructions in length. This organism could self-replicate but could not perform any logical computations. The descendants of this organism rapidly filled up each population (to its maximum capacity of 2500). During the first 100,000 updates[2] of evolution the copy mutation rate was set to 0.0075 per instruction and the insertion/deletion rate was set to 0.05 per genome. After the first 100,000 updates we turned off all mutations and let the population replicate for an additional 50,000 updates. This latter phase allowed us to identify genotypes able to stably coexist as a result of ecological interactions without the complications of new genotypes being introduced through mutation. Hereafter, we call the 100,000 updates of evolution with mutation turned on the "evolution phase" and the subsequent 50,000 updates with mutation turned off the "ecology phase".

We evolved a total of 69 populations; 30 in a limited resource chemostat environment, and 39 in an environment where all resources were infinitely abundant. In both environments 9 resources were present. These could be "metabolized" by performing logical computations. The full set of computations rewarded were: Not, Nand, And, OrNot, Or, AndNot, Nor, Xor, and Equals. To complete one of these computations, the organisms must input one or two 32-bit numbers, perform the logic operation in a bitwise fashion on them, and output the correct 32-bit result. The ability to perform one of these computations (and therefore use the associated resource) increases the speed at which an organism executes its instructions, allowing it to replicate more often. The output of a computation must be exact–a single bit in error disallows the organism to use the resource in question.

To determine the genotypic diversity present in populations we used the Shannon-Weaver index, calculated as $-\sum p_i \ln(p_i)$, where p_i is the frequency of the ith genotype in the population. The same formula was used to measure phenotypic diversity except substituting the frequency of phenotypes (based on the logic tasks each organism could perform) in a population for the frequency of genotypes. Mann-Whitney rank sum tests were used to compare measures of diversity between the two treatments because diversity values were non-normally distributed.

Results: Ecosystem Evolution

We measured the effect of competition for resources on genotypic and phenotypic diversity present in the evolving populations. If competition for limited resources creates multiple niches, we expect diversity to be higher in resource-limited populations. We found

---

[2]An update is a unit of "real time" for the organisms during which $30 \times$ population size instructions are executed globally. The number of instructions executed by a particular organism depends on its use of resources.

that both phenotypic and genotypic diversities were significantly higher in populations evolved in the environment where there was competition for resources (Mann-Whitney test: genotypic diversity, $U = 1586$, $n_1 = 39$, $n_2 = 30$, $P \ll 0.0001$; phenotypic diversity, $U = 1635$, $n1 = 39$, $n2 = 30$, $P \ll 0.0001$). A comparison of the average diversity present after 100,000 updates of evolution in the two environment types is shown in Figure 1.
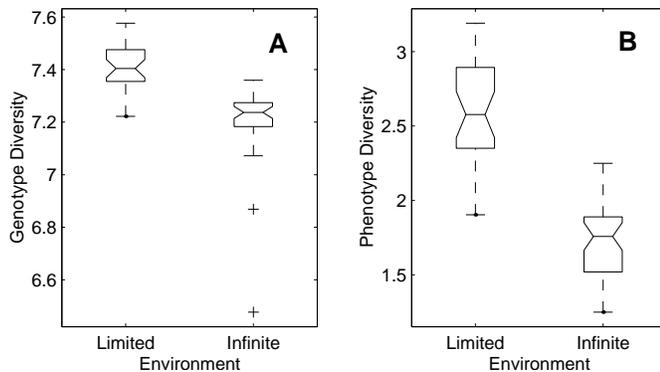
Figure 1: Diversity present in limited-resource and infinite-resource populations after 100,000 updates of evolution. (A) Genotype diversity. (B) Phenotype diversity. Whiskers and box edges represent 95% confidence intervals and quartiles, respectively. The mean is indicared by a line inside the box. Outliers are indicated by a '+' .

The high mutation rate used in the evolution of these populations causes the continual production of mutant genotypes. Although many of these mutants are destined to become extinct, they nevertheless increase the number of coexisting genotypes and thus the basal level of diversity within populations. The effect of high basal diversity is to reduce the fraction of total diversity caused by truly coexisting genotypes. For this reason, all populations were extended for a further 50,000 updates in an environment identical to the one they had experienced before, but with the mutation rate set to zero (the ecology phase). In this environment, ecological processes determine the dynamics of diversity without the complication of the continual production of new genotypes. Unfit mutant genotypes already present will be removed by population genetic processes. If the higher diversity in the resource-limited populations was caused by stabilizing pressures allowing organisms to coexist, then we expected diversity to be maintained in these populations. In the infinite resource populations we expected only the single fittest organism to be retained, decreasing diversity to zero. Consistent with these expectations, in figure 2 we show that whereas genotypic and phenotypic diversity was maintained in populations where organisms competed for resources, it dropped to near-zero in

populations grown in the infinite resource environment (Mann-Whitney tests: genotypic diversity, $U = 1635$, $n1 = 39$, $n2 = 30$, $P \ll 0.0001$; phenotypic diversity, $U = 1635$, $n1 = 39$, $n2 = 30$, $P \ll 0.0001$).
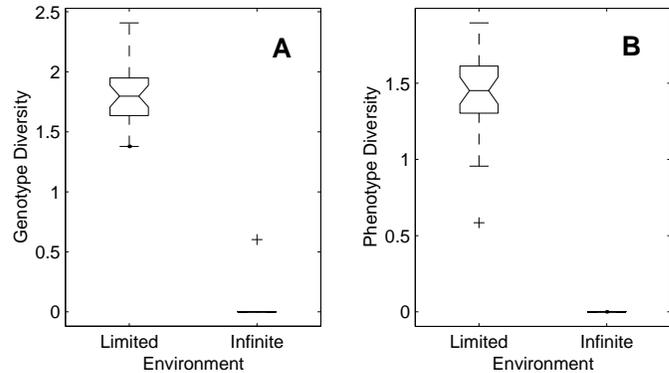
Figure 2: Diversity present in limited-resource and infinite-resource populations after 50,000 additional updates with mutations off (ecology phase). (A) Genotype diversity. (B) Phenotype diversity. Whiskers and box edges represent 95% confidence intervals and quartiles, respectively. The mean is indicared by a line inside the box. Outliers are indicated by a '+' .

Evolution of Specialists and Generalists

To examine the nature of the ecological mechanisms allowing coexistence in the resource limited populations we chose two case-study populations, one consisting of generalist organisms and one of specialist organisms, to study further. Figure 3 shows which resources were used by each genotype remaining after the ecology phase described above. In the population consisting of specialist organisms, the use of each resource was heavily dominated by only one genotype (Figure 3a). In the population consisting of generalist organisms, eight genotypes were present. These genotypes could be grouped into three distinct resource utilization groups based on the logic computations they could perform: group 1- Xor, AndNot and OrNot; group 2- Equ, And and Nand; group 3- Nor, Or and Not (Figure 3b). Two explanations could account for the continued coexistence of genotypes that shared the same phenotype. (1) Coexistence might reflect organisms that had very similar fitnesses, such that very long periods of selection would be required to separate them. (2) Subtle differences in the number of times an organism with a particular genotype performed a computation might cause oscillations in resource concentrations, allowing coexistence of organisms specialized to different levels of resource.

To distinguish between these possibilities, the generalist population collected after the ecology phase was restarted with five-fold replication in an identical envi-
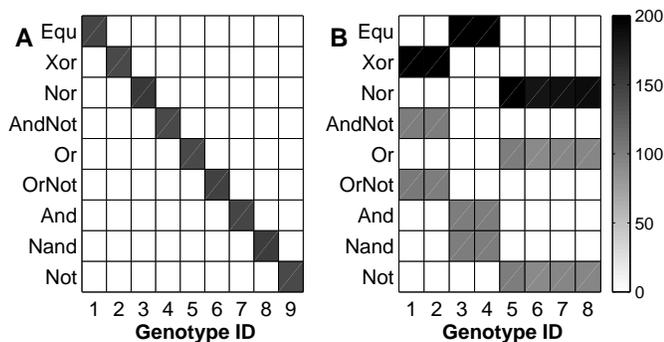
Figure 3: Map defining the phenotypes of each genotype present at the end of two runs. (A) Population consisting of specialist genotypes; (B) Population consisting of generalist genotypes. The colorbar indicates the number of times a given logical computation is performed during one generation. The more often a computation is done the more the cognate resource is used.

Figure 4: Coexistence of genotypes during the continued ecology phase with limited-resources. Genotypes are numbered as in Figure 3. (A) Genotype dynamics throughout this phase in a representative generalist population. Dark-gray, genotypes 1/2; light-gray, genotypes 3/4; black, genotypes 5/6/7/8. Genotypes 1,3 and 5 coexist at the end of the run. (B) Genotype dynamics throughout this phase in a representative specialist population. In decreasing order of abundance genotypes are: 1, 2/3, 4/5, 6/7 and 8/9. Differences in equilibrium genotype abundances result from the different rewards gained for using each resource.

ronment. If coexistence was due to the presence of genotypes of quasi-equivalent fitness, we expected the number of coexisting genotypes to decrease over time. We found that the number of coexisting genotypes had declined to three in all replicate populations by 300,000 updates. These three genotypes were then stably maintained up to the end of the experiment at 500,000 updates (Figure 4a). In all cases, only one member of each resource utilization type was maintained. Genotypes 1 and 5 from groups 1 and 3, respectively, were maintained in all of the runs. From group 2, genotype 3 was maintained in two runs and genotype 4 in three runs. In five control runs restarted from the specialist population, all nine genotypes were maintained for 100,000 updates (Figure 4b). These results suggest that the nine genotypes of the specialist population represent a stable ecosystem, whereas the eight genotypes of the generalist population can be grouped into three types by their resource use profile. A stable ecosystem is formed when only one genotype from each of these types remains.

The most likely explanation for the continuing coexistence of genotypes in the specialist and generalist populations described above is that density-dependent selection acts to favor rare phenotypes. This selection is caused by the reciprocal relationship between the benefit for using a resource and the number of organisms using it. To test this hypothesis, we took the five specialist and five generalist populations saved from the extended ecology runs described above, and restarted them in the same environment, except that resource levels were set to be infinite. In this environment there is no inherent advantage in using an underutilized resource so that the genotype most efficiently doing the most valuable task(s) is expected to exclude all other genotypes. As shown in

figure 5, all populations quickly became dominated by a single genotype. This genotype was always the one calculated as having the highest absolute fitness based on the rewards given for the tasks it was able to perform. These results indicate that density-dependent selection was responsible for the stable coexistence of genotypes during the ecology runs.

Discussion

Despite decades of study, the role of resource-based competition in promoting diversity remains unresolved (Schluter 1996). In this study, we demonstrate the evolution of high diversity in environments in which organisms were forced to compete with one another for resources. This diversity was caused by the stable coexistence of genotypes in these populations and continued to be stable for many generations. In contrast, when resources were not limiting, only one genotype was ever maintained. Coexistence did not depend on any spatial
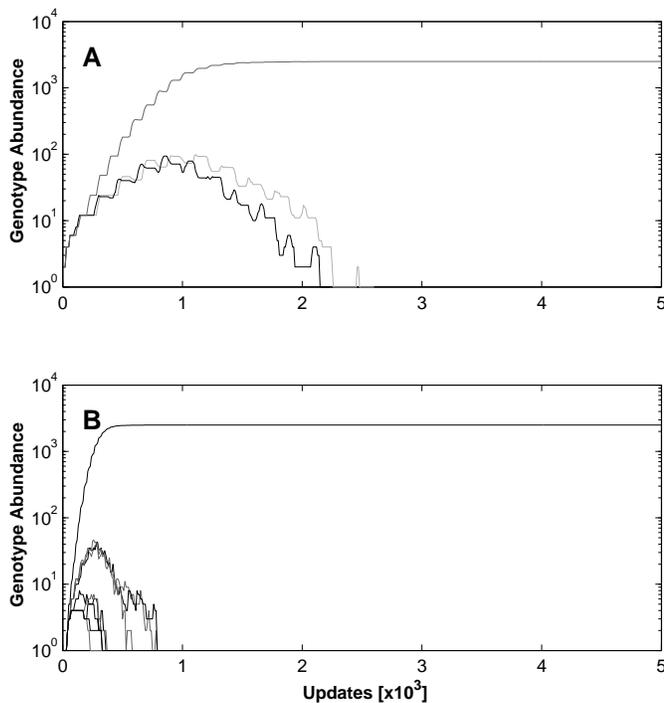
Figure 5: Coexistence of genotypes during the continued ecology phase with infinite-resources. Genotypes are numbered as in Figure 3. Note that a log scale was used for the y-axis. (A) Genotype dynamics throughout this phase in a representative generalist population. Dark-gray, genotype 1; light-gray, genotype 3; black, genotype 5. Genotype 1 remains at the end of the run. (B) Genotype dynamics throughout this phase in a representative specialist population. Genotype 1 remains at the end of the run.

or temporal heterogeneity in the environment.

The most likely explanation for the maintenance of multiple genotypes in a resource-limited environment is that density-dependent selection acts to create multiple niches. This selection is generated because the level of a particular resource depends on the number of organisms using it, and the benefit for being able to use a resource depends on its level. This reciprocal relationship creates selection for the use of underutilized resources. Two broad outcomes of density-dependent selection can be imagined. At one extreme, organisms could specialize, adapting to one resource at the expense of their competitiveness for others. The effect of this is to partition the environment such that as many species as there are resources are potentially able to coexist. At the other extreme, generalist organisms, able to use underutilized resources while retaining the ability to use those used by their progenitor, may result. To test whether density-dependent selection was responsible for the coexistence of genotypes in the resource-limited populations

we evolved, we increased the level of resources in the environment so that they were not limiting. This disrupts density-dependent selection by removing the penalty for using the same resource as other organisms and by no longer providing an advantage for being one of a few organisms using a resource. Effectively a single niche is created wherein only the fittest genotype is expected to survive (Kassen, *et. al.* 2000). When we did this, as expected, only one genotype was maintained.

The theory of competitive exclusion predicts that each environmental niche can support only one species, thus putting an upper bound on the number of species able to coexist in an environment (Hardin 1960). However, the number of species actually coexisting depends also on the genetics and physiology of the organisms. Whilst there is a general expectation that selection will tend to favor organisms which specialize on few resources (i.e. adaptive radiation), this expectation assumes that there are inherent trade-offs of adaptation such that for a given resource a specialist will be more competitive than a generalist (Futuyma & Moreno 1988). Whilst trade-offs have been observed in some studies (for example (Dykhuizen & Davies 1980; Cooper & Lenski 2000)), in others they have not (Reboud & Bell 1997; Bennett, *et. al.* 1992). Further underlining the uncertainty involved in making this assumption, in this study we observe the evolution of both specialists and generalists starting from the same ancestral genotype and evolving in the same environment (compare Figure 3 panels A and B). This finding may reflect either evolutionary contingencies in the physiology of the organisms or that evolution of one or other of the populations was stopped before a final strategy had been reached. Future work will address this issue.

As the ability of humankind to influence ecosystems increases, the search for mechanisms that underlie the diversity observed in nature has come to be of pragmatic as well as scientific interest (Tilman 2000). The Avida platform represents a uniquely tractable and powerful experimental model that has previously been used to answer evolutionary questions on a scale not possible with traditional systems (Lenski, *et. al.* 1999; Wilke, *et. al.* 2001). The extension that we introduce here will allow this system to be used to address ecological questions requiring the generation of resource dependent interactions between organisms within Avida populations. These studies may be valuable in giving heuristic insight into the workings of natural ecosystems.

Acknowledgements

References

Adami, C. 1998. *Introduction to Artificial Life.* Santa Clara: TELOS Springer-Verlag.

Adami C, Ofria C, and Collier TC 2000. Evolution of Biological Complexity. *Proc. Natl. Acad. Sci. USA* **97**:4463-4468.

Bell GAC 1997. Experimental evolution in *Chlamydomonas.* I. Short-term selection in uniform and diverse habitats. *Heredity* **78**:490-497.

Bell GAC 1999. Biodiversity of plankton by species oscillations and chaos. *Nature* **402**:407-410.

Bennett AF, Lenski RE, and Mittler JE 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. *Evolution* **46**:16-30.

Cooper VS, and Lenski RE 2000. The population genetics of ecological specialization in evolving *Escherichia coli* populations.
Nature **407**:736-739.

Dykhuizen D, and Davies M 1980. An experimental model: Bacterial specialists and generalists competing in chemostats. *Ecology* **61**:1213-1227.

Futuyma D, and Moreno G 1988. The evolution of ecological specialization. *Ann. Rev. Ecol. Syst.* **19**:207-233.

Hardin G 1960. The competitive exclusion principal. *Science* **131**:1292-1297.

Kassen R, Buckling A, Bell G, and Rainey PB 2000. Diversity peaks at intermediate productivity in a laboratory microcosm.
Nature **406**:508-512.

Lenski RE, Ofria C, Collier TC, and Adami C 1999. Genome complexity, robustness and genetic interactions in digital organisms. *Nature* **400**:661-664.

Morin PJ 2000. Biodiversity's ups and downs. *Nature* **406**:463-464.

Notley-McRobb L, and Ferenci T 1999. Adaptive *mgl*-regulatory mutations and genetic diversity evolving in glucose-limited *Escherichia coli* populations. *Env. Microbiol.* **1**:33-43.

Notley-McRobb L, and Ferenci T 1999b. The generation of multiple co-existing mal-regulatory mutations through polygenic evolution in glucose-limited populations of *Escherichia coli. Env. Microbiol.* **1**:45-52.

Ofria C, Brown, CT, and Adami, C. 1998. The avida user's manual. In *Introduction to Artificial Life* (1998).

Rainey PB, and Travisano M 1998. Adaptive radiation in an heterogeneous environment.
Nature **394**:69-72.

Reboud X, and Bell G 1997. Experimental evolution of *Chlamydomonas.* III. Evolution of specialist and generalist types in environments that vary in space and time. *Heredity* **78**:507-514.

Schluter D 1996. Ecological causes of adaptive radiation. *Am. Nat.* **148**:s40-s64.

Schluter D 2000. Ecological character displacement in adaptive radiation. *Am. Nat.* **156**:s4-s16.

Schluter D 2001. Ecology and the origin of species. *Trends Ecol. Evol.* **16**:372-379.

Tilman, D.. 1982. *Resource Competition and Community Structure.* Princeton: Pirnceton University Press.

Tilman D 2000. Causes, consequences and ethics of biodiversity. *Nature* **405**:208-211.

Travisano M, and Rainey PB 2000. Studies of adaptive radiation using model microbial systems.
Am. Nat. **156**:s35-s44.

Wilke CO, Wang JL, Ofria C, Lenski RE, and Adami C 2001. Evolution of digital organisms at high mutation rate leads to survival of the flattest. *Nature* **412**:331-333.