

Alteration of (Frequency-Dependent) Fitness in Time-Shift Experiments Reveals Cryptic Coevolution and Uncoordinated Stasis in a Virtual Jurassic Park

Abstract Among the major unresolved questions in ecosystem evolution are whether coevolving multispecies communities are dominated more by biotic or by abiotic factors, and whether evolutionary stasis affects performance as well as ecological profile; these issues remain difficult to address experimentally. Digital evolution, a computer-based instantiation of Darwinian evolution in which short self-replicating computer programs compete, mutate, and evolve, is an excellent platform for investigating such topics in a rigorous experimental manner. We evolved model communities with ecological interdependence among community members, which were subjected to two principal types of mass extinction: a pulse extinction that killed randomly, and a selective press extinction involving an alteration of the abiotic environment to which the communities had to adapt. These treatments were applied at two different strengths (Strong and Weak), along with unperturbed Control experiments. We performed several kinds of competition experiments using simplified versions of these communities to see whether long-term stability that was implied previously by ecological and phylogenetic metrics was also reflected in performance, namely, whether fitness was static over long periods of time. Results from Control and Weak treatment communities revealed almost completely transitive evolution, while Strong treatment communities showed higher incidences of temporal intransitivity, with pre-treatment ecotypes often able to displace some of their post-recovery successors. However, pre-treatment carryovers more often had lower fitness in mixed communities than in their own fully native conditions. Replacement and invasion experiments pitting single ecotypes against pre-treatment reference communities showed that many of the invading ecotypes could measurably alter the fitnesses of one or more residents, usually with depressive effects, and that the strength of these effects increased over time even in the most stable communities. However, invaders taken from Strong treatment communities often had little or no effect on resident performance. While we detected periods of time when the fitness of a particular evolving ecotype remained static, this stasis was not permanent and never affected an entire community at once. Our results lend support to the fitness-deterioration interpretation of the Red Queen hypothesis, and highlight community context dependence in determining fitness, the shaping of communities by both biotic factors and abiotic forcing, and the illusory nature of evolutionary stasis. Our results also demonstrate the potential of digital evolution studies to illuminate many aspects of evolution in interacting multispecies communities.

* Corresponding author.

Tian-tong Luo

Nanjing Agricultural University
Department of Zoology
College of Life Sciences

Jian-long Zhu

Nanjing Agricultural University
Department of Zoology
College of Life Sciences

Trond Reitan

University of Oslo
Centre for Ecological and
Evolutionary Synthesis
Department of Biology
trond.reitan@ibv.uio.no

Gabriel Yedid*

Nanjing Agricultural University
Department of Zoology
College of Life Sciences
gyedid02@gmail.com

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I Introduction

I.1 Who Holds Court in Biological Communities, the Queen or the Jester?

The stability of biological communities made up of multiple interacting species may be considered from both strictly ecological [39, 42, 48, 50] and evolutionary [17, 27, 31, 52] perspectives; namely, whether the same factors that lead to ecological stability will also produce resistance to evolutionary change, or whether these ecological interactions instead drive evolutionary change. There are two major primary contrasting views of multispecies community evolution: the Red Queen [36, 52] and the Court Jester [4]. In the former, the “environment” is defined largely by biotic interactions among community members, which are the primary drivers of evolution. Any change in the strength or type of these interactions stemming from evolutionary change in one interactor (individuals, species, etc.) must then be matched by changes in others, with the corollary that any evolutionary advance in one interactor will result in a loss of fitness in others, which must then evolve as quickly as possible simply in order to maintain fitness. Taken to its logical extreme, the concept suggests that these interactions might drive evolution indefinitely even if the external, abiotic environment could be held completely constant.

At the other end of the continuum, the Court Jester view posits that evolution is governed by chance survival of organisms in inconstant, unpredictable external environments, which may result in massive redirections of the course of evolution. Although first proposed by a paleontologist [55], the Red Queen has come to be favored by neontological biologists, particularly with regard to the evolution of sexual reproduction and host-parasite coevolution (e.g., [6, 18, 40]), while the Court Jester is favored by geoscience workers who see evolution of life on Earth as inseparable from major geological and astronomical events in Earth history, to which life can only respond passively (e.g., [35]). A third view, dubbed “ecological locking” [41], also posits a web of biotic interactions among community members, but these interactions come to impede evolution by generating community-wide stabilizing selection, rather than driving evolution through continual directional selection. Evolution may then only occur when that web of stabilizing interactions is destroyed in Court Jester-type events. Clearly, real evolution contains elements of all these views [19, 20, 22, 24, 25, 46].

I.2 Looking at Going Somewhere While Running in Place

According to the Red Queen hypothesis, biotic interactions among organisms mean that they should always be pushing each other towards ever greater states of adaptedness. Evolution may appear to be standing still by some measures, but may actually be cryptically continuing [13]. Hence, if communities were truly stable both ecologically and evolutionarily, and if communities from different points in time could be competed repeatedly against each other, we could reasonably expect resulting mixed communities in which each member that occupied a particular ecological niche would come 50% of the time from the earlier community and 50% of the time from the later community. Types taken from the later time would be able to easily “slot in” to the earlier community (and vice versa), without disrupting the performance of the rest of the community. While first proposed as thought experiments several decades ago [52], such experiments remain in the realm of science fiction, at least for vertebrate megafauna (e.g., [16]). However, they are easily achieved with microbial experimental evolution, where samples of a microbial population evolving over many thousands of generations may be kept frozen and revived as needed [14, 33]. An *in silico* alternative is digital evolution, which offers similar capabilities at much greater speed and gives the experimenter a greater degree of environmental control.

In previous work [38], we examined the tendency of cross-feeding communities of digital organisms generated by the *Avida* digital evolution platform [44] to show stability with and without mass extinction, as measured using metrics incorporating information from ecology and phylogeny. We determined that 75% of communities generated under these conditions were ecologically unstable even without mass extinction. However, about 25% of the replicate populations in our data set showed some evidence for stability using eco-phenotypic metrics. It remains to be seen, however,

whether these apparently stable communities are also stable in terms of performance, that is, whether they would satisfy the aforementioned criteria.

Here, we use digital evolution to perform exactly such experiments, using simplified versions of the communities used in previous work [38, 59, 62] to look for evidence of *performance stasis* in communities diversified into multiple coexisting ecotypes. We examine whether or not such stability exists at the whole-community or individual-niche level, whether there is any occurrence of temporal intransitivity (i.e., where an earlier-evolved type displaces a later-evolved one), and also whether mass extinctions can alter these kinds of evolutionary and population dynamics.

2 Methods

2.1 Experimental Platform

We used the digital evolution platform Avida [44] as our experimental platform, allowing for a complete record of the course of evolution and precise manipulation of environmental parameters. An Avida world consists of a grid of digital organisms (hereafter referred to as *Avidians*), each of which consists of a genome of instructions written in an assembly-like, Turing-complete computer language, along with a simple virtual computer chip that executes those instructions. Avida has been detailed at length elsewhere [44], as has our experimental setup [38, 59, 62], but some details of our implementation deserve mention. First, the ecosystem has a limited amount of space, introducing an element of drift as new organisms displace the old. Second, the environment features multiple depletable resources, linked to certain computations, where a low concentration of a resource reduces the benefit gained by performing an associated computation. This feature mimics density-dependent competition for resources, favoring organisms that most efficiently consume particular resources or target underutilized ones. Resources are globally available to all organisms, with no spatial structure. Only a limited number of resources are supplied exogenously, while the remainder can only be generated as by-products by organisms when they successfully complete certain associated computational functions (these interactions are discussed at greater length in Supplement A, and illustrated in Supplementary Figure S1, which can be found in the online supplementary materials for this article at www.mitpressjournals.org/doi/suppl/10.1162/artl_a_00305). This introduces ecological interdependence into the population dynamics, which is a feature of real communities. Configuration files for performing the experiments described here are available at [doi:10.5061/dryad.67506q4](https://doi.org/10.5061/dryad.67506q4).

We used two different versions of Avida for the work described here. The base communities were generated using v. 2.4.4, while all competition trials and fitness assays were conducted with v. 2.12. The source code for each version is available from https://www.dropbox.com/home/Luo_et_al_ALIFE_SourceCodes.

2.2 Experimental Methodology

2.2.1 Mass Extinction Treatments

We have previously used Avida [38, 59–62] to investigate the effects of two general types of mass extinction—*press* and *pulse*—on recovery dynamics and community composition. A pulse extinction is an instantaneous mass culling of individuals from the population, with survivors selected at random and providing no opportunity for adaptation; by contrast, a press extinction involves a period of altered environmental conditions—in this case, greatly reduced inflows of basal resources—that persists long enough to allow an adaptive response in the population. Each of these two treatment types was applied at two different strengths, *Strong* and *Weak*, along with a *Control* treatment in which evolution continued uninterrupted. Details of the treatment regimens have been described at length in the aforementioned publications, and are summarized in Supplement A in the online supplementary materials.

2.2.2 Generation of Ecologically Stable Communities

The base communities are those used in previous work [38, 59, 62]. Avida communities typically contain many hundreds of genotypes and dozens of Avidians with distinct functional combinations.

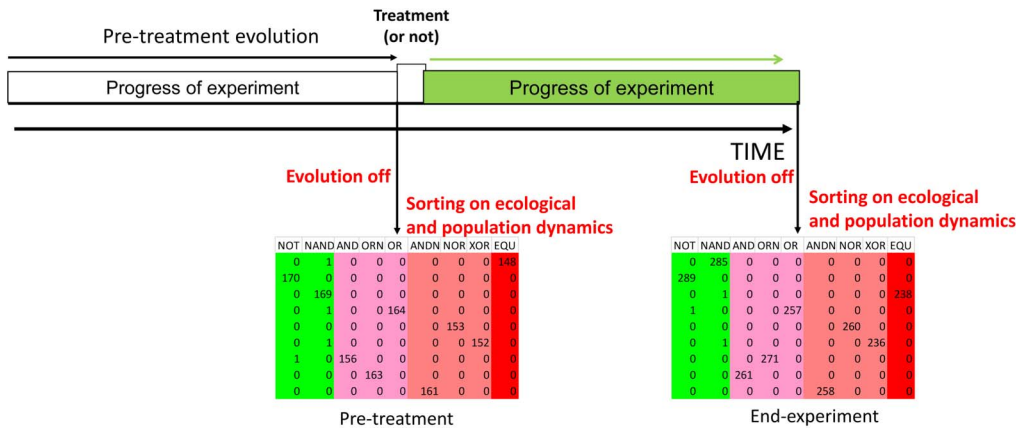


Figure 1. Schematic depiction of generation of ecologically stable communities. After an initial period of evolution and diversification, all evolutionary forces are stopped, allowing communities containing thousands of genotypes to sort on ecological and population dynamics to produce an ecologically stable community. Such communities may be produced before or after a treatment entailing a major macroevolutionary event. Each row in the community represents a single Avidian, while the number in each cell shows how many times the Avidian performs the computational function (column headings) during the course of a single gestational cycle.

To enable tractable competition experiments, we generated ecologically stable communities according to previously described methods [12, 15]. Briefly, Avida experiments were seeded and run as described previously, but, at specific times, all mutation was turned off, allowing the extant genotypes to sort on ecological and population dynamics for an additional 100,000 Avida updates. This additional step resulted in a greatly reduced set of Avidians that could coexist with minimal ecological overlap, representing the ecological “elites” of the population at the time evolution was halted (Figure 1). Each unique Avidian with a particular ecological profile (computational functions performed and corresponding resources used) is an *ecotype*. We generated such stable communities for the following times:

- 100,000 updates (representing the immediate pre-treatment state)
- Control: 105,000; 115,000; 130,000; 155,000; 180,000; 205,000 updates
- Press treatments: 205,000 updates
- Pulse treatments: 200,000 updates

2.2.3 Initial Assessment of Fitness in Stable Communities

We first used these ecologically stable communities to derive a measure of fitness for each component ecotype. We did not use the native measure of fitness in Avida, which is more like a metabolic rate. Instead, we opted for one more akin to what is used in real microbial evolution experiments, a reproductive rate. For each pre-treatment community, we conducted fitness assays in which we seeded an Avida population grid with a single individual of each stable ecotype at particular fixed positions, with ample space around each individual to avoid early extinction of ecotypes. Each ecotype was given a specific *lineage label* that would be carried by all descendants of that particular seed individual. For each assay, we recorded the total number of births (cumulative population count, or CPC [14]) of each labeled ecotype for 50,000 Avida updates (Figures 2(a), 2(c)). Each ecotype’s CPC data were then converted into *instantaneous birth rate* (IBR, i.e., the first derivative of the CPC with respect to time, see Figures 2(b), 2(d)), which was subsequently analyzed with breakpoint analysis [64, 65] in order to determine the initial increase and equilibrium phases. The measure of fitness was the average of all equilibrium-phase IBR values from the last breakpoint to the end of the data series.

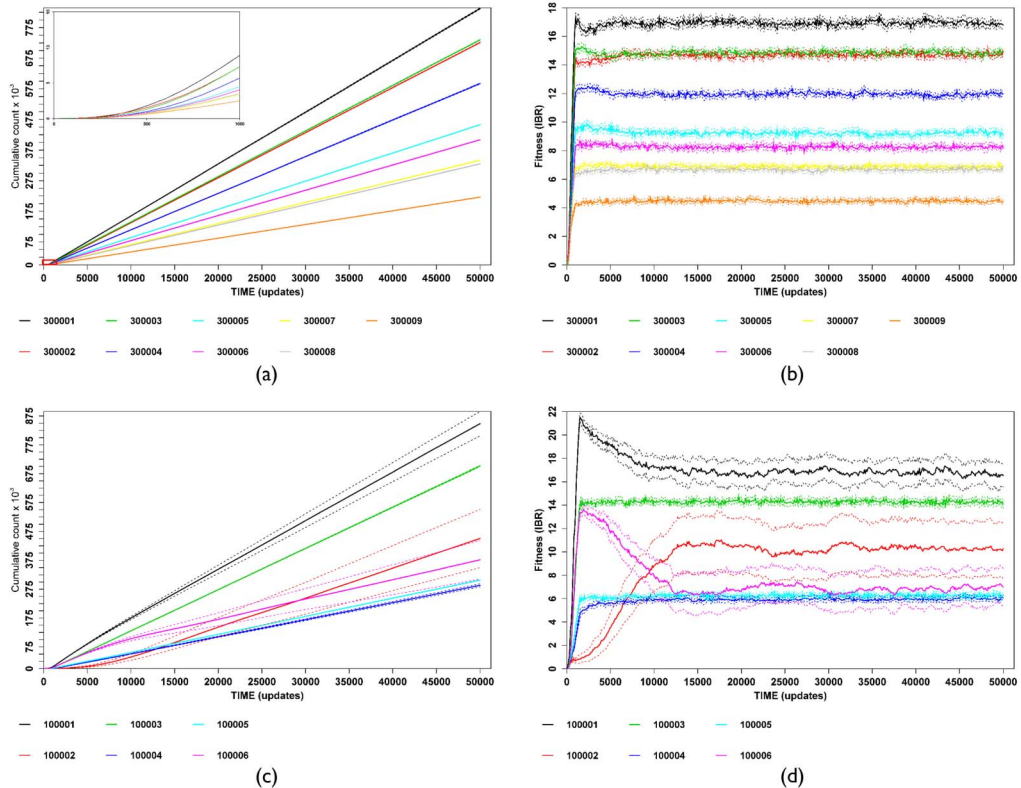


Figure 2. Illustration of conversion of cumulative population count (CPC) for each stable ecotype into instantaneous birth rate (IBR), used here as the measure of fitness. Each colored trace represents a distinct ecotype. Values are averages of ten subreplicates (thick traces) with 95% confidence intervals (thin traces). Panels (a) and (c) show the CPC, while panels (b) and (d) show the IBR. (a, b) A specialist community with nine stable ecotypes. Inset in panel (a) shows initial nonlinear growth corresponding to pre-plateau region of (b) (red rectangle in lower left-hand corner). (c, d) A more generalized community with six stable ecotypes, three of which (the black, red, and magenta traces) show substantial ecological overlap and frequency-dependent dynamics. Note the nonlinear CPC traces and the wider 95% CIs for these ecotypes.

Given the cross-feeding environment (including self-feeding potential), as well as the ability of many of the tested ecotypes to produce multiple resources, starting each trial with only a single individual of each ecotype could conceivably result in positively frequency-dependent effects before equilibrium was attained. Having reached this point, ecotypes with substantial niche overlap would then show negative frequency dependence (Figures 2(b), 2(d)).

2.2.4 Time-Shift Competition Trials

We next used the ecologically stable communities to perform the following kinds of competition trials:

- a. *Full-community competitions.* Each trial was seeded with a single individual of each ecotype represented in each replicate's pre-treatment and end-treatment stable communities. These trials were intended to test for the emergence of long-term community-level stasis as described in the Introduction, that is, whether the final community would have one source community completely eliminate the other, or would be a mixture of ecotypes from each source community, with each component ecotype coming sometimes from the earlier one and sometimes from the later one.

- b. *Replacement trials.* For communities where there was a clear phenotypic correspondence between the end-treatment and pre-treatment ecotypes, each trial was seeded by replacing one of the pre-treatment ecotypes with its end-treatment eco-equivalent (a *replacer*), noting what effect (if any) this replacement had on the equilibrium IBRs of the other Avidians in the pre-treatment community (termed *residents*). Each end-treatment ecotype was tested in this way. These trials tested for two aspects of stasis at the level of ecotype: whether the replacer had about the same fitness as the resident being replaced, and the replacer's effects on the remaining residents' fitnesses. Only 65/500 total replicates (across all treatments) could be treated as replacements.
- c. *Invasion trials.* For communities where the end-treatment community contained ecotypes not present in the pre-treatment community, or vice versa, a single individual of an end-treatment ecotype was allowed to invade against the whole pre-treatment community, without a priori removal of any resident. Again, each end-treatment ecotype was evaluated in this manner. We again noted how the invasion affected the fitnesses of the other residents, as well as whether any residents were displaced by the invader. 435/500 total replicates were treated as invasions.
- d. *Inverse invasion trials.* These were similar in nature to the invasion trials, except that they featured a pre-treatment ecotype invading against the whole end-treatment community. We performed these trials only for cases where a pre-treatment ecotype was present in the final community from a full-competition experiment, or where invariance of resident fitness was suggested by the results of a replacement or invasion trial as described above.

We performed 10 subreplicates of each trial, each of 50,000 updates in duration. The seed Avidians for each trial were again always placed at particular fixed positions on the Avida population grid, amply spaced so as to prevent early extinctions by overwrites.

2.3 Analytical Methodology

2.3.1 Determination of Fitness Effects

In order to assess the effects of replacements and invasions on the fitnesses of resident ecotypes, we used a likelihood ratio test developed previously [38] to compare the average IBR (derived from the equilibrium IBR time series data) of each ecotype in the absence and in the presence of the replacer/invader. For each resident ecotype, we first determined the fold difference between the average IBR with and without the replacer or invader. Each fold difference is interpreted as a coefficient of selection, and was tested for statistical significance through use of a chi-square test to compare the log likelihoods of a model assuming a difference between the averages of the with and without data series versus a null model assuming no difference. We then determined a mean fold difference for each replacer or invader against all (other) residents. This way of assessing relative fitness is also akin to the lag-load of [52].

2.3.2 Nomenclature and Terminology

In the results (Section 3), single ecotypes are named by the random seed used to generate their community, followed by a two-digit number that indicates their time of origin and rank-abundance, then by one or two letters that indicate which treatment generated them: PT for pre-treatment, C for Control, SP for Strong Press, SU for Strong Pulse, WP for Weak Press, and WU for Weak Pulse. Hence, 72000PT refers to the most common ecotype from replicate 7200's pre-treatment community, while 720091C refers to the most common ecotype from replicate 7200's end-Control community; similarly, 720093SP would be the third most common ecotype from replicate 7200's end-Strong press community. Identical numbers do not necessarily indicate ecologically equivalent types.

Additionally, the term “intransitivity” should be understood to mean *temporal* intransitivity, where an earlier-evolved type outperforms a later-evolved one.

3 Results

3.1 Full-Community Competitions

When pre-treatment communities were competed against end-Control communities, no evidence of full-community intransitivity was found (summary results in Table 1; example in Figures 3(a), 3(b)). Only 2/100 replicates (7200 and 8700) showed any evidence of intransitivity at the level of single ecotypes, with a single pre-treatment ecotype surviving to the end of the trial at greater frequency and higher fitness than the end-treatment eco-equivalent. Results for the two Weak treatments were similar, with only 2/100 and 4/100 replicates for Weak Pulse (2400, 7200) and Weak Press (2400, 3100, 8700, 10000), respectively, showing any evidence of single-ecotype intransitivity. In all these cases, however, the surviving pre-treatment ecotype was present at lower fitness in the mixed population than in its own fully native community.

The two Strong treatments showed markedly different results from Control and Weak treatments. Although there were again no cases of full-community intransitivity, an order of magnitude more replicates showed evidence of single-ecotype intransitivity. When communities that were not ecologically complete before the treatment were removed, Strong Press still showed the greatest number of replicates with single-ecotype intransitivity, followed by Strong Pulse. Even in these cases, there was a strong tendency for the carryover ecotypes (pre-treatment ecotypes that were present in the mixed community) to show lower fitness in the final mixed communities than in their own fully native conditions (Table 2, Figures 3(c), 3(d)). It was much less common for carryovers to have about the same fitness as in native conditions, and rare for their fitness to actually be higher (Table 2). Many communities were ecologically incomplete because the difficult XOR and EQU functions did not evolve, or, more often, failed to re-evolve after a strong mass extinction [60–62]. Even in these last cases, carryovers—the pre-treatment XOR- and EQU-bearing ecotypes—tended to have lower fitness even when they could just “slot in” to empty niches without competition (Table 2).

We next conducted a series of post hoc invasion experiments to determine the strength of the intransitivity for ecotypes from the aforementioned Control competitions. Replicate 8700 showed

Table 1. Summary for full-community competitions. Data for means are across all 100 replicates for each treatment ± 2 s.e.

Treatment	Mean no. of ecotypes in final community	Mean no. of ecotypes from pre-treatment community	Mean no. of ecotypes from end-treatment community	No. of replicates where both communities are ecologically complete (/100)	No. of replicates where both communities are ecologically complete and at least one pre-treatment ecotype is present in final community
Control	5.97 \pm 0.235	0.02 \pm 0.028	5.95 \pm 0.232	85	2
Strong Press	6.45 \pm 0.238	1.62 \pm 0.27	4.83 \pm 0.283	56	40
Strong Pulse	6.12 \pm 0.256	0.63 \pm 0.179	5.49 \pm 0.253	74	25
Weak Press	6.08 \pm 0.221	0.06 \pm 0.056	6.02 \pm 0.222	85	4
Weak Pulse	5.99 \pm 0.245	0.04 \pm 0.056	5.95 \pm 0.238	84	2

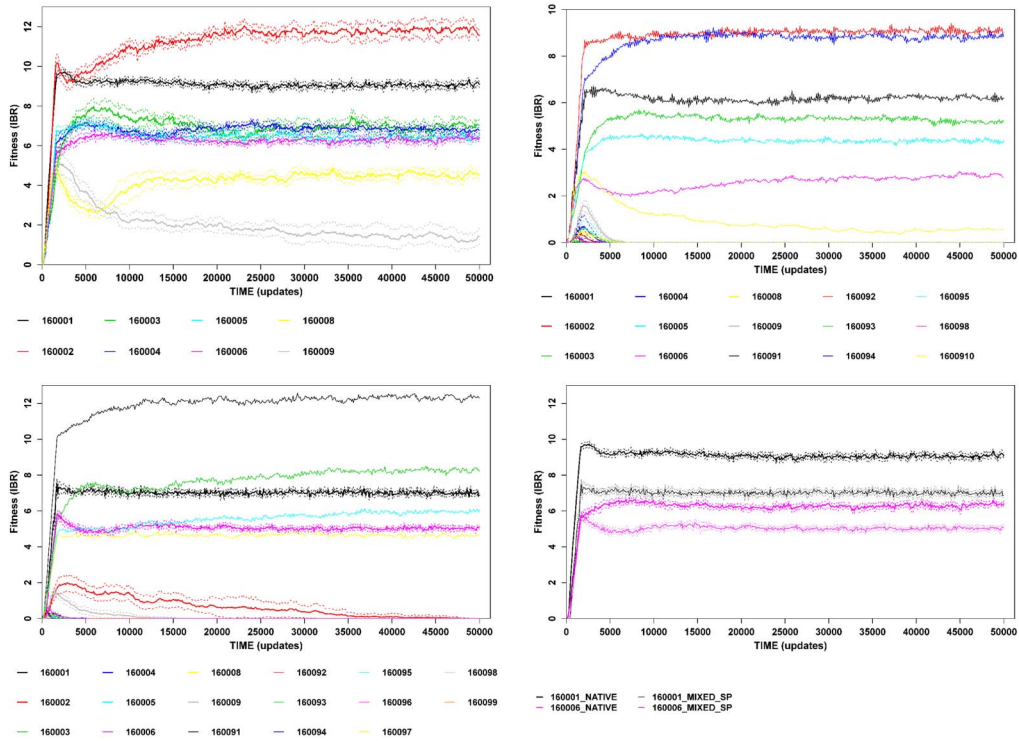


Figure 3. Example of partial evolutionary intransitivity in full-community competitions. All traces are means of ten sub-replicates. Error series (where present) are 95% confidence intervals around means. (a) IBR plot for a community (replicate 1600) with eight stable ecotypes. (b) Result of full-community competition for 1600PT (thick traces with errors) versus 1600C (thin traces with no errors). All pre-treatment ecotypes are driven extinct rapidly. (c) Result of full-community competition for 1600PT (thick traces with errors) versus 1600SP (thin traces with no errors). Eight ecotypes are present in the final mixed community, two of which are carryovers from the pre-treatment community (note also long-term persistence of a third pre-treatment type before it goes extinct). (d) Comparison of IBR for carryover ecotypes in fully native conditions (thick traces with errors) versus mixed-community conditions (thin traces with errors). The mixed-community fitnesses are well outside of the 95% CIs of the fully native values.

total intransitivity, as ecotype 870001PT always survived, while its end-treatment equivalent 870091C always went extinct. In replicate 7200, however, the intransitivity was partial, as ecotype 720001PT always survived, but its end-treatment equivalent 720092C did not always go extinct, although the latter was always present at lower frequency and fitness, where it too survived in the final mixed community.

The ecotypes that displayed intransitivity had little or no change in genome size (although there had been a great deal of sequence evolution) and tended to be quite ecologically generalized, albeit with low levels of functional expression (no function was performed more than ten times during a single gestational cycle). An interesting observation about the type that showed complete intransitivity was that the corresponding end-Control ecotype was actually missing one function, still present in the pre-treatment version, which would have allowed it to be fully self-feeding. Instead, it was rendered dependent on by-products from other community members in order to achieve its full fitness, while the pre-treatment version was fully self-feeding.

3.2 Replacement Trials

In replacement trials, where a single pre-treatment ecotype was replaced by a clear future ecotype, most such replacements resulted in substantial depression of the pre-treatment residents'

Table 2. Pre-treatment carryovers in full-community competitions and their fitness in mixed versus fully native conditions for Strong Press and Pulse extinctions.

Treatment (No. of replicates)	Number of pre-treatment carryover ecotypes (total mean \pm 2 s.e.)	Number of carryovers with <i>lower</i> fitness than native conditions (total % of total carryovers mean \pm 2 s.e.)	Number of carryovers with <i>higher</i> fitness than native conditions (total % of total carryovers mean \pm 2 s.e.)	Number of carryovers with approximately same fitness as native conditions (total % of total carryovers mean \pm 2 s.e.)
Both communities ecologically complete				
Strong Press (40)	87 2.175 \pm 0.319	69 79 1.725 \pm 0.27	6 7 0.69 \pm 0.114	12 14 0.3 \pm 0.19
Strong Pulse (25)	38 1.52 \pm 0.367	34 89 1.36 \pm 0.303	1 3 0.04 \pm 0.08	3 8 0.12 \pm 0.13
One or both communities ecologically incomplete				
Strong Press (35)	73 2.086 \pm 0.397	54 74 1.54 \pm 0.361	4 5 0.114 \pm 0.109	15 21 0.428 \pm 0.29
Strong Pulse (18)	26 1.444 \pm 0.29	23 88 1.11 \pm 0.454	1 4 0.056 \pm 0.111	2 8 0.11 \pm 0.15

fitnesses. Out of all replacement tests (65/500 total replicates across all treatments), only three (one replacer each from 2/20 Control trials and three replacers from a single Strong Press trial) showed any evidence of long-term stasis in a particular ecotype's fitness, and not for all replacers. In only these particular cases, the replacer had about the same fitness as the replaced resident, and negligible effects on the remaining residents.

Across all replacement trials, the range of fitness effects as expressed by the IBR-based selection coefficients (see Section 2) was extremely wide, as low as 0.87% (i.e., a less than 1% difference in fitness) and as high as 1161% (Figure 4(a)). Within these extremes, the median change in the selection coefficient was a reduction of 10.8%, with 2.5 and 97.5 percentiles of 1.7% and 82%. Over the entire length of the post-treatment period, ecotypes mostly did not remain static in their performance, even if their ecological profiles remained unchanged; the replacements usually showed quite substantial, measurable effects on the performance of the pre-treatment residents. Hence, long-term evolution in these replacer communities was largely fully transitive. Over shorter time scales, however, either neutrality (as shown by inconsistent survival of particular ancestral ecotypes across assay replicates) or intransitivity (consistent survival of particular ancestral ecotypes across assay replicates) could still be observed (Supplement B in the online supplementary materials).

The outcomes of the full-community competitions strongly suggested that selection coefficients did not remain static over time. We investigated this in more detail by looking at seven case study communities. Five of these showed both very high ecological and phylogenetic stability as determined previously [38] and were fairly generalized (6–7 stable ecotypes per community). The other two were composed of pure specialists (9 stable ecotypes), and showed high ecological stability but not phylogenetic stability. All chosen communities were also ecologically complete (i.e., all resources were used). Replacement trials were done using the community states at 105,000; 115,000; 130,000; 155,000; and 180,000 updates as sources of replacers. The effects of replacements are best seen in the pure specialist communities, where every end-experiment replacer had some measurable effect on the residents (Figure 5(a), Figure 6). Over the duration of the post-treatment period, there was an increase in performance for all ecotypes against the residents, but the strength of this increase was not equal for all of them (Figure 6). Some of them (e.g., the ANDN ecotype) remained closer to the pre-treatment level of performance, while the performance of others (the NOT, NAND, and

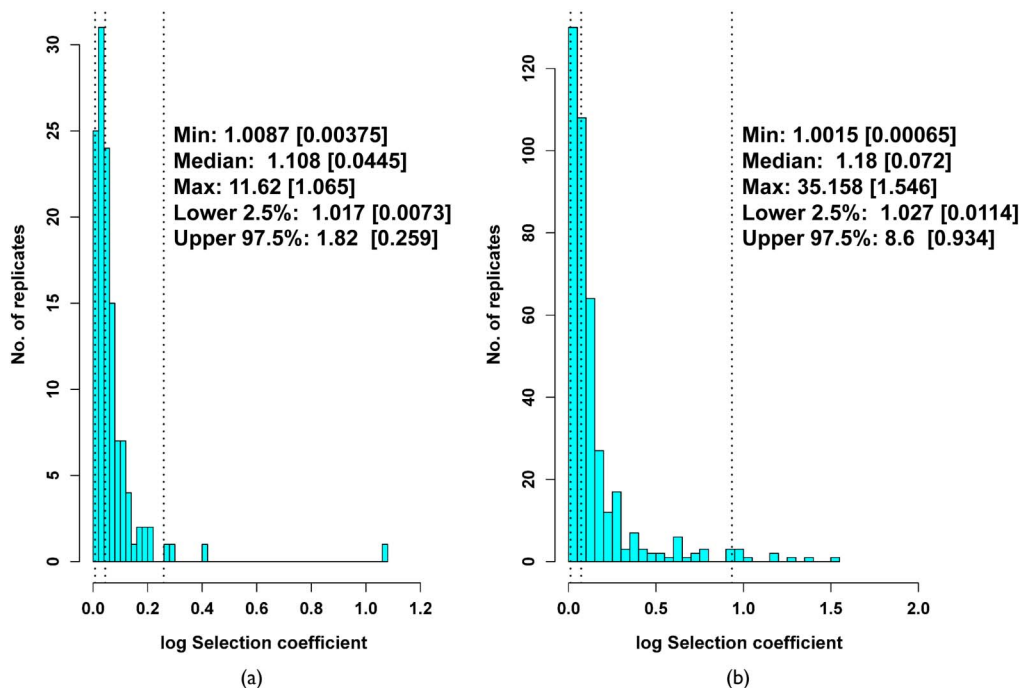


Figure 4. Distribution of selection coefficients (log-transformed) for invasion and replacement trials for end-treatment communities versus pre-treatment communities. In both plots, the vertical dotted lines are (from left to right) the 2.5th percentile, the median, and the 97.5th percentile of each distribution. Values in square brackets are log transformations to aid interpretation of the x-axis. (a) Replacement trials. (b) Invasion trials.

EQU ecotypes) increased much more over the pre-treatment community (Figure 5(a)). Certain ecotypes (particularly ANDN, AND, OR, XOR) did not evolve much over certain time periods (Figure 5(a), highlighted with red ovals), but others did during the periods of stasis for these types, and any observed periods of stasis for any particular ecotype were not permanent. Further, certain replacers had strongly negative effects on all other residents, reducing fitness well outside of the native range of variation (Figure 6(a)), while others had weaker effects, reducing fitness only to around the lower end of the native range (Figure 6(b)).

In contrast, the more generalized communities showed different behavior. While the overall trend of increasing coefficients of selection was again evident, several ecotypes showed enormous variation around each estimate (Figure 5(b)). Unlike the pure specialist communities, certain replacers tended to have extremely strong effects on one to three residents, and much weaker effects (if any) on the others, sometimes to the extent of driving another resident extinct (even if that replacer originated before the end of the experiment, as in Supplement B, in the online supplementary materials). Further, certain replacer ecotypes had only weak effects against all other residents over the entire post-treatment period, acting largely like the residents they replaced. We also found some cases where addition of the replacer actually increased the fitness of one or more residents while decreasing the fitness of others; this was not observed in either of the specialist communities, where fitness effects were always negative, even if the decrease was not significant.

3.3 Invasion Trials

The invasion trials, where a single end-experiment ecotype (*a future invader*, or FI) attempted to invade against all of the pre-treatment residents, showed an even wider range of outcomes than did the

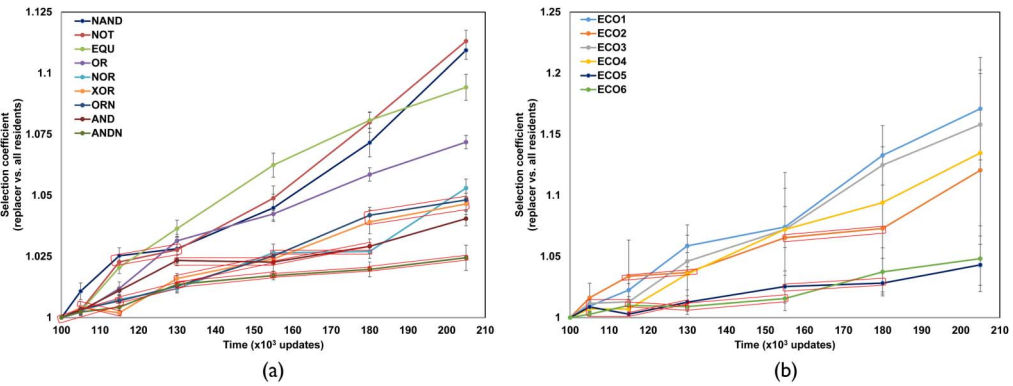


Figure 5. Increase in coefficients of selection (see Section 2 for definition) over time for replacer ecotypes against pre-treatment residents. In both plots, each trace represents the change in average selection coefficient for each replacer ecotype (shown in legend) against all non-equivalent pre-treatment residents. Each value is the mean of all coefficients across every resident for that ecotype. Errors are 95% confidence intervals. Red ovals indicate time periods where the change in selection coefficient is strictly less than 1.00 percent. (a) A pure specialist community with nine stable ecotypes where each Avidian specializes on one of the nine available resources and performs one of the nine standard Avida logic functions (shown in legend). (b) A more generalized community with six stable ecotypes where each Avidian performs two or three functions. Note the much larger errors associated with each estimate compared to panel (a).

replacement trials (Figure 4(b)). In these less ecologically stable communities, we observed a higher incidence of invariance in resident performance (Table 3).

As with the replacements, residents usually showed a measurable reduction in fitness as a result of the invasion (median 18%, 2.5 percentile 2.7%, 97.5 percentile 860%, Figure 4(b)). The most common outcome was that a FI displaced at least one resident (although it was sometimes two or more) and had strong depressive fitness effects on at least one other resident. However, there was very wide among-resident variation in the strength of these effects; typically, a FI would have strong effects on only one or two residents in a community, and much weaker or negligible effects on others. This was true across all treatments, but was particularly noticeable for Strong Press and

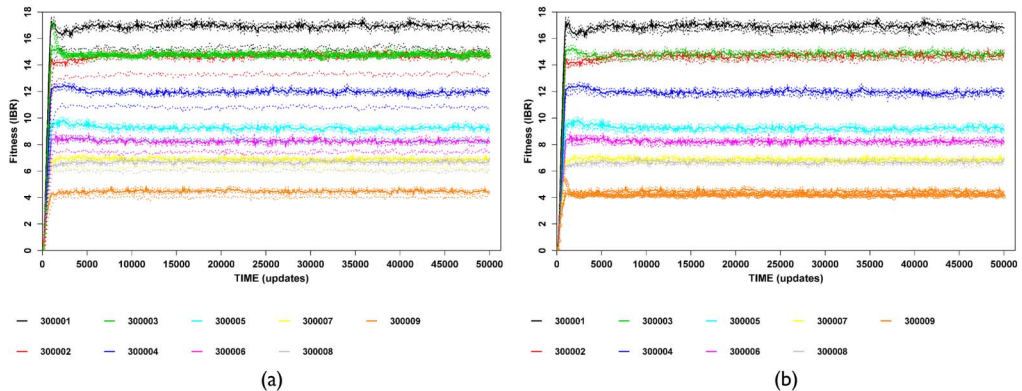


Figure 6. Fitness assay plots for the specialist community (Figure 5(a)), showing differences in fitness change using different end-treatment replacer ecotypes. Each ecotype is represented by a different color. In both plots, the pre-treatment residents' native fitnesses (averaged over ten sub-replicates) are shown by the thick traces with thin error series (95% CIs), while their fitnesses in the presence of the replacer are shown by dotted traces. The fitness of the replacer is shown as a trace of open circles, colored the same as the replaced resident. (a) Replacer is the end-control NAND ecotype (colored bright green). The other residents' equilibrium fitnesses are reduced by an average of $\approx 10.9\%$. (b) Replacer is the end-control ANDN ecotype (colored orange). The other residents' equilibrium fitnesses are reduced by an average of $\approx 2.45\%$.

Table 3. Summary data for resident performance stasis in invasion trials.

	Proportion of replicates showing resident stasis (total no. of replicates treated as invasion trials)	Mean no. of invaders with no significant effect on residents (± 2 s.e.)	Proportion of invaders that have no significant effects on residents in the most stable community
Control	4/80	1.25 \pm 0.5	2/7
Weak Pulse	7/79	1.14 \pm 0.286	2/8
Weak Press	4/81	1 \pm 0.00	1/5
Strong Pulse	26/96	1.42 \pm 0.297	4/6
Strong Press	41/99	2.024 \pm 0.346	5/7

Strong Pulse (Table 3). We did not find any cases in which *every* tested invader failed to affect the residents' performance.

In a number of cases, the results of the invasion trials conflicted with those of the corresponding full-community competitions, where particular FIs failed in every invasion attempt against the pre-treatment community when tested individually, yet were present in the final community in the full competition. We therefore performed a number of post hoc co-invasion trials, where one of these failed FIs, or a replacer with a very weak fitness effect (1% or less), was allowed to invade accompanied by a second FI or replacer from the same community that was already determined separately to have strong fitness effects on at least one member of the pre-treatment community. In every such case, the double invasion succeeded, with both FIs or replacers establishing themselves and displacing one or more residents.

3.4 Inverse Invasion Trials

The purpose of these trials was to confirm whether pre-treatment ecotypes that survived in the full-community competitions could actually invade the end-treatment community. The results matched the outcome of the full-community competitions almost perfectly, in that both series of experiments ended with almost the same mixed community for each replicate. However, even here there were two notable exceptions: Ecotypes 190004PT and 290004PT survived in the full-community competitions, but always failed as single invaders of their Strong Press end-treatment communities.

4 Discussion

4.1 The Significance of Paleocological Stasis, and the Need to See Through It

Paleontological examination of the fossil record has revealed the apparent stasis in morphology of a number of single evolutionary lineages, and even whole paleocommunities, over significant spans of geological time [26, 37]. It is unclear whether this stasis is more under abiotic or community-level control, as theoretical and simulation studies allow for the emergence of evolutionary stasis depending on the strength and type of interactions among community members irrespective of abiotic conditions [43, 52]. Further, it is unclear whether morphological stasis implies stasis in performance, particularly in fitness, especially when the biotic context around the static lineages has changed due to macroecological shifts, for example, survivors of a mass extinction existing among taxa that diversified following the extinction. In this study, we used full-community competitions and time-shift replacement and invasion trials to determine whether ecologically diversified communities of

digital organisms that showed stasis based on ecological and phylogenetic data [38] were truly evolutionarily static in terms of performance.

4.2 Ecological or Genealogical Stasis Does Not Mean Whole-Community Stasis in Performance

Our primary interest here was to determine whether these digital communities showed performance stasis in addition to the stasis implied from ecological and phylogenetic data. If this were in fact the case, predictable consequences would follow (see Introduction). Our data show that for these digital communities, these predictions were largely not fulfilled. In the absence of major ecological or population disruption, long-term evolution in this system appears to be almost completely transitive, with later-evolved communities usually eliminating all members of an ancestral community, with only rare intransitive exceptions. Even in cases of long-lasting ecological associations among community members (the appearance of *coordinated stasis*), performance does not remain constant over long spans of time, though different communities appear to evolve at different rates (Figure 5 and Supplement B in the online supplementary materials). This means that even when genealogical continuity between ecotypes can be established [38], the later versions are not truly the same as the ancestral versions, despite niche persistence. While this conclusion holds for our Control and Weak treatment communities, it is much less certain for the Strong treatment communities. Strong Press in particular showed the highest amount of intransitive outcomes for the full-community competitions, as well as the highest incidence of resident invariance in the invasion trials. Post-treatment ecotypes were not necessarily superior to pre-treatment ones, as shown by the surprisingly high number of outcomes where at least one pre-treatment ecotype was present in the mixed population rather than a post-treatment one. These results show that a strong enough disruption, particularly one that radically reshapes a community at levels going all the way from ecological relationships to genomic characteristics [38, 59, 60], can break the inherent transitivity of an evolving community. Post-treatment ecotypes that evolve in these new ecological and genetic contexts, even if they successfully occupy a particular niche and fit well with the rest of the post-treatment community, may in fact be less effective than that niche's previous occupants. This is normally difficult (if not impossible) to assess when those previous occupants have been rendered completely extinct (as happened in the original full experiment), but here we have effectively resurrected them and so the sensitivity of their post-treatment successors may be demonstrated.

Finally, with regard to the issue of coordinated stasis, we failed to find any examples that showed such for performance. Although our survey was hardly exhaustive, the most promising candidates that showed high ecological and genealogical stability (Figure 5(b), Supplement B in the online supplementary materials) did not show any periods where performance remained static for fitness effects against *all* resident ecotypes in a community. If even these best candidates failed to show coordinated stasis of performance, it is unreasonable to expect it from other replicate populations that were demonstrably unstable, as shown by shifting ecotype composition and division of labor [38]. Further, competitions with sequentially occurring communities showed shifting bouts of short-term neutrality and longer-term transitivity and intransitivity (Supplement B in the online supplementary materials). We conclude that while such stasis can occur in our experimental system, it is impermanent and uncoordinated: certain community members may remain static in performance for a particular time period, while others evolve substantially over that same time, but the community as a whole is not static despite outward appearances and does not represent an optimal fixed point [13, 45].

4.3 Fitness Is Highly Dependent on Community Context

Our results also demonstrate the extent to which an ecotype's performance is affected by the other community members around it. Several sources demonstrate this property for our digital communities. The first is the strong tendency for pre-treatment carryovers to have lower fitness in mixed communities also containing post-treatment ecotypes, which obtained with both ecologically complete and incomplete communities (Table 2). The second is the replacement and invasion trials,

where the great majority of fitness effects on residents by the invaders were negative (Table 2, Figure 4), and which sometimes resulted in failure to invade by ecotypes that were successful in the corresponding full-community trials, indicating that the presence of other ecotypes from their native communities was necessary for their success. While the great majority of fitness effects were negative, we did observe some cases where a replacer or invader increased the fitness of one or more residents (while having negative or negligible effects on others). This was seen mostly in communities where there was evidence for negative frequency dependence of interactions among two or more residents with a substantial degree of niche overlap (e.g., Figure 2(d)). We see two possible explanations for such results: (i) the replacer or invader affects one of those residents negatively, alleviating frequency-dependent interactions, or (ii) the replacer or invader actually provides a direct boost to the fitnesses of those positively affected residents through enhanced provisioning of resources (relative to the replaced resident). However, we cannot currently distinguish between these two possibilities.

While it is often politely acknowledged that a quantitative assessment of “fitness” (understood here as a rate of reproduction) applies only under particular conditions (e.g., [8]), the contribution of other community members to that determination often remains underappreciated. In our cross-feeding communities, Avidians serve as resource transformers, and the effectiveness with which a particular ecotype fulfills that role will play a large part in determining the response, and ultimately the evolutionary characteristics, of other ecotypes that depend on those resources. Substituting an ecotype with different such effectiveness (either by direct manipulation, or by evolutionary change) will alter the responses, performance, and potentially the evolutionary trajectories of other ecotypes in the community. Hence, in communities with these kinds of interactions, fitness (however measured) becomes an increasingly nebulous concept when every community member’s characteristics depends on the performance and relative abundance of every other member.

4.4 The Queen and the Jester Rule Together

One of the long-standing issues in our exploration of evolution of cross-feeding digital communities and how mass extinction and recovery affect them [38, 59–62] has been whether new and/or improved ecotypes attain ecologically prominent positions through community drift effects [23, 37] or Red Queen effects resulting from interactions among community members [18, 36]. Our data indicate that in this system, it is the latter. The results of the full-community competitions and replacement/invasion trials clearly show that without a major abiotic disruption, evolutionary transitivity usually prevails. The more detailed trials (Figure 5 and Supplement B in the online supplementary materials) demonstrate that the fitness effects of evolved ecotypes on the older pre-treatment residents tend to increase over the length of the post-treatment period. Further, these effects are generally negative (though we did observe exceptions), measurably depressing the performance of at least one resident. Our results lend support to the original, most general formulation of the Red Queen hypothesis, which can apply to any system of interactors where evolutionary effects are mediated by ecological interactions [36, 55]: Evolutionary advance in one community member that interacts ecologically with several others will result in deterioration of the fitnesses of those members, which must continue evolving or go extinct.

Although all our trials were run under ecological conditions (with no new types allowed to evolve), we argue that we still observed these predicted effects, though mostly indirectly. First, we observed a preponderance of negative fitness effects, which generally increased in strength over time. Second, we observed in the replacement and invasion trials the occasional secondary extinction of pre-treatment residents whose ecotypes both did not go extinct *and* remained ecologically prominent in the original evolutionary experiment, demonstrating that there was indeed evolutionary adjustment for these ecotypes during the post-treatment period (compare with [32]). Third, in the sequential full-community competitions (Supplement B in the online supplementary materials), the particular ecotypes that showed occasional intransitive effects sometimes changed between the assayed time points, indicating that there was actual coevolution between ecotypes in these com-

munities. These effects also help explain the results of the full-community competitions. In the replacement and invasion trials, each replacer or invader usually negatively affected at least one particular resident; hence, the older community is driven extinct not just because the later one is composed of superior competitors for all available niches, but also because most members of that later community may exert indirect negative effects on older ones through altered ecological interactions. We draw these conclusions primarily from the Control experiments (where evolution continued uninterrupted), but they also apply to the Weak treatments, which produced very similar results. However, the results of the Strong treatments seem to require a different explanation, given the fairly frequent occurrence of mixed communities containing at least one pre-treatment ecotype (Table 1), and the increased incidence of invasion trials where the invader had negligible effects on performance of the remaining residents (especially in Strong Press), even if at least one resident was eliminated (Table 3).

We believe that these results argue for a role of mixed Red Queen–Court Jester dynamics in shaping the post-treatment communities [9, 19, 24, 25]. The pre-treatment communities are heavily compromised or wiped out by massive abiotic forcing [38, 59, 62], allowing for redirection of evolution and breaking of the prevailing dynamics that would otherwise yield a transitive outcome. The communities that emerge in the aftermath of the Strong extinctions are made up of Avidians that often have altered ecotype composition and functional properties, and whose ecological structure may pass through a number of transitional stages during recovery [38]. Although we have not looked directly at community web structure [30, 48] or ecological connectance [5] here, the greater incidence of resident invariance when using post-Strong-treatment invaders suggests that those invaders interact only weakly with the residents, implying that the post-treatment ecological structures from which they are drawn differ substantially from those of the pre-treatment residents. This outcome was already suggested by larger changes in the degree of a community’s division of labor after Strong treatments [38]. Put another way, the lack of fitness effects on residents seen using these invaders is paradoxically *not* because the invaders fit seamlessly into the remaining residents’ ecological network, but because they are actually more ecologically disconnected from it. (This result should not be overemphasized, though, as there are still plenty of post-Strong-treatment invaders that *do* produce adverse effects on the residents.) We say this because the results from the Control and Weak experiments (where resident invariance is much less common) show what happens when such a network is invaded by a more evolved ecotype that matches the ecological profile of the replaced resident, and that ought to fit into the network the same way. The change in post-treatment ecological structure is also implied by the occasional persistence of pre-treatment ecotypes in the full-community competitions, indicating that the structure of post-treatment communities is sometimes less robust than the corresponding pre-treatment community’s.

4.5 Parallels with and Applications to Real Biological Systems

We have previously compared the broad qualitative similarities of the ecological and phylogenetic aspects of our results to major extinction and recovery events from Earth history [38, 59, 61]. Our present results are of general interest only if they are not system-specific. However, we argue that these results too have broad qualitative parallels with diverse systems at different levels of real-world biology and ecology.

1. “Softening up” of communities by strong invaders. Although uncommon among our results, we still observed several incidents of replacers or invaders failing when attempting invasion individually, but present in the mixed communities of full-community competitions, and able to invade when accompanied by a co-invader with strong depressive fitness effects on at least one resident. This result is broadly analogous to what is expected under the invasion meltdown hypothesis [51], where invasive species with strong ecosystem engineering capabilities can alter ecological dynamics in the recipient community, rendering them vulnerable to further invasion by species that would not

otherwise succeed. While our experiments were replacements and invasions across time rather than space, we are confident that similar dynamics would occur using spatially separated, independently evolved communities that do not include the competing residents as part of their own evolutionary history [57, 58].

2. Facilitation facilitates invasion. Related to the previous point, our cross-feeding environmental setup only allows for facilitative resource conversion: Consumed resources are transformed into by-products that then become resources themselves, but only have positive effects on Avidians that consume them (Supplement A in the online supplementary materials). However, these kinds of facilitative dynamics may also result in increased susceptibility to invasion [3, 34, 54], which in the present context may also contribute to the relative paucity of stable results for most of our digital communities [38]. Our model does not include direct negative interactions such as predation, parasitism, or allelopathy, though such interactions are in principle possible with this digital system [10, 21, 63] and thus present intriguing possibilities for future work, particularly if inclusion of them enhances ecological and evolutionary stability [41, 42, 50].
3. Microbial evolution population dynamics. The conventional paradigm that emerged for interpreting the results of early microbial evolution experiments emphasized a periodic selection process where successive, cumulative substitution of beneficial mutants in a continuous lineage ought to produce a quite deterministic evolutionary process that agreed well with theoretical predictions. Some experiments produced results highly inconsistent with this expectation [1, 2], leading to an alternative interpretation of chemostat populations as mixtures of genotypes that could possibly modify culture conditions and alter the relative fitnesses of others [7]. This has been demonstrated directly even for serial transfer experiments started from single clones in which different ecotypes emerge in both mixed [11, 49, 53] and static cultures [47]. Here, we have shown that the fitness of any one type is dependent on the characteristics of other types, and evolutionary advances in one particular lineage can alter the fitnesses of coexisting lineages that remain invariant. Results from our replacement and invasion trials involving extinction of resident ecotypes that persisted throughout the source experiment are qualitatively comparable to those shown previously for a two-ecotype microbial model system [32]. In this latter case, early clones of a second ecotype failed in reciprocal invasion experiments against later-evolved clones of the first ecotype, yet this second ecotype persisted across the original experiment, demonstrating that it did evolve to avoid extinction in the intervening time. We believe our results will apply to any evolving, closed-community system (see [56] for a critique of the generality of the assumptions under which Red Queen dynamics will emerge) that develops some degree of niche differentiation and/or capability for niche construction, regardless of whether they are in chemostat-like or serial-transfer contexts.
4. Negative effects on performance of pathways by temporally displaced members. While we have treated the Avidian communities as analogues for real natural communities, they may also be seen as analogous to enzymes in a metabolic pathway, and the virtual metabolism of resources as analogous to the modification of substrates by one enzyme, which are then passed to the next one in the pathway. (The analogy here is not entirely accurate, because the Avidians often use more than one resource and may consume their own by-products.) It has recently become possible to produce reconstructed ancestral sequences of particular enzymes, insert them into extant living cells, and analyze the physiological and evolutionary responses of the unmodified remainder of the pathway [28, 29]. An inferred ancestral variant of the essential gene *tufB* (which in bacteria encodes elongation factor Tu), when inserted into a modern *E. coli* cell, was not immediately lethal, but did cause a twofold decrease in fitness due to reduced protein dosage. This resembles the reduction in fitness seen in multiple ecotypes (sometimes the whole community) in the presence of a

temporally displaced replacer or invader. These qualitatively similar results suggest that both our digital communities and real metabolic pathways are made up of mutually co-adapted elements, and that the context as a whole suffers when one element is out of evolutionary sync with the others. Our results also suggest another interesting avenue for future work by allowing evolutionary responses [29] in the kinds of experiments we have performed here, rather than just ecological-scale experiments where selection does not act on either the replaced ecotype or the rest of the community. Better still would be experiments where the invader is kept evolutionarily frozen while the rest of the community is allowed to evolve.

5 Concluding Remarks

Our results show the potential for digital evolution to contribute to studying dynamics of multispecies community interactions, combining them with aspects of macroevolution for a fuller understanding of how community composition and ecological and evolutionary responses are shaped over time. While digital organisms and the communities they form lack many details of real-world analogues, our results bear a number of similarities to what is known from real communities, particularly microbial ones, and suggest a number of promising avenues for future research. Here, we have shown that “fitness” (measured as a reproductive rate as for real organisms) in cross-feeding communities of digital organisms can depend heavily on community context. We have also shown that evolution continues in a cryptic but discontinuous manner even when eco-phenotypic data would imply stasis, and that major macroevolutionary events such as mass extinctions can produce unforeseen redirections of prevailing evolutionary tendencies, leading to unexpected evolutionary intransitivity when pre- and post-extinction communities meet. Remarkably, our results obtain without any “sophisticated” biotic interactions, such as predation and parasitism; rather, they arise from relatively simple facilitative resource dynamics. Further development of digital evolution platforms (for example, to allow lineage-specific “freezing” of evolution), when combined with measurement approaches developed for experimental evolution and community ecology, make digital evolution a capable alternative to more traditional modeling and biological approaches for elucidating general principles of evolution in interacting multispecies communities.

Although we have already highlighted commonalities between our results and previous real biological ones, perhaps the greatest implications of our results are for still-speculative areas of science. Here, we have been able to resurrect organisms lost during mass extinctions and compete them against post-extinction ones, often revealing the inadequacy of the latter. While still the domain of science fiction novels and movies, advances in modern biotechnology could conceivably resurrect extinct megafauna within the next century. While great caution must be exercised in extrapolating results from a digital system like ours to the real world (or even a still-fictional extension of it), we believe that our results have bearing on the wisdom of actually attempting this. Unless resurrected species are kept in tightly contained artificial environments, our results suggest that, if released into the modern natural world, such species might well have unforeseen negative interactions with members of the modern biota, perhaps to the point of driving those modern organisms extinct. Our results also imply that even if resurrected species succeed in establishing themselves in the modern world, it is quite likely that, due to mismatched ecological interactions, their performance will be reduced relative to what it was in their own fully native community prior to their initial extinction.

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