Genome size and the extinction of small populations

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Abstract

Species extinction is ubiquitous throughout the history of life. Understanding the factors that cause some species to go extinct while others survive will help us manage Earth's present-day biodiversity. Most studies of extinction focus on inferring causal factors from past extinction events, but these studies are constrained by our inability to observe extinction events as they occur. Here, we use digital experimental evolution to avoid these constraints and study "extinction in action". Previous digital evolution experiments have shown that strong genetic drift in small populations led to larger genomes, greater phenotypic complexity, and high extinction rates. Here we show that this elevated extinction rate is a consequence of genome expansions and a concurrent increase in the genomic mutation rate. High genomic mutation rates increase the lethal mutation rate, leading to an increase the likelihood of a mutational meltdown. Genome expansions contribute to this lethal mutational load because genome expansions increase the likelihood of lethal mutations. We further find that an increased phenotypic complexity does not contribute to an increased risk of extinction. These results have implications for the causes of extinction in small populations and suggest that complexity increases in small populations may be limited by high rates of extinction.

Keywords: Extinction, Genome Size, Small Populations, Digital Experimental Evolution

Introduction

The ubiquity of extinction events throughout the history of life (Jablonski 1986), and the increasing realization that the biosphere may be experiencing a sixth mass extinction (Barnosky et al. 2011) drives interest in determining the factors that cause certain species, but not others, to go extinct (Maynard Smith 1989). It is accepted that a combination of genetic (Spielman et al. 2004; O'Grady et al. 2006), demographic (Matthies et al. 2004; Melbourne and Hastings 2008), environmental (Lindsey et al. 2013; Urban 2015), and ecological (Clavero and García-Berthou 2005; Pedersen et al. 2007; Dunn et al. 2009) factors

contribute to species extinctions. Beyond those deterministic factors, chance events also likely influence certain extinction events (Raup 1992; Turner et al. 2015). Here, we focus on the genetic factors influencing extinction, specifically the role of small population size and genetic drift (Lynch and Gabriel 1990).

In small populations, weakened purifying selection leads to increased fixation of small-effect deleterious mutations (Whitlock et al. 2004). As multiple deleterious mutations fix, the absolute fitness of the population may decrease, resulting in a decrease in population size. This decreased population size further weakens selection, leading to the fixation of additional deleterious mutations and a further decrease in population size. This process continues until the population goes extinct. This positive feedback loop between decreased population size and deleterious mutation fixation is known as a mutational meltdown (Lynch et al. 1993). Mathematical models of mutational meltdowns suggest that even intermediate-sized asexual populations can quickly go extinct (Gabriel et al. 1993; Lynch et al. 1995). Likewise, small sexual populations are also vulnerable to fast meltdowns (Lande 1994). The detection of mutational meltdowns in natural populations is difficult; other non-genetic factors (i.e., environmental or ecological factors) can obscure the role of mutation accumulation in extinction. However, laboratory experiments with microbes confirm that mutational meltdowns can occur in small asexual populations with elevated mutation rates (Zeyl et al. 2001).

While the concept of a mutational meltdown provides a population-genetic mechanism for extinction, it is still uncertain what factors beyond population size influence the likelihood of a meltdown. For example, if deleterious mutation accumulation drives mutational meltdowns, then species with a greater genomic mutation rate should be at a greater risk of extinction (Singh et al. 2017). Therefore, genetic mechanisms that increase the genomic mutation rate may also increase the likelihood of species extinction. One such genetic mechanism that could increase the mutation rate are genome expansions (i.e., mutations that increase genome size) because species with larger genomes (but similar point mutation rates) have greater genomic mutation rates. Indeed, there is some evidence that genome size positively correlates with extinction risk in certain clades of multicellular organisms (Vinogradov 2003; 2004). Furthermore, increased genome size in multicellular organisms is often attributed to selfish genetic elements (Doolittle and Sapienza 1980; Orgel and Crick 1980) that also increase the mutation rate and perhaps the extinction rate (Arkhipova and Meselson 2005; Dolgin and Charlesworth 2006).

It is difficult to experimentally test the role of genome size in extinction in both natural and laboratory model systems. Here, we use digital experimental evolution to test whether genome expansions can drive population extinction. Digital experimental evolution is the computational counterpart to microbial experimental evolution (Hindré et al. 2012; Kawecki et al. 2012; Batut et al. 2013). Instead of a population of microbes evolving in a flask (or other physical microcosm), digital evolution experiments instantiate a population of self-replicating computer programs that reproduce and mutate in a digital world (Adami 2006). Most digital evolution systems do not try to emulate any specific biological system. Instead, these systems implement populations with heritability, variation, and differential fitness (i.e., the three requirements for Darwinian evolution) but composed of organisms and genomes significantly simpler than those in biological populations (Pennock 2007). Digital evolution experiments are often performed to test hypotheses difficult to test in biological systems (Lenski et al. 1999; Adami et al. 2000; Yedid and Bell 2002; Knibbe et al. 2007;

Goldsby et al. 2012; Covert et al. 2013; Goldsby et al. 2014; Zaman et al. 2014), and allow for significant advantages when large numbers of replicate populations are needed to ascertain statistical significance.

In a previous digital evolution study on the role of population size in the evolution of phenotypic complexity (measured in terms of the number of phenotypic traits), LaBar and Adami found that the smallest populations (just 10 individuals) evolved the largest genomes and the most novel traits, but also had the greatest extinction rates (LaBar and Adami 2016). Here, we use this experimental setup as a model system to test the role of genome size in the extinction of small populations. We find that extinction in these small populations is to a large extent driven by genome expansions, and that an increased genome size not only leads to an increase in the genomic mutation rate, but also to an increase in the likelihood of lethal mutations. The combination of increased genomic mutation rates and increased likelihood of lethal mutations increases the lethal mutation rate, eventually leading to population extinction. We also show that the evolution of novel traits does not increase the extinction rate. Instead, phenotypic complexity and extinction are correlated because the same casual factors drive both the evolution of novel traits and high extinction risk.

Methods

Avida

For the following experiments, we used the digital experimental evolution platform Avida (Ofria et al. 2009). In Avida, simple computer programs ("avidians") compete for the resources required to undergo self-replication. Each avidian consists of a genome of computer instructions drawn from a set of twenty-six available instructions in the Avida genetic code. A viable asexual avidian genome must contain the instructions to allocate a new (offspring) avidian genome, copy the instructions from the parent genome to the offspring genome, and divide off the offspring genome into a new avidian. During this copying process, mutations may occur that introduce variation into the population. These novel mutations can then be passed onto future generations, resulting in heritable variation. This genetic variation causes phenotypic variation: avidians with different genomes may self-replicate at different speeds. As faster self-replicators will outcompete slower self-replicators, there is differential fitness between avidians. Therefore, given there is heritable variation and differential fitness, an Avida population undergoes Darwinian evolution (Adami 1998; Pennock 2007). Avida has previously been used to test hypotheses concerning the evolution of genome size (Gupta et al. 2016; LaBar and Adami 2016), the role of population size in evolution (Misevic et al. 2004; Elena et al. 2007; LaBar and Adami 2016; 2017), and the consequences of population extinction (Yedid et al. 2008; 2009; 2012; Strona and Lafferty 2016).

The Avida world consists of a toroidal grid of N cells; each cell can be occupied by at most one avidian. Thus, N is the maximum population size for the Avida environment. Here, N is set to 10 in each experiment to study the effects of extinction in small populations. While Avidian populations are usually at carrying capacity, the presence of lethal mutations can reduce their effective population size below this maximum size. In traditional Avida

experiments, the geometry of the environment can alter evolutionary dynamics, as offspring our placed into the environment in one of nine cells neighboring their parent's cell (including the parent cell) (Lenski et al. 2003). Here, offspring can be placed into any cell in the environment, simulating a well-mixed environment (i.e., no spatial structure). If a cell is occupied by another avidian, the new offspring will overwrite the occupant. The random placement of offspring avidians adds genetic drift to Avida populations, as avidians are overwritten without regard to fitness.

Time in Avida is structured in discrete units called "updates". During each update, $30N$ genome instructions are executed across the population. The ability for an avidian to execute one instruction in its genome is called a SIP, or Single Instruction Processing unit. In a population consisting of N individuals with the same genotype, each avidian will receive approximately 30 SIPs, and thus execute 30 instructions each update. However, in a population with multiple genotypes, some genotypes may be allocated more SIPs than others, depending on a value called "merit"; genotypes with greater merit will receive proportionally more SIPs than genotypes with lesser merit.

An avidian lineage can evolve increased merit through two means. First, an increase in genome size will increase merit by a proportional amount. Merit is set to be proportional to genome size in order to offset the decrease in replication speed, and thus the decrease in fitness, caused by increasing genome size. The second way to increase merit is through the evolution of certain phenotypic traits. Avidians can evolve the ability to perform Boolean logic calculations. If an avidian can input random numbers from the environment, perform a calculation using these numbers and output a correct result, its offspring's merit will be altered by a preset amount. The performance of calculations of greater complexity will result in a greater merit improvement. In the experiments here that select for trait evolution, we used the so-called "Logic-9" environment (Lenski et al. 2003). In this environment, the performance of NOT or NAND multiplies merit by 2, the performance of ORNOT or AND multiplies merit by 4, the performance of OR or AND NOT multiplies merit by 8, the performance of NOR or XOR multiplies merit by 16, and the performance of EQUALS multiplies merit by 32. If a genotype can perform multiple calculations, the merit multiplier is multiplicative (i.e, a genotype that can perform NOT and NAND for example has its merit multiplied by 4).

Fitness for an avidian genotype is estimated as the genotype's merit divided by its gestation time (the number of instruction executions needed for reproduction). Thus, fitness is the ratio of the number of instructions a genotype can execute in a given time to the number of instructions it needs to execute to reproduce. Therefore, there are two avenues for a population of avidians to increase fitness: increase their merit or decrease the number of instruction executions needed for self-replication.

Experimental Treatments

The first experiment we performed repeated the experimental treatment from the original study (LaBar and Adami 2016); we will refer to this as the *Variable Mutation rate* treatment. We evolved 100 populations of 10 individuals for 2.5×10^5 generations. Each population was seeded with 10 copies of the default Avida ancestor with all excess instructions removed; this resulted in an ancestor with a genome of 15 instructions (only those needed for repli-

cation). These populations could evolve the nine phenotypic traits (i.e., logic functions) mentioned above. Point mutations occurred during the copying of instructions at a rate of 10^{-2} mutations per instruction copied. Single instruction insertion and deletion mutations each occurred upon division at a rate of 5×10^{-3} mutations/genome/generation. Therefore, while the genomic point mutation rate varies with genome size in this treatment, the genomic insertion/deletion rate is fixed. In addition, genome duplications can occur at a non-specified rate; these duplications are deterministic and the result of genotype-specific replication errors.

We next performed a sequence of experiments with different treatments to test for the cause of population extinction. First, we evolved 100 populations under the *Fixed Genome Size* treatment to test whether genome expansions were required for extinction. This treatment was the same as the *Variable Mutation Rate* treatment, except genome size could not change over the experiment. Then, we evolved 100 populations under the *Fixed Mutation Rate* treatment. This treatment used the same parameters as the *Variable Mutation Rate* treatment, except point mutations happened at division, not during the instruction copying process. These mutations occurred at a rate of 0.15 mutations/generation/genome, fixing the genomic mutation rate at the ancestral value. This removed the link between genome size and genomic mutation rate and tested the effect of genome size on extinction independent of the genomic mutation rate. Next, we evolved 100 populations under the *Low Mutation Rate* treatment. Here, all parameters were the same as in the *Variable Mutation Rate* treatment except we set the copy mutation rate to 10^{-3} per instruction copied. This treatment tested the role of high mutation rates in extinction while eliminating the possibility that extinction occurred due to mutations arising during the copying process, not during the division process. Finally, we evolved 100 populations under the *No Trait Evolution* treatment. Here, all parameters were the same as in the *Variable Mutation Rate* treatment except we did not select for the evolution of the nine phenotypic traits (i.e. they did not increase merit). This treatment tested the role of increased phenotypic complexity in driving extinction.

Next, we evolved an additional 20 populations under the *Variable Mutation Rate* treatment and isolated the most abundant genotype from each population in order to discover the exact mechanism that causes complex genotypes to undergo extinction. We selected, based on their genome size, four of these genotypes to test the relationship between the lethal mutation rate (U_{Lethal}) and the extinction rate. We selected two genotypes with large genomes (208 instructions and 176 instructions) and two genotypes with smaller genomes (16 instructions and 50 instructions) to seed populations and evolved them across a range of point mutations ($\mu = \{2.5 \times 10^{-3}, 5.0 \times 10^{-3}, 1.0 \times 10^{-2}, 2.0 \times 10^{-2}, 4.0 \times 10^{-2}\}$ mutations per copied instruction for the large-genome genotypes and $\mu = \{1.0 \times 10^{-2}, 2.0 \times 10^{-2}, 4.0 \times 10^{-2}, 8.0 \times 10^{-2}, 1.0 \times 10^{-1}\}$ mutations per instruction copied for the small-genome genotypes). For each point mutation rate and genotype combination, we evolved 100 populations of 10 individuals for 10^4 generations. In order to constrain the evolution of U_{Lethal}, we fixed the genome size in these experiments at the ancestral size. We then repeated this experiment for another genotype with a small genome of 32 instructions to see if the trend seen with the genotype with a genome size of 50 instructions was an outlier (see Results). For this genotype, we used the small-genome point mutation rates.

Data Analysis

For the five main experimental treatments (*Variable Mutation Rate*, *Fixed Genome Size*, *Fixed Mutation Rate*, *Low Mutation Rate*, and *No Trait Evolution*), we collected genome size data by saving the characteristics of the most abundant genotype every 10^3 generations. For the additional 20 populations evolved to test for the genetic mechanism behind extinction, we also saved the data of the current populations and all of its ancestors every 10^3 generations. Therefore, all data shown here represent the state of the population, or its most abundant genotype, at most 10^3 generations before extinction.

In order to calculate the lethal mutation rate and other relevant statistics for a genotype, we generated every single point mutation for that genotype and measured these mutants' fitness using Avida's Analyze mode. To obtain the rate of lethal mutations (p_{Lethal}), we counted the number of these mutations that were lethal (n_{Lethal}) and divide that by the total number of mutations ($25 \times L$, where L is the genome size and 25 is the number of alternative alleles per locus). We then used these results to calculate the lethal mutation rate, $U_{\text{Lethal}} = \mu \times L \times p_{\text{Lethal}}$, where μ is the point mutation rate.

All data analysis was performed using the statistical programming language R version 3.3.1 (R Development Core Team 2016); figures were generated using the R package ggplot2 (Wickham 2009). All Avida scripts, data analysis scripts, and data used to generate the figures and tables are available at (). The Avida software is available for free at (https://github.com/devosoft/avida).

Results

Relationship between Extinction and Genome Size, Mutation Rate, and Phenotypic Complexity

In order to examine the role of genome size, mutation rate, and phenotypic complexity in driving extinction, we first repeated the previous digital evolution experiment for only small populations (LaBar and Adami 2016). For this *Variable Mutation Rate treatment*, 50% of the populations went extinct (Table 1). Extinct populations evolved larger genomes (median = 122.5 instructions) compared to surviving populations (median = 30 instructions; Two-sample Wilcoxon Rank Sum Test: W = 2307.5, n = 50; one-sided $p < 1.66 \times 10^{-13}$; Fig. 1). To further test if genome size contributes to extinction risk, we evolved 100 populations in an environment where genome size could not change (the *Fixed Genome Size* treatment). Under these conditions, no population went extinct, further supporting the hypothesis that genome expansion is required for extinction in this environment (Table 1).

There are two broad mechanisms that can in principle connect genome expansion and population extinction in Avida. First, genome expansions could directly lead to extinction by decreasing their bearer's replication speed, leading to decreased population growth and eventual extinction. Second, genome size could indirectly lead to extinction by influencing another trait directly responsible for extinction. One such trait that genome sizes influences is the genomic mutation rate: larger genomes lead to greater genomic mutation rates. Therefore, the previous results suggest a possible mechanism for extinction in these populations:

Table 1: Number of extinct populations (out of 100) across the main experimental treatments. See Methods for descriptions of treatments.

Treatment	Extinct Populations
Variable Mutation Rate	50
Fixed Genome Size	0
Fixed Mutation Rate	0
Low Mutation Rate	0
No Trait Evolution	49

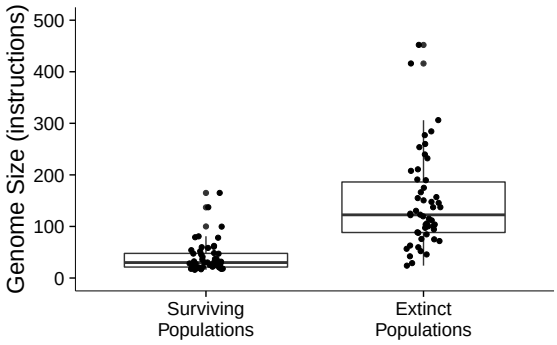

Figure 1: Final genome size of extinct versus surviving populations in the *Variable Mutation Rate* treatment. The center bar shows the median value and the edges of the box are the first and third quartile. Whiskers are at most 1.5 times the interquartile range. Each data point represents the final genome size for the most abundant genotype from one population.

an increased genomic mutation rate leads to an increased mutational load and eventually the start of a mutational meltdown (Zeyl et al. 2001; Singh et al. 2017).

To test whether genome size directly or indirectly drove population extinction, and whether the increased genomic mutation rate was the trait that directly drove these extinctions, we performed two additional experiments where we altered the relationship between genome size and the genomic mutation rate. When we eliminated the relationship between genome size and the genomic mutation rate (the *Fixed Mutation Rate* treatment) or decreased the point mutation rate by an order of magnitude (the *Low Mutation Rate* treatment), no populations went extinct (Table 1). The results from both of these "decreased mutation rate" treatments demonstrate that it is not directly a larger genome that cause population extinction. Instead, larger genomes results in increased genomic mutation rates and these elevated mutation rates cause population extinction.

Next, we tested the role of phenotypic complexity in the rate of extinction in small populations. We evolved another 100 populations under the *No Trait Evolution* treatment where increased phenotypic complexity was not under positive selection. In this treatment, the extinction rate was similar to the *Variable Mutation Rate* treatment (Table 1); 49 populations went extinct in the *No Trait Evolution* treatment. Additionally, these populations evolved similar genome sizes to those that evolved under the *Variable Mutation Rate* treatment (Two-sample Wilcoxon Rank Sum Test: W = 4609.5; median of 61.5 instructions vs. median of 59.5 instructions; n = 100; two-sided $p > 0.34$). Together, these results indicate that the evolution of phenotypic complexity does not enhance extinction, but simply occurs under the same demographic conditions.

Lethal mutational load drives population extinction

Next, we explored why an increased mutation rate enhanced the extinction rate. One possible explanation is that an elevated genomic mutation rate increases the likelihood that any given offspring will receive a lethal mutation. In this scenario, genome expansions lead to an increase in the lethal mutation rate, explaining the relationship between genome size and extinction. To test this possible relationship, we evolved a further 20 populations under the *Variable Mutation Rate* treatment, independent from our previous populations. Eleven of these populations went extinct, in accordance with the earlier results (Table 1). Similar genome size dynamics were also seen in these populations (Fig. 2).

Next, we calculated the expected number of lethal mutations per generation (the genomic lethal mutation rate) for the most abundant genotype for each population at the end of the experiment. If the population went extinct, these genotypes were selected at most 10^3 generations before the extinction event. We estimated the genomic lethal mutation rate as $U_{\text{Lethal}} = \mu \times L \times p_{\text{Lethal}}$, where μ is the point mutation rate, L is the genome size, and p_{Lethal} is the fraction of all single point mutations that are lethal. Genotypes from extinct populations had a greater U_{Lethal} than genotypes from surviving populations (Two-sample Wilcoxon Rank Sum Test: W = 82; median of 0.27 mutations/generation vs. median of 0.18 mutations/generation; n = 20; one-sided $p < 6.2 \times 10^{-3}$; Fig. 3). These results suggest that genotypes with larger genomes and high genomic mutation rates go extinct because a high genomic mutation rate increases the genomic lethal mutation rate.

To further test the relationship between U_{Lethal} and extinction, we performed experiments

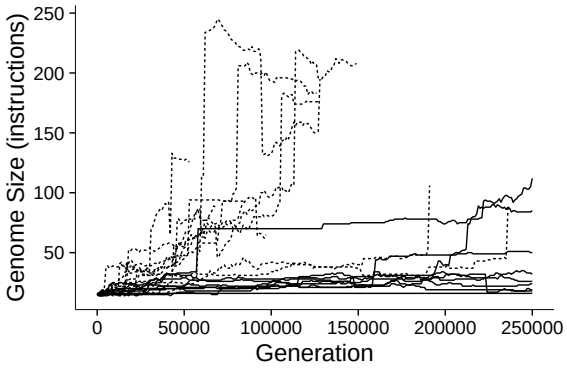

Figure 2: Genome size over time for the 20 additional populations evolved under the *Variable Mutation Rate* treatment. Dotted lines represent populations that eventually went extinct and solid lines are populations that survived. Genome size of the most abundant genotypes was measured every 10^3 generations.

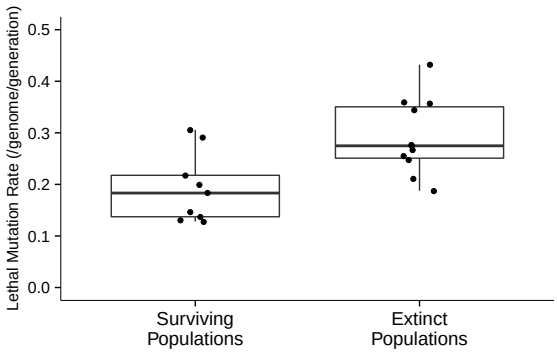

Figure 3: Final lethal mutation rate (U_{Lethal}) for surviving and extinct populations. Data shown are from the most abundant genotype from each of the 20 additional populations evolved under the *Variable Mutation Rate* treatment. Boxplots as in Figure 2.

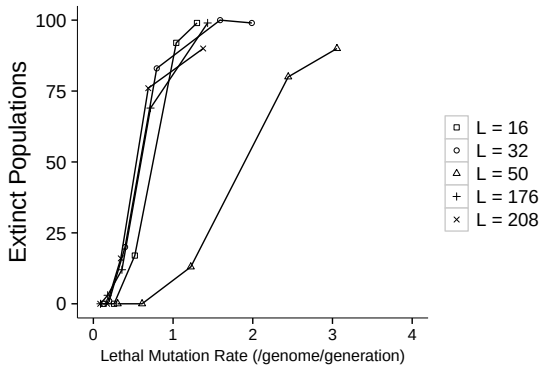

Figure 4: The relationship between the lethal mutation rate and population extinction/survival. The number of extinct populations as a function of lethal mutation rate for five of the genotypes shown in Figure 3. L is the genome size for a given genotype in number of instructions. One hundred replicate experiments were performed for each point mutation rate and genotype combination.

where we manipulated U_{Lethal} for different genotypes and measured how increases in U_{Lethal} altered the extinction rate. We took the two genotypes with the largest genomes (genome sizes of 208 instructions and 176 instructions, respectively) and the three genotypes with the smallest genomes (genome sizes of 16, 32, and 50 instructions, respectively) from the twenty populations and evolved them for 10^4 generations under a range of different point mutation rates. We fixed their genome sizes at these ancestral values in order to control U_{lethal}. However, one element of U_{Lethal} (p_{Lethal}) could still evolve. The extinction rate increased as U_{Lethal} increased for every genotype, further demonstrating that the lethal mutational load drives population extinction (Fig. 4). Four out of the five genotypes showed a similar relationship between U_{lethal} and the extinction rate. However, one genotype showed a drastically different trend than the others, suggesting that it is possible to evolve genomic architecture robust to extinction.

The above results demonstrate that genotypes with large genomes have an increased genomic lethal mutation rate that directly causes population extinction. However, it does not explain why these large-genome genotypes have an increased genomic lethal mutation rate. While it is true that increasing genome size will increase the genomic mutation rate, it does not follow that the lethal mutation rate will also increase. For example, if an individual's genome doubles in size, its genomic mutation rate will also double. However, if the additional genome content has no effect on fitness, then p_{Lethal} will be reduced by half and U_{Lethal} will remain constant. This can be further seen by re-writing $U_{\text{Lethal}} = \mu \times L \times \frac{n_{\text{L}}}{25 \times L}$, where n_{L} is the number of lethal point mutations (among all genotypes one mutation away from the wild-type genotype) and 25 is the number of alternative alleles per locus for Avida genomes. Thus, $U_{\text{Lethal}} = \mu \times \frac{n_{\text{L}}}{25}$. In other words, in order for U_{Lethal} to increase, n_{L} must increase. Therefore, for an increased genomic mutation rate to cause extinction by increasing the lethal mutation rate, the total number of lethal mutations must also increase. This equation then predicts that if there is a positive correlation between U_{Lethal} and genome size, there will also

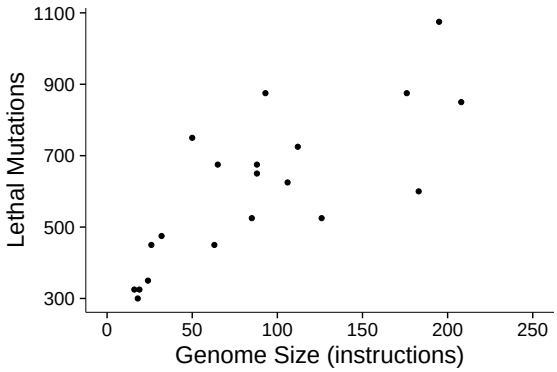

Figure 5: The relationship between genome size and the number of lethal point mutations (n_{L}) in a genotype's immediate mutational neighborhood. Each data point represents data from the most abundant genotype from one of the 20 additional populations evolved under the *Variable Mutation Rate treatment*.

be a positive correlation between genome size and the number of possible lethal mutations.

To test why larger genome size led to a greater lethal load, we took the 20 genotypes previously used to measure U_{Lethal} (Fig. 4) and measured the correlation between the number of lethal point mutations (n_{L}) and genome size. These two variables were positively correlated (Pearson's $r = 0.77; p < 6.08 \times 10^{-5}$; Fig. 5). These results suggest that it is the combination of an increased genomic mutation rate and an increased number of lethal point mutations that results in an increased lethal mutation rate and the relationship between genome size and an elevated extinction risk.

Discussion

We explored the role of genome size in the extinction of small populations and found that small populations went extinct because weakened selection allowed for the fixation of genome expansions. These genome expansions both increased the genomic mutation rate and the total number of lethal point mutations in a genotype's mutational neighborhood. Together, increases in these traits resulted in an elevated lethal mutation rate, which caused a greater risk of extinction. These experiments demonstrate that genome expansions that fix due to weakened selection can increase the risk of extinction in small populations.

It was previously observed that small population size drives the evolution of greater genome size, greater phenotypic complexity, and greater extinction risk in the Avida experimental setup used here (LaBar and Adami 2016). Here, we further tested whether the evolution of novel complex traits contributed to extinction risk. In other words, are more complex organisms at a greater risk of extinction? We found no direct influence of phenotypic complexity on extinction risk. Instead, we found that the same factors that drove the evolution of increased phenotypic complexity indirectly increased extinction risk: small population size, increased genome size, and increased genomic mutation rate. This relationship further suggests that gains in phenotypic complexity in small populations may be limited by

extinction.

The genetic mechanism behind extinction we observed here was that genome expansions lead to: 1) increased genomic mutation rates, and 2) an increased likelihood of lethal mutations, likely due to epistatic interactions between original genome content and novel genome content. Together, these factors lead to an increased lethal mutational load and drive population extinction. While the Avida genetic system has no obvious parallel in biology, it is suggestive that similar mechanisms behind extinction operate in natural populations. For instance, selfish genetic elements were originally proposed as the mechanism behind the correlation between genome size and extinction risk due to enhanced transposable element (TE) activity (Vinogradov 2003; 2004). These selfish genetic elements are absent from avidian genomes by design. However, in both cases, the consequences are similar. An expansion of the number of TEs increases the chance that essential genome content will be mutated, thus increasing the lethal mutation rate. Similarly, an increase in genome size also increases the likelihood of a lethal mutation in Avida, although by point mutations, not mobile elements. Likewise, it was recently proposed that genome duplications can cause increased mutational fragility at some loci (Diss et al. 2017), in addition to increased mutational robustness at other loci (Gu et al. 2003). Both of these genetic mechanisms may provide a causal link between genome size, the lethal mutation rate, and population extinction in biological populations, just as we have shown occurs in digital populations.

Traditional models of mutational meltdown predict that both asexual and sexual populations with up to 10^3 individuals can go extinct quickly on a macroevolutionary timescale (Lynch et al. 1993; 1995). However, we found that only very small populations underwent extinction, that these extinctions occurred only after a long survival time, and that these populations only went extinct due to the fixation of genome expansions. Why the discrepancy? One factor is that the fixation of deleterious genome expansions only occurs in very small Avida populations due to the strong deleterious effects of insertion mutations (LaBar and Adami 2016). Furthermore, in Avida, the fixation of slightly-deleterious point mutations does not directly decrease absolute fitness, only relative fitness. The fixation of slightly-deleterious genome expansions indirectly decreases absolute fitness by increasing genome size and increasing the lethal mutation load. However, the lack of a direct pressure on absolute fitness in Avida likely limits the rate of extinction. The lethal mutation rate never evolves above 0.5 mutations/generation/individual (Fig. 3) and does not continuously increase throughout the experiment. In models of mutational meltdown, slightly-deleterious mutations will directly impact absolute fitness by either decreasing a genotype's growth rate or its offspring's probability of survival to adulthood (Lynch and Gabriel 1990; Gabriel et al. 1993). This direct link allows for a larger absolute-fitness decrease in mutational meltdown models than seen in Avida, and is likely behind the differences in extinction rates seen between these two models.

It is worth remarking on the one outlier genotype that appeared robust to extinction compared to some of the other genotypes (Fig. 4). We did not determine which characteristics of this genotype resulted in this "extinction robustness". One possible population-genetic hypothesis to explain extinction robustness could be that this genotype has a high likelihood of suppressor mutations. In high mutation-rate conditions, genotypes often receive multiple mutations. Therefore a mutation that is lethal by itself may be ameliorated in the presence of a second mutation. In other words, the second mutation suppresses the lethal phenotype of

the first mutation. We recently proposed the concept of *drift* robustness as an evolutionary response to the problem of fitness maintenance in small populations (LaBar and Adami 2017). It is not inconceivable that small populations could evolve extinction robustness through a similar mechanism. Only lineages from small populations that happen to evolve extinction robustness would be maintained over time because only these lineages would survive. While such a scenario would not manifest itself in a single small population, a metapopulation of small populations could lead to such an evolutionary outcome. Future work should investigate whether the presence of suppressor mutations explains the trend we saw here and whether extinction robustness may be a common evolutionary outcome in small populations.

Given that extinctions seen in this experimental setup only occur at very low populations sizes (LaBar and Adami 2016) and that these extinctions only occur after tens, if not hundreds, of thousands of generations (Fig. 2), it is worth asking if the likelihood of extinction occurring due to genome size is greater than extinction due to other factors. Small populations often have a smaller adaptive potential than large populations due to decreased genetic variation (Willi et al. 2006). This limits a small population's ability to survive sudden environmental changes (Bell and Gonzalez 2009). Our experiments here could not test whether sudden environmental shifts cause greater extinction rates than genome expansions. While it is likely that environmental change is responsible for more extinction events than the accumulation of deleterious and lethal mutations, we would argue that the lethal load from genome expansions still alters the survival probability of small populations. A population with a large lethal load is likely less able to adapt to an environmental stressor due to the reduction in effective population size. Furthermore, an increased lethal mutational load may drive an already-stressed population beyond a tipping point to eventual extinction. Future exploration of the relative likelihood of different factors in driving extinction are needed to firmly establish genome expansions as a mechanism behind extinction in small populations.

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