Investigating the consequences of the mating system for drug resistance evolution in *C. elegans*

Trubenová, B., Hellinga, J., Krücken, J.,
von Samson-Himmelstjerna, G., Schulenburg, H., Regoes, R.

November 7, 2024

Abstract

Helminth infections, a significant global health concern, are effectively treated with anthelmintic drugs. However, the rise of anthelmintic-resistant strains in livestock threatens both animal and human health. Understanding the factors influencing anthelmintic resistance is crucial to mitigate the threat posed by these parasites. Due to difficulties in studying parasitic worms in the laboratory, the non-parasitic nematode *Caenorhabditis elegans* is used as a model organism to investigate anthelmintic resistance evolution. However, the suitability of this free-living nematode as a model for parasitic worms is also debatable due to its unique androdioecious reproductive mode, which enables hermaphrodites to reproduce by selfing or mating with males. While selfing offers reproductive assurance and efficient selection, it can reduce genetic diversity and lead to harmful mutations. Conversely, outcrossing with males introduces new genetic variation, aiding adaptation but potentially slowing the spread of beneficial mutations. The androdioecious reproductive system is rare among nematodes, raising questions about the generalizability of findings from *C. elegans* to other species with different mating systems. In this study, we aim to evaluate the suitability of *C. elegans* as a model organism for investigating the evolution of antihelmintic resistance in parasitic nematodes. We developed a polygenic model incorporating various reproductive modes and drug resistance to better understand the complex interplay between reproductive mode and resistance evolution. Combining a population genetic perspective on anthelmintic resistance evolution with pharmacodynamic modelling, we investigated the effects of reproductive strategy and other aspects, such as dominance, mutational effects, the number of loci, and population size, in determining the dynamics and outcome of evolutionary processes. We found that androdioecious populations exhibited rapid initial adaptation, typical for hermaphrodites, alongside the ability to endure high drug concentrations, which was observed in dioecious populations. Androdioecious populations also exhibited the highest diversity and shortest time to fixation of the beneficial allele. These results suggest that androdioecious populations can harness the advantages of both selfing and outcrossing, optimizing their reproductive strategy in response to drug selection. Both population size and the specific genetic architecture of resistance influence the ideal balance between these reproductive modes.

1 Introduction

Helminths, particularly parasitic nematodes, are diverse parasites of plants, animals, and humans. Despite advances in sanitation, helminth infections are among the most common infections worldwide, affecting large human populations, mainly in low-income countries and in the poorest and most deprived communities. They cause a significant burden on the population, drastically decreasing the quality of life and life expectancy, especially when combined with malnutrition. Nevertheless, many helminthiases belong to the so-called

Neglected Tropical Diseases group because they are almost absent from the global health agenda and research into these diseases is severely underfunded.

Anthelmintic drugs are generally very efficient, clearing infections within days. However, extensive use of these drugs in grazing animals led to the evolution of drug-resistant strains [Schwab et al., 2006, Schulz et al., 2018, Charlier et al., 2022, Mohammedsalih et al., 2024]. Their prevalence has increased globally in recent years, representing a severe problem in various livestock sectors [Kotze and Prichard, 2016, Sangster et al., 2018], and becoming an increasingly severe threat to agriculture and human health [Charlier et al., 2022, Wolstenholme et al., 2004, Von Samson-Himmelstjerna, 2012, Tinkler, 2020]. Indeed, the reduced sensitivity of *Ascaris* populations to benzimidazole has already been detected in children in Rwanda [Krücken et al., 2017]. As many parasitic worms are zoonotic, anthelmintic resistance in worms is a significant focus of One Health initiatives.

Understanding the factors that influence the evolution of anthelmintic resistance in parasitic worms is essential to mitigate their threat. Unfortunately, conducting laboratory experiments involving parasitic worms poses challenges due to the requirement of an experimentally accessible host organism, the lack of genetic tools for analysis of the parasites, and the long life cycle of many parasites. Consequently, these studies are relatively rare. Therefore, the intensively studied nematode *Caenorhabditis elegans* serves as a model organism for helminth infections despite not being a parasite, mainly due to its short life span and easy maintenance in the laboratory setting, well-annotated genome and similar response to anthelmintics [Bürglin et al., 1998, Geary and Thompson, 2001]. It has been used successfully as a model for parasitic worms in the past, yielding important insights into the evolution and genetics of resistance against anthelminthic drugs [Driscoll et al., 1989, Dent et al., 2000, James and Davey, 2009, Ménez et al., 2019, Hellinga et al., 2024].

However, the suitability of the free-living *C. elegans* as a model organism for parasitic worms has also been challenged [Geary and Thompson, 2001]. Beyond its free-living lifestyle, one of the key distinctions lies in its reproductive mode. *C. elegans* is androdioecious, with populations consisting of self-fertilizing hermaphrodites and a small proportion of males. While nematode reproductive modes are remarkably diverse [Singaravelu and Singson, 2011], most of the parasitic worms reproduce by mating between males and females (dioecy). One of the few known exceptions is *Phasmarhabditis hermaphrodita*, a nematode parasite of slugs, that is a hermaphrodite [Wilson et al., 1993, Rae et al., 2010]. Overall, androdioecy is relatively rare, with only a few examples [Weeks, 2012, Kanzaki et al., 2013].

This diversity in reproductive strategies raises crucial questions for understanding adaptation. Theoretical studies in population genetics have emphasized the potential impact of reproductive mode on the rate and trajectory of evolution [Orr and Otto, 1994, Kondrashov, 1994, Otto and Lenormand, 2002, Kleiman and Tannenbaum, 2009, Gerstein et al., 2011, Colegrave, 2012, Hartfield and Glémin, 2016, Hartfield et al., 2017, Uecker, 2017]. However, these studies often focus on the broad divide between sexual and asexual reproduction [Melián et al., 2012], rather than the finer distinctions among mating systems like dioecy, hermaphroditism, and the complex dynamics of androdioecy. As a rare exception, Husse et al. [2013] developed a one-locus model to explore the dynamics of androdioecious species, finding that a slight reproductive advantage of males can maintain high male frequencies. Weeks et al. [2006] conducted a comparative analysis of androdioecy in animals, suggesting that it may have evolved as a response to reproductive assurance in species with episodic low densities.

Due to the limited number of studies on androdioecy in animals (see Yamaguchi and Iwasa [2021] for exceptions), our understanding of the androdioecious systems and their consequences on evolutionary adaptation come primarily from plant research, where androdioecy is more common. Androdioecy requires specific conditions to evolve, and its origin and presence are still puzzling scientists [Wegewitz et al., 2008, Chasnov, 2013]. Some researchers suggest that androdioecy is an intermediate state between hermaphroditism

and dioecy (see Weeks [2012] for a detailed review). It has been shown that selfing by hermaphrodites provides reproductive assurance, allowing populations to persist through periods of low density when mates are scarce [Chasnov, 2013]. In addition, selfing increases homozygosity, exposing new mutations (both beneficial and deleterious) to natural selection, thereby increasing its efficacy [Ellis and Lin, 2014]. However, selfing may reduce genetic variation over time, limiting the potential to adapt to novel conditions. In contrast, when hermaphrodites outcross with males, new genetic variation can be introduced into the population, which may be especially suitable for adaptation to changing environments [Yamaguchi and Iwasa, 2021, Ellis and Lin, 2014]. Furthermore, outcrossing is expected to compensate for lower fitness caused by inbreeding depression [Frankham et al., 2010].

In summary, the androdioecious reproductive mode presents a complex landscape of potential evolutionary advantages and disadvantages. However, due to the limited number of studies, the evolutionary consequences of androdioecy remain poorly understood and the generalizability of findings from *C. elegans* to other nematode species with diverse reproductive modes remains an open question.

To address this gap, this study investigates the suitability of *C. elegans* as a model organism for studying the evolution of antihelmintic resistance in parasitic nematodes. We aim to improve our understanding of the evolutionary consequences of androdioecy using a mathematical modelling approach. For this, we developed a polygenic population genetic model of a nematode population with dioecious (mating), hermaphroditic (selfing), or androdioecious (combined selfing and mating) reproduction. We investigate how these reproductive modes influence the rate and outcome of adaptation to an anthelmintic-containing environment. Moreover, we investigate how other factors, such as dominance, population size and the number of loci and their effects, influence the consequences of the mating system. To incorporate the selective pressures of this environment, we utilize a modified pharmacodynamic framework from Regoes et al. [2004], which captures the varying effects of drug concentrations on populations with different mutations, thus enabling us to model anthelmintic resistance in diploid organisms. This approach, previously successful in studying polygenic adaptation in bacterial biofilms [Trubenová et al., 2022], promises novel insights into the role of reproductive strategy in drug resistance evolution among parasitic worms.

2 The model

Below, we describe our compartmental, polygenic, population genetic model. Inputs of the model are various parameters determining the genetics, (e.g., number of loci), the translation to phenotype (e.g. fitness effects of individual mutations), and the details of the experiment (e.g. length of the treatment, drug concentration, etc., see Table 1 for details). The model compartments represent a particular class of individuals defined by their sex and genotype. We assume that the organisms are diploid.

How each genotype manifests depends on whether the mutations are dominant or recessive, which determines the genetic values of the genotypes. Then, each genotype is associated with a basal fitness (in the absence of the drug), and it has an EC_{50} value, as explained in detail below. Hermaphrodites from each compartment can self-fertilize or mate, laying eggs according to the origin of sperm, their fitness, and genotype. These eggs may have various genotypes, assuming that all genetic locations are not linked and are carried at different chromosomes. Additionally, mutations may occur. Finally, offspring generation replaces the parental one and may be reduced to a required size depending on the modelled scenario. All of these processes are explained in detail below. See Figure 1 for a schematic representation of the overall simulation process.

The model's output is a complete record of the population sizes of all compartments over time. Therefore, it is possible to observe population dynamics (e.g., changes in population sizes, allele frequencies, or accumulation of the mutations), the timing of events (e.g., when a mutant appears or reaches a certain frequency), and

to calculate the emerging population properties (e.g., diversity) and observe their changes over time. The model is implemented in Python, and simulations are stochastic, allowing us to determine the probability distributions of the outcomes.

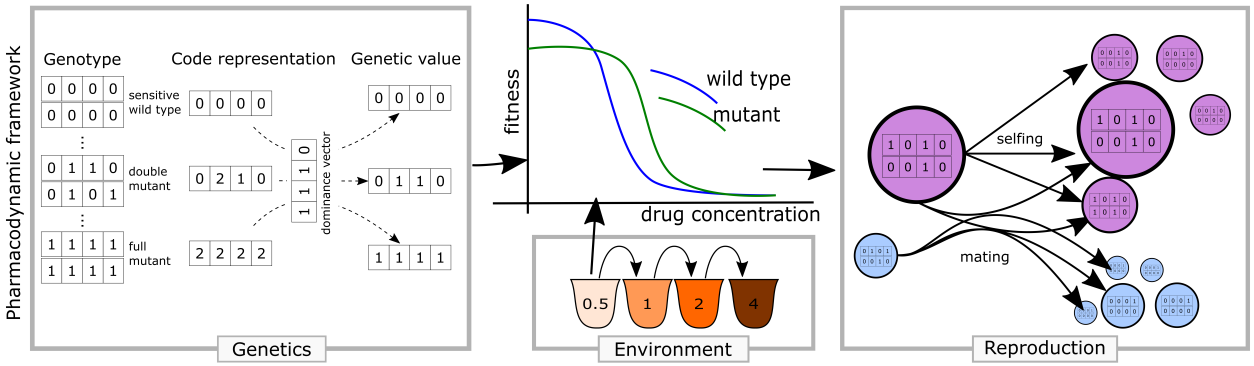

Figure 1: Modelling evolution of resistance in diploid organisms. The panel on the left shows diploid genomes with 4 loci, each with two alleles (0 or 1), whereby 1 confers drug resistance. The middle panels shows drug resistance for wild-type and mutant, indicated through the relationship between drug concentration (X axis) and egg count as a proxy for fitness (Y axis). The right panel highlights different reproduction alternatives between a female/hermaphrodite (in purple) and a male (blue). The panel at the bottom represents a serial passage experiment with increasing drug concentration.

Genotypes As parasitic worms, as well as *C. elegans*, are diploid organisms; we represent their genotype, consisting of k unlinked loci relevant for the modelled adaptation process, as a string of length k. Each position describes the number of mutated alleles at that particular locus: 0 represents a sensitive/wild-type homozygote (at the particular locus), 1 represents a heterozygote, and 2 represents a homozygote with two mutated alleles. With k loci, there are 3^k possible genotypes. These represent sub-populations (classes) with possibly distinct pharmacodynamic properties.

As we assume a diploid genome, the relationship between the alleles of the same locus must also be defined. Vector D (length k) determines dominance for individual loci. If $D = 1$, all mutated alleles (at all loci) are considered dominant, and genotype 1 (with alleles 0 and 1) has the same phenotype p as genotype 2 (with two mutated alleles). If $D = 0$, mutated alleles are considered recessive, and genotype 1 has the same phenotype as genotype 0. If $0 < D < 1$, we assume co-dominance and heterozygotes will differ from both homozygotes.

Sexes From each genotype, it is possible to have individuals who are capable of contributing their genetic information to the next generation but do not lay eggs, further referred to as males. In addition, we also include individuals that contribute their genetic information and are capable of laying eggs. Depending on the simulated scenario, these individuals may be able to self (hermaphrodites) or require mating in order to lay eggs (representing females). However, due to simplicity, we will refer to all egg-laying individuals as hermaphrodites throughout this manuscript, and the ability to lay eggs with or without male presence will be modulated by a parameter ξ, as explained below. All individuals may have any possible genotype, altogether creating 2×3^k of sub-populations (compartments) that are simulated.

Pharmacodynamics and fitness We assume that the main effect of the environment is demonstrated in the number of offspring a particular genotype can produce. While biologically, this is undoubtedly a simplification, it is fitness in its narrow sense as it describes a number of offspring that survive until adulthood and can undergo reproduction. The pharmacodynamic can encompass various processes - from decreased survival of the genotype in focus, with subsequently reduced offspring production and decreased mobility, thus affecting the ability to feed/find a mating partner(s), or just having fewer eggs per individual.

In the presence of an anthelmintic, the fitness of all individuals is reduced. The concentration of the anthelmintic required to produce a specific effect (such as killing, paralyzing the worms, or preventing reproduction) is higher for the mutated worms, indicating an increase in the EC_{50} compared to the wild type. However, we also assume that mutations conferring drug resistance come with a cost in the absence of the drug, leading to a reduction in fitness.

Therefore, in our model, each resistant allele is associated with a benefit increasing the carrier's fitness in the presence of drugs and a cost-reducing fitness in its absence. Costs and benefits are given as vectors of length k, each element corresponding to the respective locus. Mutational effects of resistance-conferring mutations can be of various sizes and combined together [Das et al., 2020].

In our model, we assume that costs combine in a multiplicative manner, while benefits in an additive manner [Trubenová et al., 2022, Knopp and Andersson, 2018, Wistrand-Yuen et al., 2018].

The total cost associated with phenotype i is

$$C_i = 1 - \prod_j (1 - p_{ij} c_j) \tag{1}$$

and the total benefit associated with phenotype i is:

$$B_i = \mathbf{p_i} \cdot \mathbf{b} = \sum_j g p_{ij} b_j. \tag{2}$$

The total cost associated with a particular genotype (strain) determines its fitness in the absence of the drug $\phi_i(0) = 1 - C_i$, where the wild-type fitness is 1. The total benefit captures the increase of the EC_{50} of this strain as $EC_{50i} = EC_{50wt} + B_i$, where EC_{50wt} is the EC_{50} of the sensitive wild-type. The fitness in the absence of the drug and EC_{50} define pharmacodynamic curves that allow us to determine the expected fitness of any strain i in any concentration A (see Figure 2:

$$\phi_i(A) = (1 - C_i) \left(1 - \frac{\left(\frac{A}{EC50_i}\right)^{\kappa}}{\left(\frac{A}{EC50_i}\right)^{\kappa} + 1} \right) \tag{3}$$

where A is the drug concentration.

Egg count Apart from fitness, the number of eggs the hermaphrodite can lay is also influenced by the origin of sperm: whether it comes from mating or selfing. In line with the experimental observations, for *C. elegans*, we will assume that mated, wild-type individuals can lay, on average, 1000 eggs in a drug-free environment when out-crossed but only 300 when selfing [Kimble and Ward, 1988]. Therefore, the number of eggs that the individual lays is $300\phi_i(A)$ if the individual selves, or $1000\phi_i(A)$ if mated.

Reproduction Depending on the simulated scenario, a fraction of hermaphrodites in the population engage in mating with males, while the remainder reproduces through self-fertilization. Mating between hermaphrodites and males is random with respect to male genotype. Thus, the frequency of mating between a hermaphrodite and a male of a given genotype is proportional to the frequency of that male genotype in the population. Offspring are generated with probabilities calculated using Punnet squares. Half of the offspring (see the definition of egg count) in each genotype class are males, while the other half is allocated to be hermaphrodites and merged with the populations calculated in the selfing step outlined below.

Due to recombination and/or chromosomal shuffling, selfing individuals can also produce offspring with different genotypes, but these are always hermaphrodites. As the emergence of males from selfing is rare,

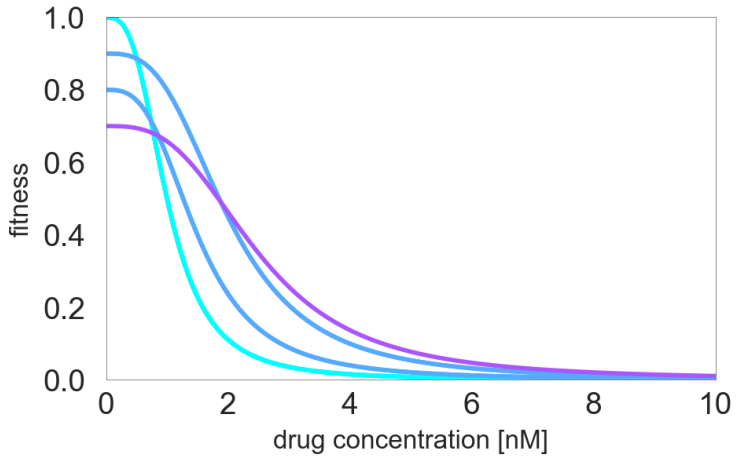

Figure 2: Fitness function of different strains.
In this illustrated example, resistance is encoded by two independent recessive mutations. Wild type (cyan) has the highest fitness in the absence of the drug but the lowest EC_{50}. Two single mutants (with homozygous mutations at one of the loci, shown in blue) have lower fitness but higher EC_{50}. The double mutant (homozygous for mutations at both loci, shown in purple) has the lowest fitness of all the strains in the absence of the drug but the highest EC_{50}. Mutations are recessive, thus heterozygous loci do not contribute to resistance, nor do they reduce fitness.

in our model, we assume that males are only generated by mating. A probability of a hermaphrodite that belongs to the genotype i to generate eggs/offspring that belongs to the sub-population with genotype j is calculated based on the Mendelian rules using Punnet squares for k loci. The linkage between loci is not currently considered; any alleles can be inherited independently from another allele at a different locus.

Mutations: During reproduction, mutations may occur. We assume that one mutation in the genome occurs directly after fertilization (either by mating or selfing) with probability μ, changing one locus in the whole genome from 0 to 1, 1 to 0 or 2, or from 2 to 1. Only a single mutation is allowed per individual, so approximately (sampling from Poisson distribution) μN_i individuals from genotype class i can mutate into any genotype class in the mutational distance 1. If there are multiple target genotype classes, μN_i individuals are divided into all of them in equal proportions.

Environment The experiment, or the treatment, consists of any number of cycles (length of the cycle corresponds to a generation time), starting drug concentration, changes in drug concentrations and their schedule, initial population sizes, the drug degradation rate, conditions for the environmental change (e.g. increase of concentration), and the dilution of the final population before it enters the next cycle. See Table 1 for details. By setting these parameters, many different treatment regimes can be modelled by adjusting these parameters: from a single treatment through serial passages commonly used in evolutionary experiments to a chemostat environment.

3 Simulated scenarios

We take advantage of this study's computational nature and investigate a wide range of parameters and aspects that would not be feasible in a laboratory setting. For each scenario, we conducted 100 repeats. Moreover, we followed the populations for 500 generations, which would correspond to more than 4 years of

Table 1: Symbols used in the model

Variable	Explanation
N	Initial number of worms in the simulated population
l	Number of loci
μ	Mutation rate per genome (not locus)
f_m	Fraction of males
egg_s	Mean selfing fecundity/ offspring number
egg_m	Mean mating fecundity/ offspring number
C	Cost vector
B	Benefit vector
D	Dominance (0 for recessive, 1 for dominant)
ξ	a fraction of hermaphrodites that mate

continuous evolution experiments with *C. elegans*. Below, we explain the different aspects we focused on and the parameters that we used.

3.1 Mating systems

In our simulations, the fraction of hermaphrodites that mate (ξ) is independent of the fraction of males in the population. This corresponds to a biological scenario where availability of males is not a limiting factor, but rather, the mating fraction is determined by avoidance/ propensity (inclination) of hermaphrodites to mate. This means that if any males are present in the population, the fraction of hermaphrodites ξ will mate, with the frequency of matings contributed by males of different genotypes proportional to the frequency of those genotypes in the population. By varying the parameter ξ, we can simulate different mating systems: from hermaphroditic through androdioecious to dioecious.

In all cases, the initial fraction of males is 0.5. However, after a single iteration, the fraction of males is independent of the starting fraction but depends on ξ, as well as on the fecundity of hermaphrodites when selfing and mating:

$$f_m = \frac{0.5\xi egg_m}{\xi egg_m + (1-\xi) egg_s}. \tag{4}$$

To simulate the **hermaphroditic mating system**, we set the fraction of hermaphrodites that mate to $\xi = 0$. This means that males are purged from the population after a single generation, and the population remains hermaphroditic.

On the other hand, to simulate the **dioecious mating system**, we set the fraction of mating hermaphrodites to $\xi = 1$. This means that all the egg-laying individuals reproduced only by mating, essentially representing females. The fraction of males fluctuates around 0.5.

When $0 < \xi < 1$, some hermaphrodites reproduce by mating, while others by self-fertilization, representing an **androdioecious mating system**. We simulated a range of values of ξ in increments of 0.1, where $0.1 \leq \xi \leq 0.9$. The fraction of males fluctuates around f_m given by equation 4

3.2 Genetic factors

Number of loci First, we assume two loci with relatively small effects, resulting in nine possible genotypes. Then, to investigate evolutionary dynamics in more complex scenarios, we assume six independent loci, three with small and three with large effect. It allows the generation of 729 different genotypes, reflecting the complexity found in nature.

Dominance We investigate adaptation when all loci are dominant or recessive.

Table 2: Performed simulations
We performed simulations for dominant (value 1) or recessive (value 0) mutations, four population sizes, and 11 values of ξ (hermaphrodites, 9x androdioecious populations with an increment of 0.1, dioecious populations). All simulations were run for 500 generations, with 100 repeats. Altogether, we performed 8 800 simulations.

Loci	Dominance	N	ξ
2	0	200	0-1
		2000	0-1
		20000	0-1
		200000	0-1
2	1	200	0-1
		2000	0-1
		20000	0-1
		200000	0-1
6	0	200	0-1
		2000	0-1
		20000	0-1
		200000	0-1
6	1	200	0-1
		2000	0-1
		20000	0-1
		200000	0-1

3.3 Size and timing

Population size Size of the population is known to play an important role in the rate of adaptation. Therefore, we performed simulations with 200, 2000, 20000 and 200000 individuals.

Time scale In addition, it is important to consider time scale when evaluating the evolutionary consequences of the mating strategy. While some may encourage short-term survival and rapid adaptation, others find long-term survival and slower adaptation beneficial in the long run. Therefore, we performed 100 simulations of 500 generations for each parameter set. Having complete information about the dynamics (population sizes of all compartments at all times), we also investigated what happens in the earlier stages that could be achieved in experiments. In particular, we looked at populations after 10, 25, 100, 250, and 500 generations.

3.4 Environment

Inspired by *in-vitro* evolution experiments performed in *C. elegans* to investigate drug resistance evolution, we simulated a gradually increasing drug concentration (e.g. ivermectin). The population starts in an environment with no drug concentration, which increases over time when the population is deemed sufficiently adapted (having an average egg count of at least 30 per individual). Populations are passaged from one generation to another, increasing the drug concentration but allowing constant growth (no limits imposed by carrying capacity). This approach allows the evolution of high resistance while limiting the probability of extinction.

4 Results

4.1 Mating facilitates sustained adaptation in large populations with recessive mutations

Our findings demonstrate that a gradual drug concentration increase enables populations to adapt to relatively high drug concentrations (Figure 3). Population size is a critical factor, with larger populations exhibiting faster adaptation. As simulations are initiated with wild-type populations (no mutations are present), it suggests that mutational supply limits adaptation in smaller populations, and mutations are less frequent in smaller populations.

However, the interplay between population size and adaptation is more complex, as is particularly evident in Figure 3 C, for mating populations. Here, larger populations (200 000 individuals) initially lag behind smaller ones (2000 and 20 000 individuals) in progressing to higher concentrations. A similar effect is also seen in androdioecious populations ($\xi = 0.5$), where populations of 2 000 individuals are the first to overcome the 24 nM threshold (Figure 3 E). This indicates that while the mutation supply limits small populations, once the mutation appears, it takes longer to outcompete the others in larger populations.

Furthermore, Figure 3 highlights distinct adaptation patterns across the three mating strategies. Strictly selfing populations (hermaphrodites only) rapidly adapt to 24 nM but fail to exceed this threshold within the simulation timeframe (Figure 3 D). In contrast, strictly mating populations exhibit slower initial adaptation but then continue adapting to 28 nM (Figure 3 F). Androdioecious populations display a rapid initial adaptation, akin to hermaphrodites, followed by a slower progression to higher concentrations. Nonetheless, they ultimately adapt to a greater extent than hermaphrodites (Figure 3 E). Moreover, adaptation of the smallest androdioecious populations in the given timeframe exceeded that of the smallest dioecious populations. This suggests that androdioecious populations may be able to reap the benefits of both mating and selfing strategies. Therefore, evolution experiments with *C. elegans* might overestimate the adaptation to antihelmintics, especially at low population sizes.

4.2 Rate of adaptation to higher concentration does not directly reflect genetic changes.

The increasing ability to survive in higher concentrations is enabled by the gradual accumulation of resistant mutations. However, the rate of adaptation does not necessarily reflect the rate of mutation accumulation, as other differences between different reproductive strategies are also present.

Figure 4 illustrates the average number of homozygous loci within the population over time, considering that resistant mutations are recessive, with six loci potentially contributing to resistance. Hermaphroditic populations exhibit a faster accumulation of homozygous mutations compared to strictly mating populations, which demonstrate the slowest adaptation. Furthermore, hermaphroditic populations display a stepwise increase in homozygous loci, whereas other mating systems exhibit a more gradual progression.

While Figure 4 shows that hermaphrodites reach the maximum of six homozygous loci within the simulated period, this is not observed in dioecious populations. Only three (or fewer) loci with large effects are homozygous for the mutated allele in strictly mating populations by the end of the simulations. Androdioecious populations display a similar pattern, remaining stagnant at three homozygous mutated loci for an extended duration. However, Figure 4 B shows that this threshold is eventually crossed, and these populations continue to accumulate mutated loci.

Initially, this appears to contradict the earlier observation of dioecious populations achieving resistance to the highest concentrations. Therefore, it is important to note here that in the same conditions, dioecious

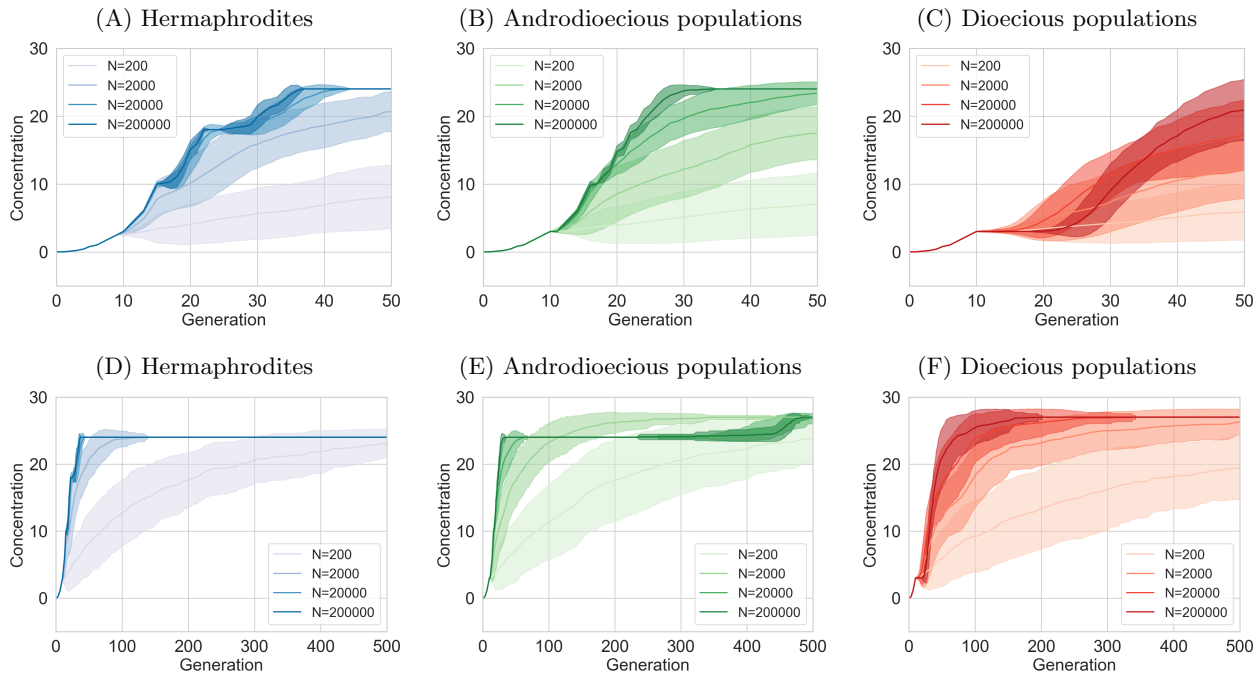

Figure 3: Adaptation in increasing drug concentration, assuming recessive mutations and six loci. Mean and standard deviation of 100 simulation trials. In androdioecious populations, 50% of hermaphrodites reproduce by mating, 50% by selfing. A-C: first 50 generations. D-F) all 500 generations.

populations produce, on average, a greater number of offspring (500 per individual) than selfing hermaphrodites (300 per individual; see Reproduction section) in a drug-free environment. This difference in average egg count persists across genotypes and drug concentrations, with hermaphrodites always laying more eggs after mating compared to self-fertilization. It allows dioecious populations to have satisfactory fertility to meet the requirements for increasing drug concentration. Consequently, the selection pressure on dioecious populations for further evolution is reduced, decelerating the accumulation of mutations. At the same time, hermaphrodites, while having all six loci adapted, do not progress to higher concentrations. This is caused by the small effects of three of the loci. Even their combined effect is not sufficient to allow them to reach the egg count threshold required to proceed to higher concentrations (see the section on Genetics and sections below). The androdioecious populations also slow down mutation accumulation after the loci of large effect are fixed, but eventually accumulate also small mutations, faster than dioecious populations, as selection pressure is higher.

These observations have implications for the relevance of evolution experiments using *C. elegans* as a model organism for dioecious parasitic worms. Our results suggest that these experiments might overestimate the number of resistance mutations that would evolve in natural parasitic populations.

4.3 Androdioecy accelerates fixation of beneficial mutations

If the environment changes rapidly, the beneficial allele can increase in frequency quickly. Figure 5 depicts the time to fixation (defined as reaching 95% frequency) of the first homozygous mutation across various mating systems, with the proportion of hermaphrodites engaging in mating represented by ξ. This ranges from exclusive selfing in hermaphrodites ($\xi = 0$, shown in blue), through androdioecious populations ($0 < \xi < 1$, green), to dioecious populations ($\xi = 1$, red). Multiple panels within the figure illustrate results for different population sizes.

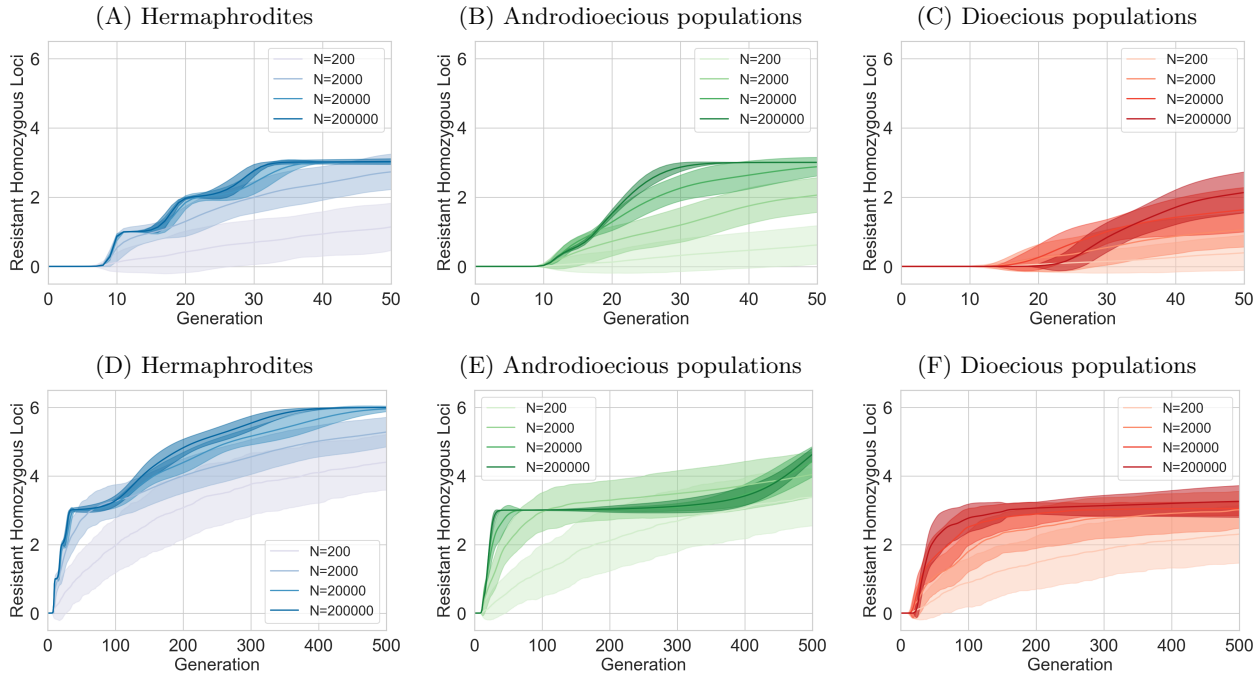

Figure 4: Number of homozygous loci over time when mutations are recessive, with six loci potentially encoding resistance. Mean and standard deviation of 100 simulation trials. A-C: first 50 generations. In androdioecious populations, 50% of hermaphrodites reproduce by mating, 50% by selfing. D-F) all 500 generations.

Figure 5 demonstrates that while selfing results in the fastest fixation of the mutated allele in small populations, a combination of mating and selfing proves advantageous in larger populations. The proportion of mating that appears beneficial increases with population size.

Furthermore, this relationship is influenced by dominance. For equivalent population sizes, a considerably lower mating frequency led to the shortest fixation time when mutations are dominant. The shortest time was achieved in populations, where only 10% of hermaphrodites mated with males, when mutations were dominant, compared to 60 and 70% for recessive mutations in populations of 20 000 and 200 000 individuals, respectively.

4.4 Dominant mutations lead to faster adaptation to higher drug concentration.

Figures 3 and Supplementary figure S1 demonstrate that adaptation to higher concentrations is faster when mutations are dominant. This is expected, as dominant mutations are exposed to selection even in the heterozygous state, conferring an immediate fitness advantage.

Time to fixation of the first mutation (Figure 5) is also generally longer for recessive mutations, especially in dioecious populations. This difference is less pronounced in androdioecious and hermaphrodite populations. Figure 5 further reveals an unexpected interaction between population size and dominance. While recessive mutations require more time to fix in smaller populations, the opposite is true for dominant mutations. This seemingly paradoxical observation can be explained by two opposing factors. In smaller populations, mutations take longer to appear due to reduced mutation supply. However, once a beneficial mutation arises, it can reach prominence more rapidly due to stronger genetic drift. This effect is particularly pronounced for dominant mutations, which are immediately visible to selection, unlike recessive mutations that require homozygosity for phenotypic manifestation.

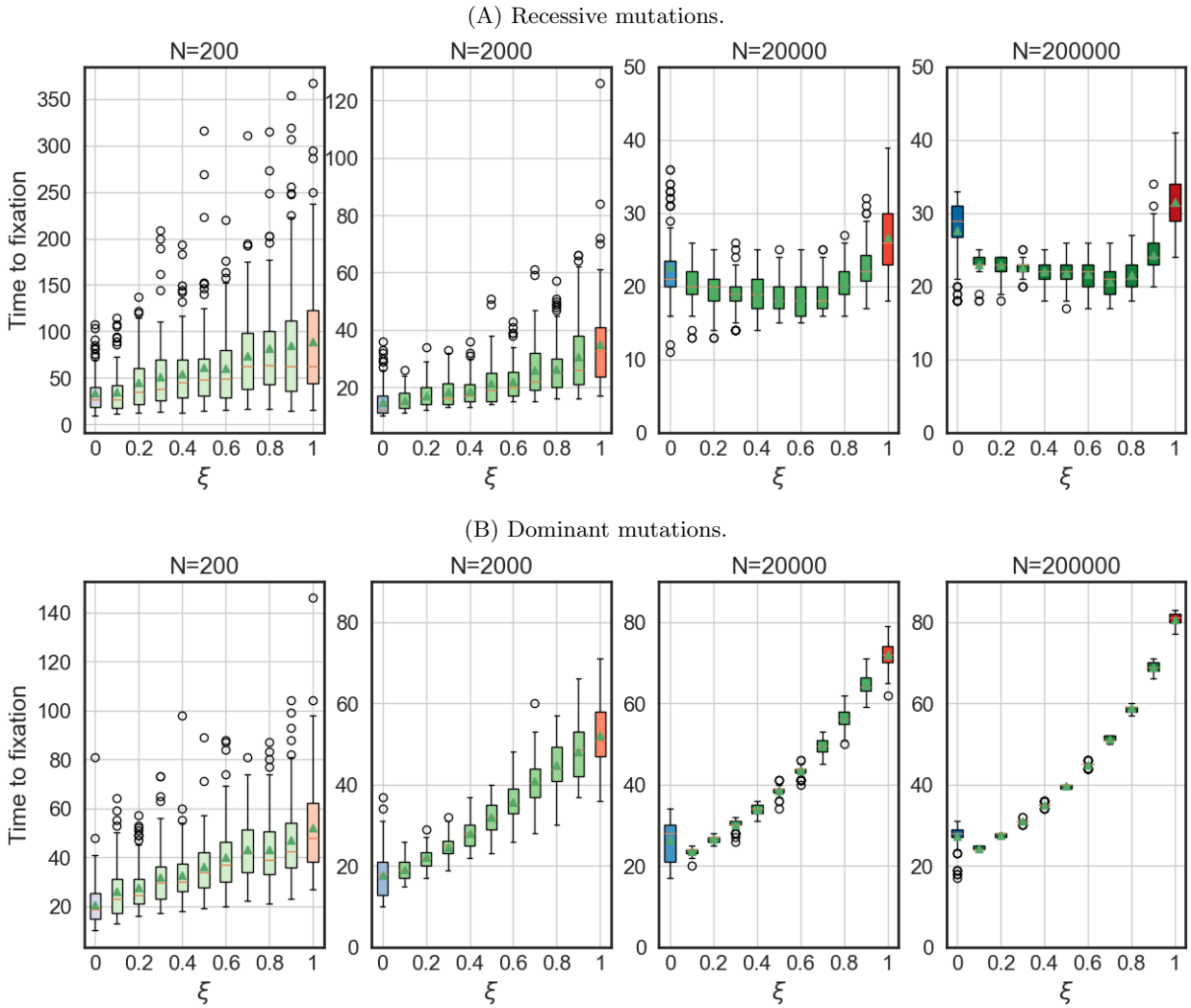

Figure 5: Time to fixation of the first beneficial allele for various fractions of mating hermaphrodites ξ. Resistance is encoded by six loci. Note the various scales on the Y-axis in recessive mutations.

4.5 Mating, dominance and large population sizes prevent the fixation of mutations with small effects

As discussed previously, dioecious populations exhibited limited fixation of recessive mutations, with an average of only three loci homozygous for the recessive allele by the simulation's end. To further investigate this phenomenon, we analyzed the identity of the locus where the mutated allele first reached fixation (95% frequency). Supplementary Figures S2 - S3 reveal that in small, hermaphroditic populations, any of the six loci can be the first to reach fixation, suggesting a stochastic process driven by the random appearance of mutations. However, in larger populations, fixation consistently occurs first at one of the loci with a large effect. This indicates that in larger populations, the evolutionary trajectory is primarily shaped by competition between mutants, where those with larger effects have a selective advantage. Furthermore, both dominance and the presence of mating increased the likelihood of large-effect loci reaching fixation first, highlighting the influence of these factors on the adaptive process.

4.6 Mating increased the diversity

Mating is known to have significant impacts on genetic diversity, with selfing leading to much lower diversity than mating [Ingvarsson, 2002, Burgarella et al., 2024]. Diversity is also expected to be lower in smaller populations. Figure 6 depicts diversity, calculated as Shannon index, over time (see Supplementary figure S4 for a zoomed-in version of the first 50 generations). In agreement with expectation, it shows that diversity is the lowest in the strictly selfing populations and in the smallest populations. However, it shows that in androdioecious populations, diversity is as high as in mating populations. In simulations where only two loci encode variance, the diversity is even higher in androdioecious populations than in the dioecious ones (Supplementary Figures S5 and S6).

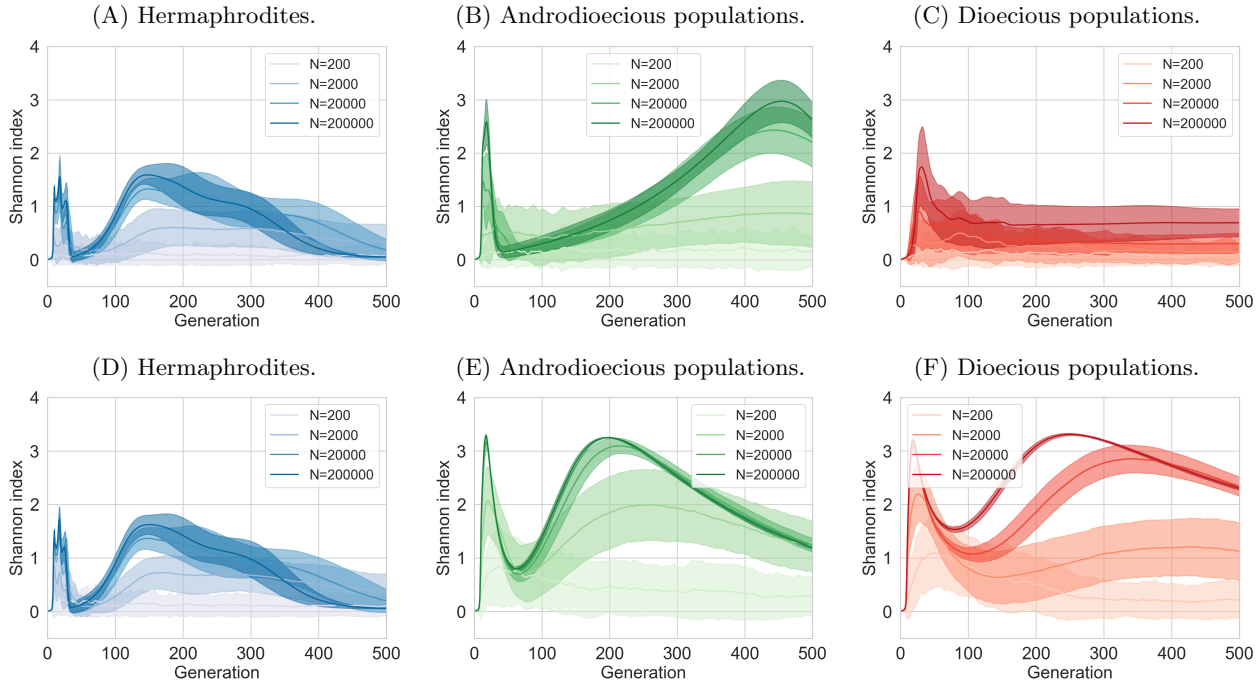

Figure 6: Shannon Index: Recessive and dominant mutations, six loci encoding resistance. A-C: receive mutations, D-F) dominant mutations. In androdioecious populations, 50% of hermaphrodites reproduce by mating, 50% by selfing.

4.7 Short-term observations can contradict the long-term ones.

Simulations were conducted to investigate population dynamics over 500 generations, equivalent to more than 4 years of continuous *in vitro* experimentation, and at least 80 years of continued *in vivo* selection of parasitic nematodes. In addition to being time-consuming, such experiments are likely to be extremely costly and unlikely to be conducted often. In practice, experiments containing approximately 50 or fewer generations are often more feasible [Hellinga et al., 2024].

However, our findings demonstrate that observations from shorter timeframes may yield conclusions that differ significantly from those derived from longer-term investigations. As illustrated in Figure 7, the adaptive advantage conferred by various mating systems (ranging from hermaphroditism, $\xi = 0$, to dioecy, $\xi = 1$) varies considerably across different time points and population sizes.

Specifically, at early time points, hermaphroditic populations appear to exhibit the most rapid adaptation, with sexual reproduction seemingly conferring a disadvantage across all population sizes. This trend, however,

reverses over time, as the benefits of outcrossing become increasingly evident, particularly in populations with recessive mutations (see Supplementary Figure S7 for dominant mutations). These results highlight the critical importance of incorporating a temporal dimension into evolutionary studies, especially when considering the interplay between reproductive strategies and multi-locus evolution.

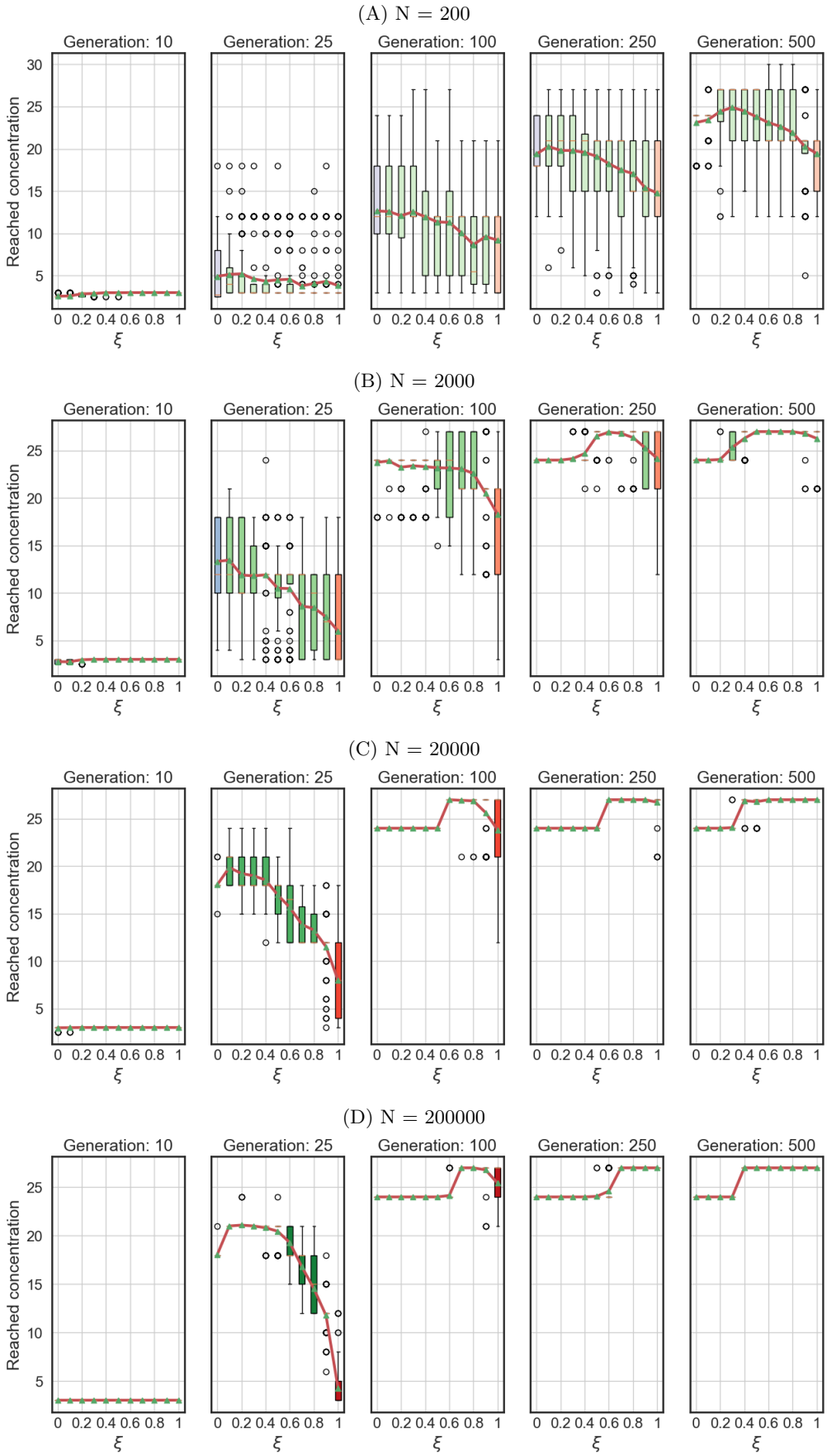

Figure 7: Drug concentration reached by populations at different time points, recessive mutations.

5 Discussion and conclusion

Our study investigated the evolution of drug resistance in worm populations with varying mating systems, ranging from dioecy to hermaphroditism, including the intermediate state of androdioecy. Our simulations, based on the biology of *C. elegans* and experiments spanning 500 generations revealed a strong influence of mating systems on evolutionary trajectories.

Hermaphrodites, capable of self-fertilization, exhibited faster adaptation under recessive mutations. Selfing promotes homozygosity, effectively exposing and fixing new mutations [Ellis and Lin, 2014]. This is due to the increased probability of generating homozygous offspring from a single mutant individual, allowing for rapid fixation of the beneficial allele. In contrast, dioecious populations, reliant on outcrossing, faced a greater challenge in establishing recessive mutations, as these require the rare occurrence of mating between two carriers of the allele.

In the long term, however, dioecious populations were able to survive and reproduce in higher drug concentrations. Interestingly, the classic "cost of males" does not apply in our model, as the presence of males leads to increased egg production per individual on average. This deviation from traditional theoretical scenarios results in altered selective pressures, with dioecious populations exhibiting higher offspring numbers compared to hermaphrodites. This increased reproductive output contributes to the ability of dioecious populations to reach higher drug concentrations despite their slower initial adaptation and slower accumulation of mutations.

Androdioecious populations, characterized by a combination of selfing and outcrossing, appear to be able to access the advantages of both reproductive modes. Androdioecious populations adapted to increasing drug concentrations with the speed initially comparable to hermaphrodites and exceeded the speed of dioecious populations. While hermaphrodites lagged in their ability to reach higher concentrations, androdioecious populations, after a delay, successfully adapted to it. This pattern suggests that the flexibility afforded by androdioecy allows populations to rapidly exploit beneficial mutations through selfing while maintaining sufficient genetic diversity through outcrossing to overcome more long-term selective pressures. These results align with previous empirical observations of lower extinction rates in androdioecious lineages of *C. elegans* during exeprimental evolution in a new environment, supporting the adaptative benefits of combining selfing and outcrossing Morran et al. [2011], Masri et al. [2013], Chelo and Teotónio [2013].

Our study allowed us to investigate a range of reproductive strategies, ranging from pure selfing to pure mating, with intermediate stages. We showed that the optimal balance between mating and selfing depends both on genetics - whether the mutations are dominant or recessive, as well as on the population size. A small fraction of males and mating may lead to the fastest adaptation.

This is consistent with three independent studies that all identified an advantage of outcrossing of androdioecious *C. elegans* populations during coevolution with pathogenic bacteria. Two of these studies included coevolving *B. thuringiensis* as an antagonist and demonstrated that the favored outcrossing rate never reached 100%, but rather remained at an intermediate level of 20-60% [Masri et al., 2013, Schulte et al., 2010]. In these cases, outcrossing with males was directly shown to increase offspring resistance against the pathogen [Masri et al., 2013]. The third coevolution experiment, which included a *C. elegans* population with less genetic diversity than in the above studies, and also a different pathogen, *S. marcescens*, found a steady increase in outcrossing rates from 20% to above 70% [Morran et al., 2011]. Overall, these findings strongly suggest an advantage of the intermediate outcrossing rates facilitated by the androdioecious reproductive system.

Our findings further highlight the role of population size in shaping adaptative outcomes. We observed that in small populations, any locus could be the first to reach fixation, indicating a stochastic process driven by the chance emergence of beneficial alleles. Conversely, in larger populations, adaptation becomes

more deterministic, with loci harbouring large-effect mutations consistently reaching fixation first. This shift suggests that competition between mutants, rather than mere chance, dictates the evolutionary trajectory in larger populations.

Our modelling study further highlights the necessity of complementary theoretical approaches, such as our model, to capture the full complexity of evolutionary dynamics. Mathematical models and simulations are especially valuable when conducting laboratory experiments is impractical or unfeasible [Opatowski et al., 2011]. In this study, our long-term simulations underscore the limitations of empirical studies when investigating the evolutionary processes driving long-term adaptation. While practical constraints often limit *in vitro* and *in vivo* experiments to a feasible timeframe of 50 or fewer generations, our model reveals that different outcomes can be observed over much longer periods. This is particularly evident in the context of reproductive mode, where the apparent short-term advantage of hermaphroditism can give way to the long-term benefits of outcrossing.

It is important to acknowledge certain limitations of our model. We assumed unlinked, autosomal genes, which may not fully reflect the complexities of real-world genomes. Furthermore, the influence of drug concentration on egg count was modelled solely through hermaphrodites, neglecting any potential role of male fitness. Future studies could address these limitations by incorporating linked genes, exploring the impact of male fitness on adaptation, and extending the model to encompass other reproductive modes, such as asexual reproduction observed in some parasitic worm species.

In conclusion, our study demonstrates the complex interplay between mating systems, population size, and genetic architecture in driving the evolution of drug resistance in worms. While hermaphroditism initially facilitates rapid adaptation through selfing, dioecy and androdioecy ultimately prevail in high drug concentrations due to increased genetic diversity and offspring production. Notably, androdioecy, by combining selfing and outcrossing, appears to offer the most robust adaptive strategy. These findings underscore the importance of long-term evolutionary perspectives and integrated theoretical approaches for understanding and predicting drug resistance dynamics in parasitic worms, with implications for effective control strategies.

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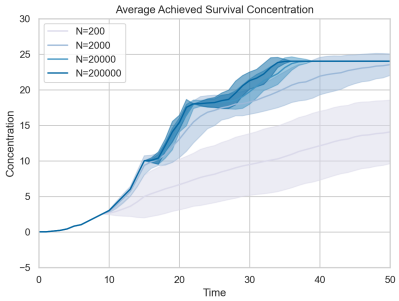
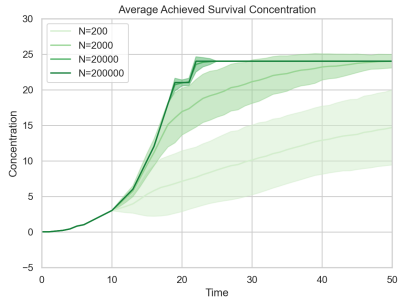
Supplementary figures



(A) ξ =0.0, dominant mutations.



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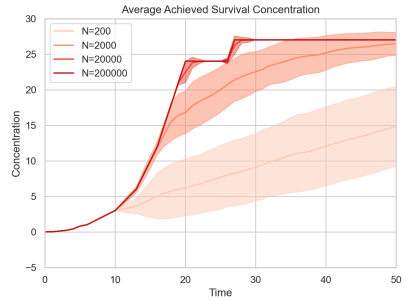

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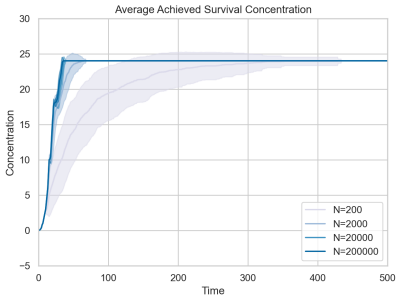

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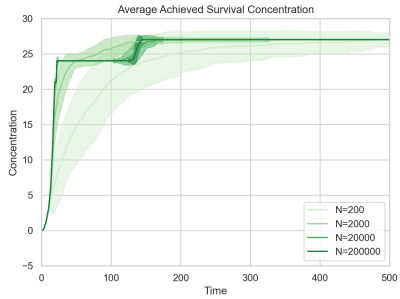
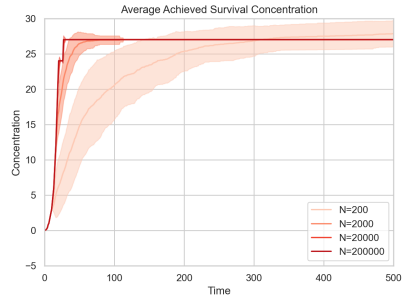


(E) ξ =0.5, dominant mutations.



(F) ξ =1.0, dominant mutations.

Figure S1: Adaptation in increasing drug concentration for dominant mutations. A-C) first 50 generations, D-F) all 500 generations. Mean and standard deviation of 100 simulation trials.

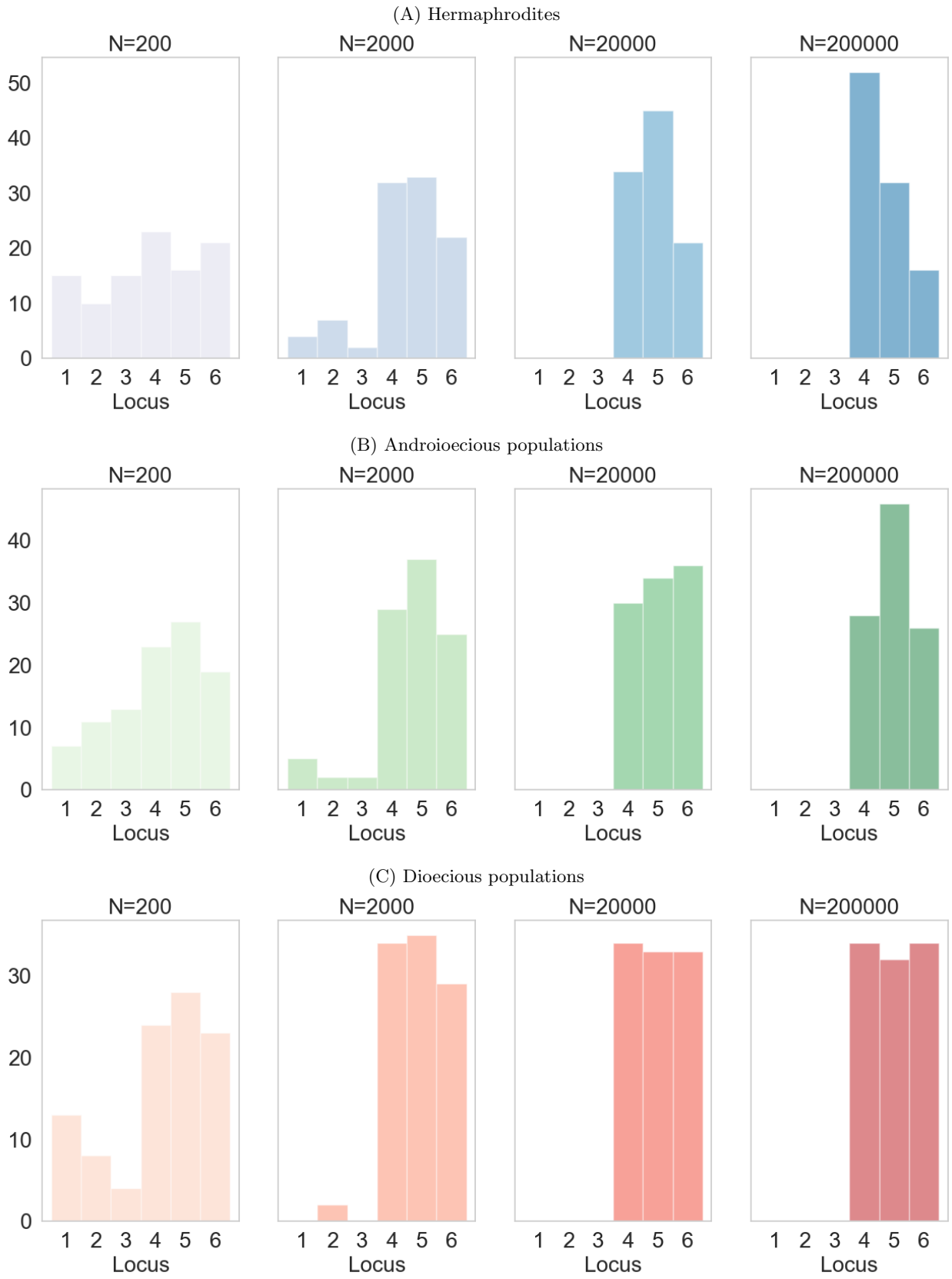


Figure S2: Time to fixation of the first mutation, recessive mutations. Note that there are different axes.

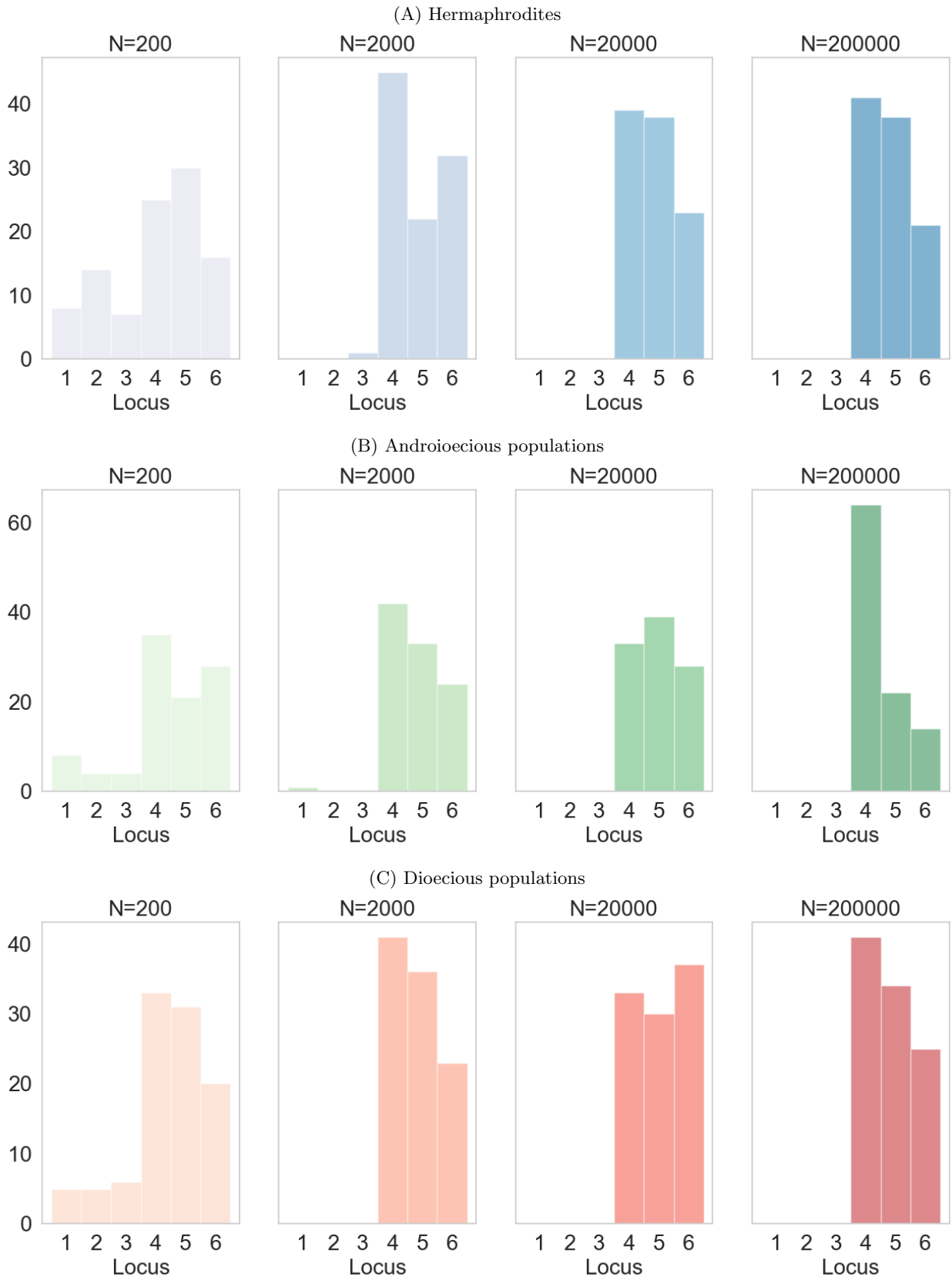


Figure S3: Time to fixation of the first mutation, recessive mutations. Note that there are different axes.

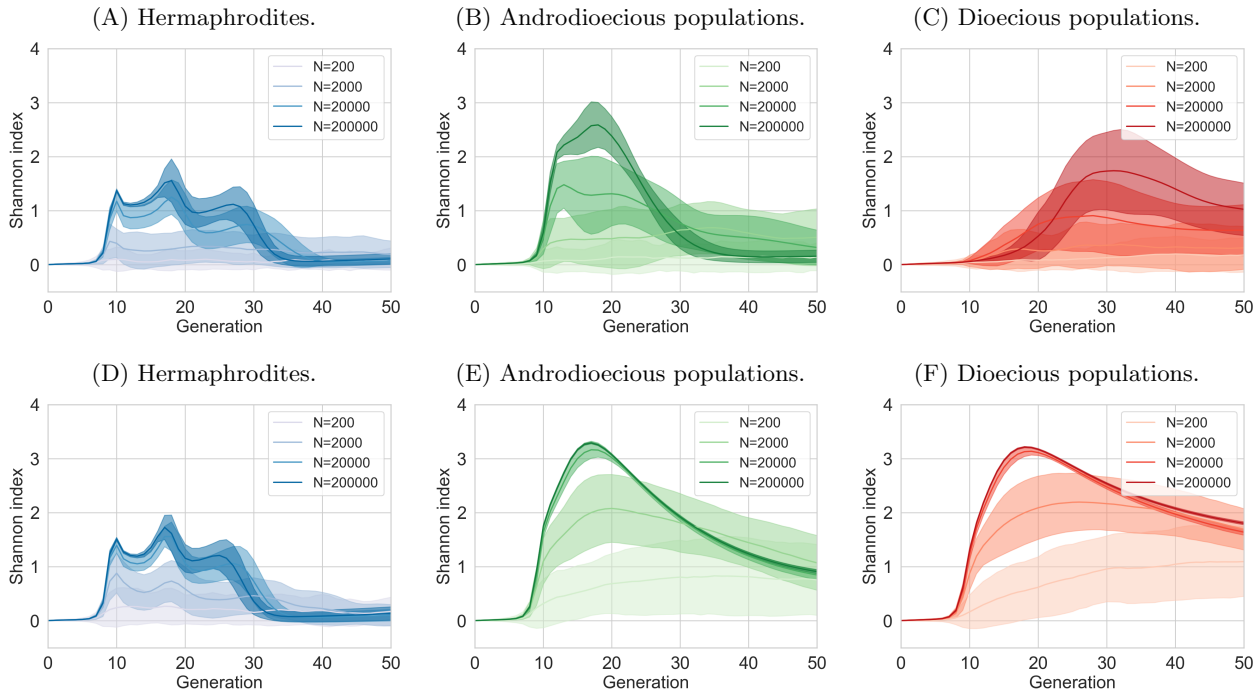


Figure S4: Shannon Index: Recessive and dominant mutations, six loci encoding resistance. A-C: receive mutations, D-F) dominant mutations. In androdioecious populations, 50% of hermaphrodites reproduce by mating, 50% by selfing. First 50 generations.

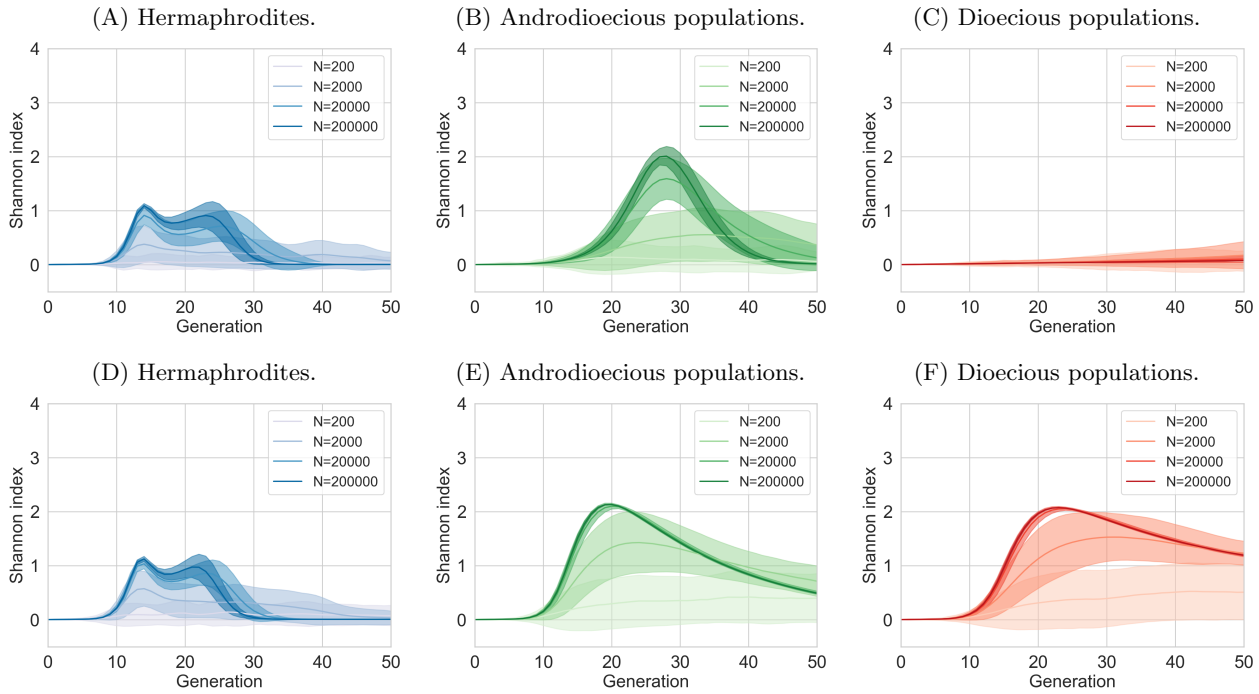


Figure S5: Shannon Index: Recessive and dominant mutations, two loci encoding resistance. A-C: receive mutations, D-F) dominant mutations. In androdioecious populations, 50% of hermaphrodites reproduce by mating, 50% by selfing. First 50 generations.

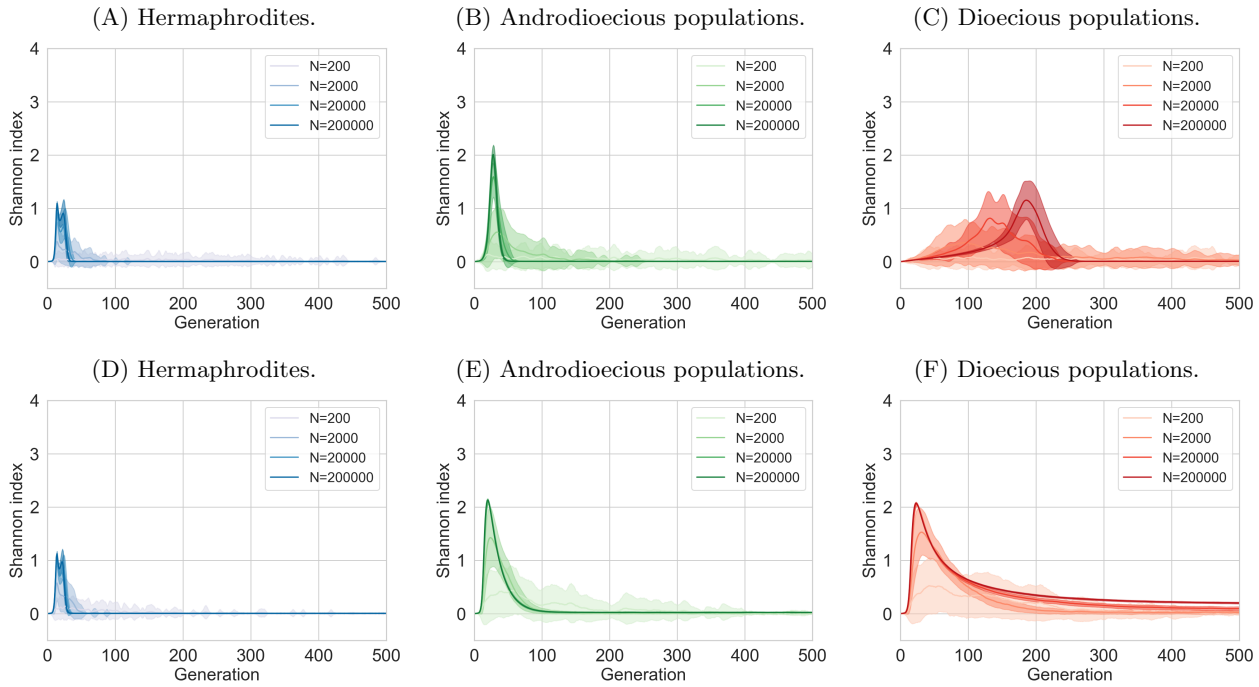
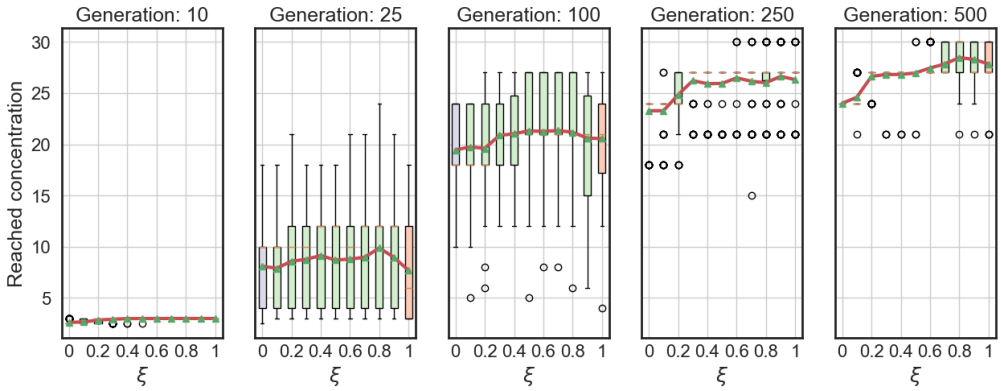
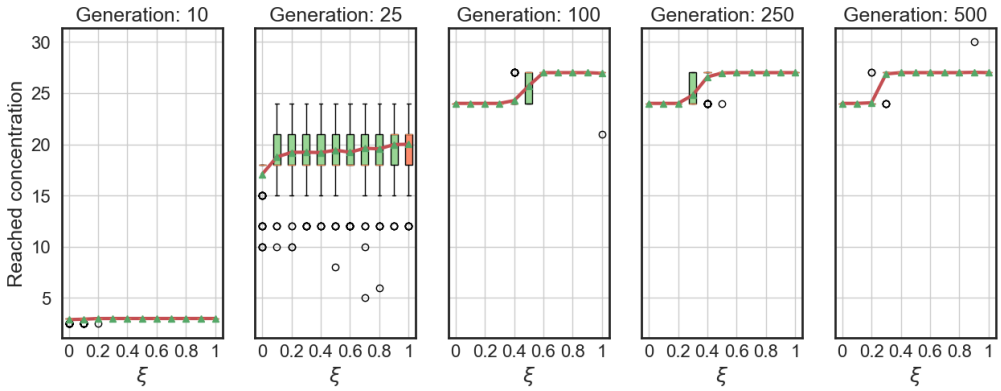


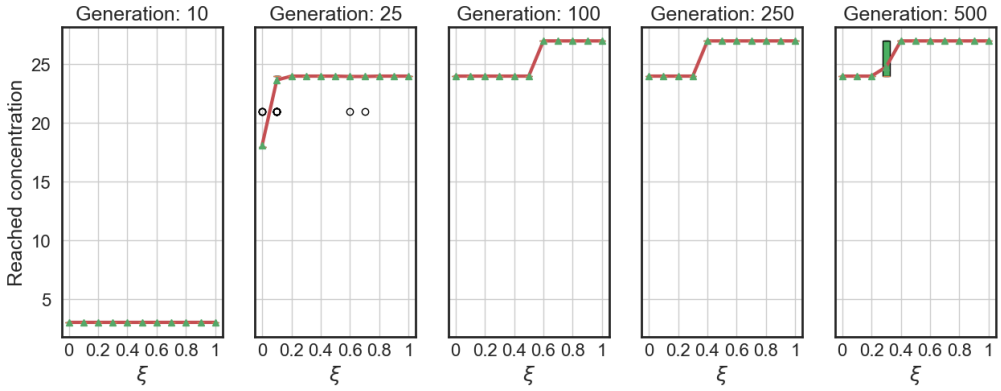
Figure S6: Shannon Index: Recessive and dominant mutations, two loci encoding resistance. A-C: receive mutations, D-F) dominant mutations. In androdioecious populations, 50% of hermaphrodites reproduce by mating, 50% by selfing. All 500 generations.



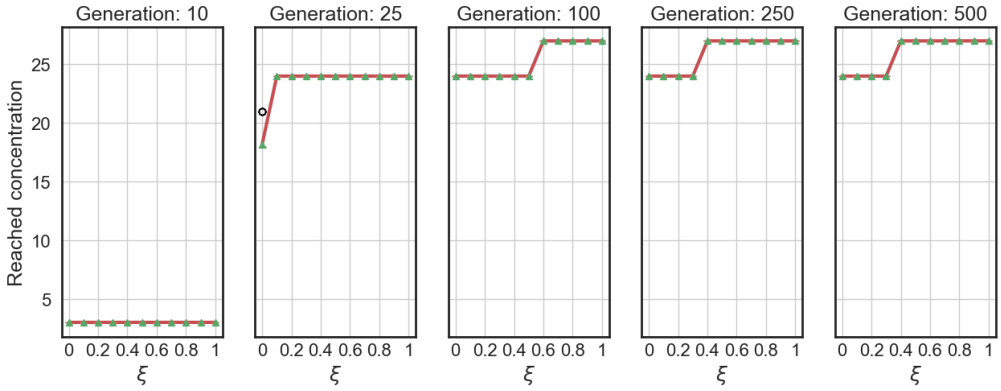
(A) N = 200



(B) N = 2000



(C) N = 20000



(D) N = 200000

Figure S7: Drug concentration reached by populations at different time points, dominant mutations.