The evolution of genetic load in the threatened species

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Abstract

The detrimental environmental impacts of human activity are driving a profound biodiversity crisis, potentially leading to a sixth mass extinction. Even if these negative impacts are successfully mitigated, the contraction of populations is expected to reduce their fitness. This reduction occurs as population declines increase the frequency of rare deleterious alleles, which could have long-term adverse effects persisting even after populations recover to a viable size. The survival of numerous species may hinge on their ability to tolerate or purge this increased genetic load, which depends on the nature of selection—whether hard or soft.

Hard selection is absolute, independent of population density and frequency, whereas soft selection is relative and influenced by density and frequency. Essentially, soft selection favours the survival of the fittest, while hard selection favours the survival of the sufficiently fit. This thesis explores these two modes of selection, revealing that they produce qualitatively different outcomes for key life-history traits such as fecundity and variables like effective population size, which are partially influenced by the environment. These divergent outcomes present a significant challenge, implying very different prospects for the survival of threatened species. To address this challenge, I develop a novel modelling approach that integrates elements of both hard and soft selection, termed compound selection. Furthermore, the final chapters of this thesis present simulations demonstrating how functional genomics and computational models can be used to design effective captive breeding programs and optimise genetic rescue efforts.

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1 Introduction

1.1 Haldane’s dilemma and Neutral theory

Understanding the mechanisms that shape evolutionary processes and drive adaptive evolution is a fundamental goal in evolutionary biology. J.B.S. Haldane's theoretical work introduced the concept that selection imposes a cost on populations, as the fixation of beneficial alleles requires the loss of individuals carrying alternative variants (Haldane, 1957). Consequently, the number of loci under selection at any given time is limited to prevent population diminishment (Smith, 1968; Hickey and Golding, 2019). This limitation poses a constraint on the rate of adaptive evolution, especially among K-strategists with low offspring production, making them vulnerable to the risk of extinction (Cook and Turner, 2020). However, the discovery of allozymes challenged Haldane's theoretical limit by revealing a significantly greater allelic diversity than predicted (Nei, 2005), leading to the formulation of Haldane's dilemma (Hurst, 2019). Haldane’s dilemma posits that the number of polymorphic loci greatly exceeds the theoretical limit that can be under selection.

Kimura's neutral theory of molecular evolution provided an explanation for the observed genetic variation, suggesting that most molecular variation is selectively neutral (Kimura, 1983). According to neutral theory, few polymorphisms are under positive selection, although widespread purifying selection acts against deleterious variation (de Jong *et al*., 2024). The fate of many new variants is therefore determined primarily by genetic drift rather than selection (Jensen *et al*., 2019). Neutral theory offers a potential resolution to Haldane's dilemma by accounting for abundant molecular variation without negating the role of natural selection in phenotypic evolution (Kimura, 1968).

1.2 Hard and soft selection

An alternative response to Haldane's dilemma was Wallace's distinction between hard and soft selection (Wallace, 1975). The selection coefficient quantifies the fitness effect of an allele. Under hard selection, absolute fitness changes the expected number of individuals that

survive or reproduce. Under soft selection, a density-limited number of opportunities is allocated according to relative fitness. Both modes can therefore determine which individuals die or reproduce, but soft selection need not add mortality beyond the demographic limit; it changes which individuals obtain the limited opportunities.

More formally, hard selection refers to a form of selection that operates independently of the density and frequency of the phenotype (or genetic variant) in the population. It concerns the likelihood of an individual to survive and reproduce successfully, independently of their relative fitness compared to the fitness of conspecifics (Reznick, 2016). An example would be salmon jumping up waterfalls in order to reach the location in which it spawns (Connor *et al*., 2019). Assuming no interference from conspecifics, an individual's survival is due only to whether it is fit enough to jump each waterfall. The ability to jump a waterfall is not affected by its fitness relative to that of other individuals within the population. Providing it is fit enough to jump the waterfall, it will survive to the next stage of its life cycle, regardless of whether it was the best at jumping the waterfall or the worst without failing. In other words, fitness is absolute. Hard selection can be thought of as "*the survival of the sufficiently fit*".

In contrast, soft selection incorporates density and frequency dependence, accounting for an individual's chance to survive and reproduce when in competition with conspecifics (Bell *et al.*, 2021). In the salmon example, once the fish have swum upstream and reached their spawning sites, individuals tend to fight to the death for the opportunity to reproduce (King *et al.*, 2023). An individual may miss out on the chance to reproduce if it is not among the fittest of its population. Without its competitors, a relatively poor-quality individual might have been sufficiently fit to reproduce. Soft selection is therefore "*the survival of the fittest*", and fitness is relative. The distinction between hard and soft selection holds significance as soft selection can potentially exert more intense selective pressure without diminishing the population size. Soft selection allows for a greater number of loci to be under selection simultaneously (Charlesworth, 2013). It is likely that most taxa undergo both hard selection and soft selection at varying times in their life cycle.

The essential distinction is biological rather than monetary. Under hard selection, an individual's outcome depends on whether its absolute fitness is sufficient to meet an environmental challenge. Under soft selection, the same outcome depends on its fitness relative to competitors for limited survival or reproductive opportunities (Wallace, 1975).

Wallace's conceptual framework predicts that small differences in absolute fitness can have much larger consequences when success depends on rank relative to conspecifics. Under soft selection, even modest fitness differences can therefore strongly affect survival or reproductive success (Wallace, 1975).

These differences also affect whether alleles are lost or fixed. Soft selection can strengthen purifying selection against deleterious variants, and can also favour beneficial variants, without necessarily adding deaths beyond those imposed by the limited number of opportunities (Charlesworth, 2013). This thesis focuses on purifying selection against genetic load. Stronger linked selection may reduce neutral variation around selected loci (Ellegren and Galtier, 2016).

1.3 Genetic load and extinction

The efficacy of soft selection to purge deleterious mutations also carries implications for genetic load. The analysis of the genetic load started with studies by Muller and Haldane in the 1950s (Haldane and Jayakar, 1965; Muller, 1950). The term genetic load is often used to represent the difference between the fitness associated with the average genome within a population compared to that of an ideal theoretical genome (Barret and Charlesworth, 1991; Dussex *et al*., 2023). Traditionally, this definition emphasises the phenotypic effects of genes that are partially expressed, but it does not include the contribution of recessive allelic variants that remain unexpressed when present in heterozygotes. Such recessive deleterious alleles may result in a fitness reduction in future generations if they become expressed as homozygotes. The potential fitness effects of deleterious alleles that are not yet expressed will be referred to here as "masked load", and it is also known as the “inbreeding load” (Morton *et al*.,1956) and “potential load” (Mathur and DeWoody 2021). In contrast, the combined effects of deleterious mutations that reduce fitness will be referred to as the "realised load" (Bertorelle *et al*., 2022). The realised load also includes the fitness effects of partially dominant alleles (i.e., alleles with a positive dominance coefficient, $h>0$) that are present in heterozygous genotypes.

In this thesis, the term “genetic load” refers to the sum of "masked load" and "realised load" (Bertorelle *et al*., 2022). An increase in genetic load within a population can result in

reduced fitness during population contraction and inbreeding because more loci become homozygous (Bell *et al*., 2019). During inbreeding and drift, the masked load may transition into realised load, reducing fitness and potentially initiating an extinction vortex. During an extinction vortex, the population size declines further, resulting in more genetic drift and inbreeding, which exposes even more deleterious mutations in homozygous loci. Eventually, this process may drive a population to extinction (Nordstrom *et al*., 2022). Similarly, a population bottleneck and a founder event can trigger the conversion of masked load to realised load through genetic drift and increased probability of inbreeding. While most deleterious mutations are likely lost during a population bottleneck due to drift or selective purging, those that endure may increase in frequency, potentially becoming fixed (Bortoluzzi *et al*., 2020; Dussex *et al*., 2023). This increase in realised load can threaten the viability of populations by reducing fitness, even during demographic recovery.

Currently, numerous taxa face substantial declines in population size due to anthropogenic activities like climate change, deforestation, and habitat loss (Cowie, Bouchet, and Fontaine, 2022). Consequently, the ramifications of genetic load are poised to impact conservation efforts for endangered species. To effectively address this challenge, understanding the processes governing genetic load is crucial (van Oosterhout 2020). This knowledge helps to assess the magnitude of the genetic load before population contraction, its post-contraction impact on population fitness, and the capacity of recovering populations to prevent the accumulation of additional genetic load. To predict the extinction risk and recovery potential of threatened species, it is of paramount importance to determine whether mutations that comprise the genetic load are removed by soft selection or hard selection.

1.4 Mode of selection in population modelling

Biological fitness is commonly considered in absolute and relative terms. Evolutionary genetics has often emphasised relative reproductive success and allele-frequency change, whereas ecology and conservation have often emphasised absolute demographic performance and population viability (Brady *et al*., 2019). This is a difference in emphasis rather than a strict division between fields, and it partly reflects the types of demographic and genetic data historically available.

In the realm of simulating populations, the choice between hard and soft selection is often influenced by the specific goals of the study, particularly whether the focus is solely upon estimating genetic load or also assessing population viability. Research aimed at understanding genetic load typically leans towards soft selection, utilising modified Wright-Fisher (WF) models (Dussex *et al*., 2023; Kardos and Luikart, 2021; Sachdeva, Olusanya, and Barton, 2022). In the Wright-Fisher model, each generation's size is predetermined, which thereby avoids extinction. Furthermore, generations do not overlap, restricting individual lifespans to a single generation (Haller and Messer, 2019). The relative fitness of each individual is used to determine their contribution to the next generation (Haller and Messer, 2019). WF therefore employs soft selection.

In contrast, studies primarily concerned with population size and viability tend to build models around absolute fitness, either employing hard selection (Caballero *et al*., 2017; Grossen *et al*., 2020; Perez-Pereira *et al*., 2022) or, more commonly, density dependent selection (Beichman *et al*., 2020; Dussex *et al*., 2021; Kyriazis, Wayne, and Lohmueller, 2021; Pinto *et al*., 2023; Xie *et al*., 2021). In both modes of selection, an individual's survival probability is determined solely by its fitness, and it is independent of the fitness of other individuals in the population. In density-dependent selection models, the survival probability is rescaled by a factor involving the carrying capacity (*k*) and population size (*N*). The formula often used is k/N, which effectively regulates population sizes. The survival probabilities decrease when N exceeds k, and they increase when *N* is below *k* (Haller and Messer, 2019). In the case of “true” hard selection (as defined by Wallace 1975), individual survival is density independent. In such models the number of selection survivors may exceed k, requiring ($N - k$) survivors to be randomly killed in order to prevent an exponential rise in the population (Caballero, Bravo, and Wang, 2017). Compared to soft selection, both these types of hard selection are likely to be less efficient at discerning subtle fitness differences between individuals because they are constrained by selective deaths.

The lack of a consensus regarding the mode of selection used in simulations gives rise to two significant challenges. Firstly, it hinders the ability to compare studies; soft selection is more efficient in purging deleterious mutations than hard selection and density-dependent selection. Secondly, each type of selection has its own limitations that compromise their realism in relation to natural populations. Hard and density-dependent models, for example, may lack the capacity to efficiently purge genetic load, increasing the risk of extinction in studies that

simulate this type of selection (Charlesworth, 2013). Conversely, soft selection models, which are modified Wright-Fisher models, do not allow key biological factors such as fecundity and generational overlap to be varied, limiting their applicability to many key species. Furthermore, such soft selection models are not able to assess the extinction risk, thereby lacking realism in certain scenarios (Kyriazis, Wayne, and Lohmueller, 2021). These issues will be explored in greater depth in the subsequent review of how selection modes are employed in population modelling.

1.5 Hard selection models

Forward simulation individual-based models using hard selection have been a primary experimental method for investigating the masked load (inbreeding load) and purging in small populations (Caballero, Bravo, and Wang, 2017). For example, hard selection models have been used to assess the viability of various effective population sizes (Pérez-Pereira *et al*., 2022), and to evaluate the impact of genetic supplementation and purging of the genetic load in populations of ibex that underwent severe bottlenecks (Grossen *et al*., 2018).

Caballero, Bravo, and Wang (2017) tested reproductive rate in small populations using hard selection at both survival and reproduction. A fixed modelling assumption assigned approximately one-third of fitness loci to viability fitness (W_V) and two-thirds to fecundity fitness (W_F). W_V determined survival probabilistically, while W_F affected offspring number; if survivors exceeded the target population size, individuals were sampled down before reproduction. Offspring inherited alleles from their parents, and the combined effects of those alleles determined genotype-derived W_V and W_F in each generation. Their lower mean load among surviving low-fecundity replicates should not be interpreted as stronger within-population purging, because high-load replicates were more likely to become extinct.

Pérez-Pereira *et al*. (2022) likewise selected on viability and fecundity. They scaled W_V and W_F by their mean values at the start of the simulation. This fixed-baseline scaling changes absolute survival and reproductive probabilities and may reduce extinction risk, but it does not make fitness depend on the current competitors and is therefore not soft selection in Wallace's relative-fitness sense.

1.6 Density-dependent hard selection

Density-dependence is employed in numerous conservation genetic and genomic studies, with applications ranging from assessing the effects of source population size in genetic rescue efforts (Kyriazis, Wayne, and Lohmueller, 2020) to evaluating the extinction risk in sea otter populations recovering from population bottlenecks (Beichman *et al*., 2021). It is also used in examining the consequences of habitat loss and population fragmentation (Pinto *et al*., 2023), exploring the effects of bottlenecks and genetic rescue on the pink pigeon (Jackson *et al.*, 2022), and understanding the purging of genetic load during past population contractions in endangered lizards (Xie *et al*., 2021).

At the core of density-dependent selection is the rescaling of individual fitness, achieved by dividing the carrying capacity (k) by the population size (N), resulting in a scaling factor (k/N). This uniform scaling factor applies to all individuals within a population across generations. This typically has the effect of keeping N below k without the necessity of either artificially removing excess survivors, as in the case of hard selection, or capping the number produced every generation, as in Wright-Fisher models. Importantly, the relative fitness differences among individuals remain the same. To illustrate, in a population of 1000 individuals with a carrying capacity of 100, individuals with fitness values of 0.7 and 0.5 would be rescaled to 0.07 and 0.05, respectively. While this process significantly reduces the survival probability of all individuals, the relative fitness differences between individuals remains unchanged.

Density-dependent hard selection is not strictly hard selection as defined by Wallace (1975), because the expected number of survivors depends on density. When $N > k$, survival probabilities are reduced by k/N; when $N < k$, the factor is capped so that probabilities do not exceed one. The same factor is applied to all individuals, so survival remains independent of their fitness relative to competitors.

Modelling density-dependent hard selection yields a more realistic representation of competition between individuals for limited resources occurring in natural populations. It reduces the population size to a realistic carrying capacity through the selective removal of individuals, still keeping the relative fitness differences into account. This is more realistic than true hard selection, which models the random removal of an excess of individuals. However, density-dependent hard selection assumes that the impact of population density uniformly

affects survival probability across all individuals in a population. This approach, therefore, does not consider the possibility that the impact of density dependent selection may differ between individuals depending on their relative fitness. Fitter individuals are likely to outcompete their less-fit counterparts more efficiently as resource competition intensifies. This would augment the initial differences in fitness between individuals and amplify the differences in survival probabilities relative to each other by making the less fit even worse off. I will refer to this as the "rich-get-richer" model. I argue that density-dependent hard selection without a soft selection component is likely to be not representative of the selective purging of harmful mutations at most genes in natural populations.

The choice of the selection model can dramatically affect the conclusions drawn from computer simulation studies. For instance, Kyriazis, Wayne, and Lohmueller (2020) integrated density-dependent hard selection into their models to investigate the success of genetic rescue based on the size of the source populations (large, moderate, or small). Their findings revealed a higher likelihood of extinction when a large population (N=10,000) served as the source population compared to moderate (N=1000) or small (N=100 or N=25) populations. This outcome arose from large populations harbouring a greater number of strongly deleterious segregating mutations that led to a more rapid decline in fitness upon introduction to the rescue populations. These mutations were present in the large donor population at a low frequency, and they formed part of the masked load. Hence, even though the realised load and the fitness in this large population was superior to that of the smaller populations, the total genetic load was larger in this large population, rendering it a poorer candidate for genetic rescue. The paper received considerable critique from conservation biologists as it is argued that the propensity of evidence from wild and experimental populations shows that large outbred populations with high genetic diversity are a superior source for genetic rescue (Ralls *et al.*, 2020). If the models employed by Kyriazis, Wayne, and Lohmueller (2020) had included soft selection, they may have better matched natural populations in their capacity to purge deleterious mutations. This may have dramatically altered their results.

The critical limitation of density-dependent hard selection models is their inefficiency in purging mutations (compared to models that simulate soft selection). Consequently, these mutations tend to drift and increase in frequency over time, a scenario that may not have occurred had they been efficiently purged though competition between individuals and soft selection. Thus, the choice between soft and hard selection modes may yield different

outcomes, warranting further investigation, as it has the potential to impact decision-making processes in conservation efforts.

1.7 Soft selection

Several studies use variations of Wright-Fisher (WF) models to investigate the impact of demographic events and selection on the genetic load (Dussex *et al.*, 2023; Grossen *et al*., 2018; Hledík, Barton, and Tkačik, 2022; Kardos and Luikart, 2021; Krukov and Gavel, 2021; Sachdeva, Olusanya, and Barton, 2022). In standard WF models, the population size for the next generation is predetermined. The reproductive process involves the random selection of mating pairs or individuals (including selfing or clonal reproduction if applicable), with their reproductive success contingent on a fitness assessment. This process involving reproduction and selection continues until the predetermined number of individuals for the next generation is reached. Although mating pairs are randomly picked, there is an element of soft selection because an individual's likelihood of reproductive success is bolstered by its fitness relative to its peers. In populations with a substantial proportion of unfit individuals, the chances of selecting unsuccessful mating pairs or individuals increase, necessitating more attempts to reach the desired number of individuals. Consequently, individuals with higher fitness are more likely to be chosen more frequently, leading to increased reproductive success. Hence, this process in WF models is akin to soft selection.

While WF models represent a form of soft selection, potentially facilitating the more efficient purging of genetic load compared to hard selection models, they also come with significant limitations. Their inflexibility in accommodating variations in fecundity, generational overlap, and assortative mating restricts their applicability in modelling diverse biological scenarios (Haller and Messer, 2019). Furthermore, WF models often assume a fixed population size, precluding the assessment of population viability and extinction risk.

To address these issues, Dussex *et al*.,(2023) introduced modifications to WF models by implementing selection based on survival rather than reproduction. In this modified approach, each individual's survival probability is determined by its absolute fitness. Survivors are then randomly chosen as mating pairs without further fitness testing, continuing until the predetermined number of offspring for the next generation is produced. This model behaves

similarly to WF models, as an individual's chance of reproducing multiple times is influenced by their fitness relative to conspecifics. If their conspecifics are relatively less fit, fewer are likely to survive, increasing the opportunities for fitter individuals to reproduce. Unlike standard WF models, extinction is possible if all or all but one individual fail to survive. However, if extinction is avoided, the predetermined population size remains unchanged, regardless of the number of survivors or the underlying fitness that determined them. In cases where extinction is narrowly avoided, the population will experience significant genetic drift because only a few individuals contribute to the next generation, resulting in reproductive sweepstakes success.

In natural populations, population size is likely influenced by fitness. Modified Wright-Fisher models by Kardos and Luikart (2021) and Sachdeva, Olusanya, and Barton (2022) incorporate mean fitness when determining the size of the next generation. Mean fitness alone, however, does not describe the full distribution of survival and reproduction, and neither the mean nor median by itself determines demographic viability. Models should therefore retain the number and distribution of individuals that survive and reproduce.

The only research paper I am aware of that directly compares hard and soft selection as defined by Wallace (1975) is a study that simulated the effects of population expansion and range shifts under these different selection regimes (Gilbert, Peischl, & Excoffier, 2018). Their simulations indicate that populations subjected to hard selection are driven to extinction by expansion load when undergoing range shifts. Expansion load refers to the accumulation of genetic load during expansion into new habitats. This is primarily driven by genetic drift, either due to the founder effect—when a small number of individuals colonize a new area—or at the margins of a population's viable range where death rates exceed birth rates, and the population is sustained by migrants (Peischl & Excoffier, 2016). In contrast, populations under soft selection are better able to maintain fitness during range shifts. The authors speculate that highly fecund species are more affected by soft selection because the large number of offspring increases competition. In the following chapter, the impact of fecundity under hard, density-dependent hard, and soft selection will be explored.

1.8 Simulation approach and choice of SLiM

SLiM was chosen because the questions in this thesis require prospective, individual-based simulations in which genotype affects fitness, survival, reproduction, demography and extinction. Its non-Wright-Fisher framework allows population size, fecundity, density dependence and overlapping generations to be modelled explicitly while retaining complete genealogies (Haller and Messer, 2019). The main disadvantages are computational cost, dependence on simplifying assumptions and sensitivity to uncertain parameters. Coalescent methods are highly efficient for reconstructing genealogies and neutral demographic history backwards from samples, but are less suited to the forward eco-evolutionary feedbacks examined here.

The distinction and workflow used in this thesis are summarised in Figure 1.1.

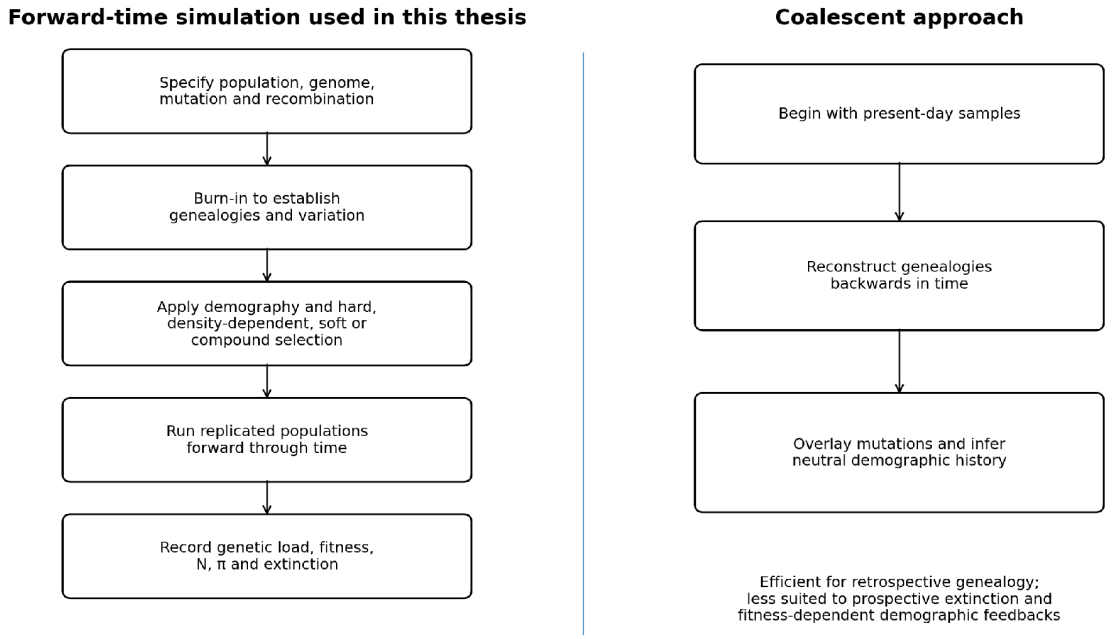


Figure 1.1 - Comparison of the prospective forward-time workflow used in this thesis with a retrospective coalescent approach.

The principal notation used throughout the thesis is summarised in Table 1.1. Values vary among experiments and are reported in the relevant methods and figure legends.

Table 1.1 - Principal symbols and parameters used in the simulations.

Symbol	Meaning	Typical value or range	Used in
N	Census population size	Variable; model outcome	Throughout
N_e	Effective population size	Not assumed equal to N or K	Diversity and drift
k	Carrying capacity or target survivors	Usually centred on 500; chapter-specific	Chapters 2-5
μ	Mutation rate per base per generation	Chapter-specific; about $2e^{-8}$ to $4e^{-7}$	Throughout
ρ	Recombination rate	Chapter-specific; often $1e^{-7}$ or $1e^{-6}$	Throughout
s	Selection coefficient	Sampled distribution of deleterious effects	Throughout
h	Dominance coefficient	Sampled jointly with s	Throughout
w	Genotype-derived fitness	0-1 survival scale in hard selection	Throughout
W_V, W_F	Viability and fecundity fitness	Defined in cited models	Chapter 1
π	Mean pairwise nucleotide diversity	Model output	Chapters 2-7
LE	Lethal equivalent	Unit of genetic load	Chapters 2-7
h^2	Narrow-sense heritability	Estimated from variance components	Chapter 3
α	Adjustable frequency-dependence strength	Proposed sensitivity parameter	Chapter 4

1.9 Aims, objectives and thesis structure

The overarching aim is to determine how alternative modes of selection affect the evolution of genetic load and the viability of threatened populations. The objectives are to: (1) test how fecundity changes purging and extinction under hard, density-dependent hard and soft selection; (2) evaluate how population size and genotype-environment association limit soft selection; (3) develop and test compound selection; and (4) explore applications to conservation investment, captive breeding and genetic rescue.

Chapter 2 compares fecundity across hard, density-dependent hard and soft selection using genetic load, population size and π as response variables.

Chapter 3 tests how population size and environmental variation alter selection efficiency and load.

Chapter 4 introduces compound selection and compares its effects on load, diversity and extinction with the other selection modes.
Chapter 5 examines parental investment, conservation investment and overlapping generations.
Chapter 6 tests captive-breeding regimes that minimise predicted realised load and/or relatedness.
Chapter 7 models captive-to-wild genetic rescue in the Arabian leopard.
Chapter 8 synthesises the results, limitations and empirical tests required to distinguish the selection modes.

All SLiM scripts used in this thesis are available through the accompanying GitHub repository at https://github.com/TBirley/UEA_Thesis_SLiM

2 The effect of fecundity under hard and soft selection

2.1 Introduction

In Chapter 1, theoretical evolutionary biologist Bruce Wallace introduced the distinction between hard selection and soft selection to address Haldane's dilemma (Wallace, 1975). Soft selection is "survival of the fittest ", and it is distinct from hard selection, which can be considered "survival of the sufficiently fit". Soft selection thereby offers the potential to discern individuals with slight differences in fitness, and the allelic variants under selection are not constrained by limits on the number of selective deaths (Charlesworth, 2013). Therefore, soft selection holds the potential of greater efficiency compared to hard selection. Different modes of selection have been implemented in computer models, including density-independent hard selection (Caballero, Bravo and Wang; 2017; Dussex *et al*.,2023; Pérez-Pereira *et al*., 2022), density-dependent hard selection (Beichman *et al*., 2020; Dussex *et al*., 2021; Kyriazis, Wayne and Lohmueller, 2021; Pinto *et al*., 2023; Xie *et al*., 2022), and soft selection (Krukov and Gravel, 2021; Hledík, Barton, and Tkačik, 2022; Sachdeva, Olusanya,and Barton, 2022). However, each type of selection has its limitations. While hard selection may lack the efficiency to purge deleterious mutations and sustain populations, soft selection may not always be suitable for models investigating extinction risk, as the population size of each subsequent generation is predetermined. This chapter aims to explore the distinctions between hard, density-dependent hard, and soft selection concerning the accumulation of genetic load, neutral diversity, and extinction risk. (The impact on extinction risk is analysed only when comparing both forms of hard selection). Fecundity serves as the key variable for investigating these selection forms, as varying the number of offspring should lead to variations in population density and the frequency distribution of fitness. Consequently, populations with differing fecundity are expected to evolve differently depending on the mode of selection, and in particular, whether selection is dependent or independent on density and frequency of other genotypes in the population.

2.2 Methods

We used computer simulations in SLiM version 3 (Haller and Messer, 2019) to examine the impact of different modes of selection on their ability to purge the genetic load. We furthermore examined the interaction with variation in lifetime fecundity across a large range of lifetime fecundity values, thereby distinguishing between r and K-strategists. Fecundity serves as an ideal variable for this purpose as it influences both population density, with increased offspring leading to heightened competition within a population of the same carrying capacity, and frequency, as a greater number of offspring introduces individuals with differing fitness levels. Consequently, it should yield distinct outcomes among the various modes of selection, depending on their respective reliance on density or frequency.

All models were forward-time, non-Wright-Fisher (nonWF) simulations run in SLiM version 3 (Haller and Messer, 2019). The models compared hard, density-dependent hard and soft selection. Generations were non-overlapping and individuals were hermaphroditic, diploid and sexually reproducing, with random monogamous mating. K was drawn from a normal distribution with mean 500 and standard deviation 35 to represent temporal environmental variation in resource availability, rather than sampling variation in census population size *N*.

Each mating pair produced a Poisson-distributed number of offspring with a mean of 8, 32, 128, 512 or 2048. Populations had a 1-Mb coding genome divided into 1,000 genes. Recombination occurred between genes at genome-wide rates of $1e^{-7}$ or $1e^{-6}$. The simulation mutation rates were deliberately varied over a broad range to span persistence and mutational meltdown in this reduced genome; they are inputs to the model, not literal mammalian genome-wide substitution-rate estimates. Selection and dominance coefficients were sampled from the heterogeneous joint distribution generated by van Oosterhout *et al.* (2022). This distribution represents comparative fitness effects in viable simulated populations rather than species-specific empirical estimates.

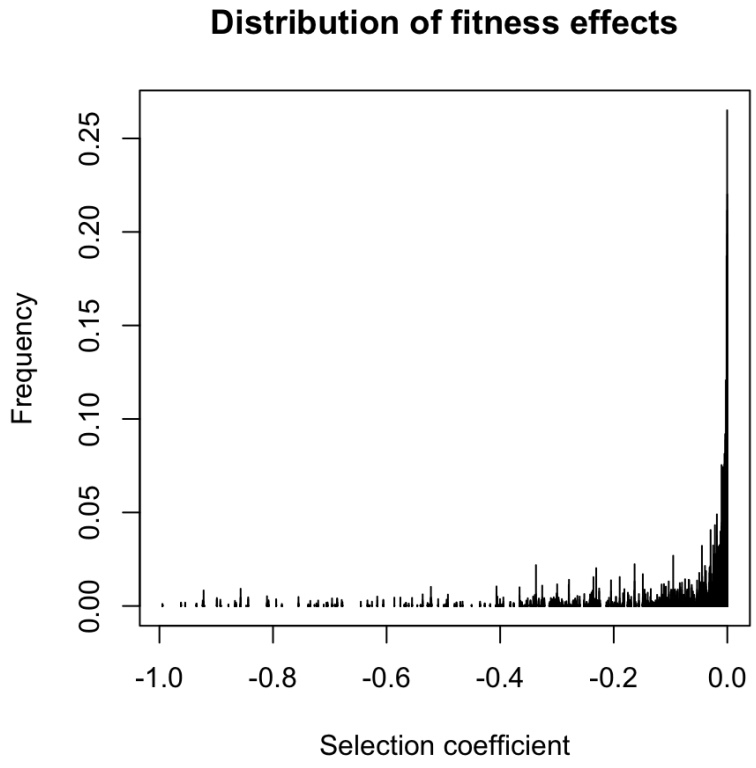


Figure 2.1 - The frequency of mutations of differing selection coefficients used in the simulations in this thesis.

There were 100 replicate runs per model. Burn-in populations were first run for 40,000 generations with neutral mutations to establish genealogies and nucleotide diversity (π) near the neutral expectation. Deleterious mutations were then introduced for 1,000 generations using the sampled selection (s) and dominance (h) coefficients. The subsequent experiments used mutation rates (μ) spanning the values stated in each figure legend and recombination rates (ρ) of $1e^{-7}$ or $1e^{-6}$.

2.2.1 Modes of Selection

SLiM uses a multiplicative fitness model in which each individual's genotype-derived fitness (w), from a starting value of 1.0, is recalculated for each mutation it carries:

$w = w * (1 + s)$ if homozygous, or,

$w = w * (1 + hs)$ if heterozygous.

In nonWF models in SLiM, survival is determined in each generation between the early and late stages. All individuals with *w* equal to 0.0 die and all those equal to or above 1 survive. For those with a score between 0.0 and 1.0, *w* is interpreted as a survival probability and used to perform a random draw to determine survival (Haller and Messer, 2019).

When SLiM's genotype-derived fitness w is used directly as the survival probability, without density- or frequency-dependent rescaling, selection is density- and frequency-independent and therefore hard selection (Bell *et al*., 2020; Wallace, 1975). Thus, under hard selection, $S_h = w$.

For density dependent selection our models are based on those used by Beichman *et al*., 2020, Dussex *et al*., 2021, Kyriazis, Wayne and Lohmueller, 2021, Xie *et al*., 2021. The survival probability (S_d) was multiplied by *k*, divided by the number of offspring *N*. The density dependent factor *k/N* reduces S_d as follows:

$S_d = w * k/N$

The soft-selection model followed the rank-based approach of Bell *et al*. (2021), in which individuals compete for a fixed number of opportunities. For each individual, w was halved and combined with a random value drawn from $U(0, 0.5)$, and the *k* highest scores survived. This procedure adds environmental noise but does not by itself guarantee a realised narrow-sense heritability of 0.5; realised heritability depends on the genetic and environmental variance components. Whenever at least *k* offspring were available, N after selection equalled *k*. If fewer than *k* offspring were produced, all available offspring survived and competition weakened.

2.3 Results

2.3.1 The effect of fecundity under different modes of selection

The study examined genetic load accumulation rates across various levels of fecundity within different selection models: hard selection, density-dependent hard selection, and soft selection. The results showed that there were no significant differences in the rate of genetic load accumulation between varying fecundity levels in the hard and density-dependent hard selection models. However, in the context of the soft selection models, the higher fecundity of r-strategists reduced the rate of load accumulation (Fig 2.2).

Under soft selection, more offspring increased competition and strengthened purifying selection. The largest difference occurred between fecundities of 8 and 32. Differences above 128 became small because selection saturated: once offspring number greatly exceeded *k*, each treatment supplied a large pool from which the highest-ranked *k* individuals were selected, so further increases added little discriminatory power.

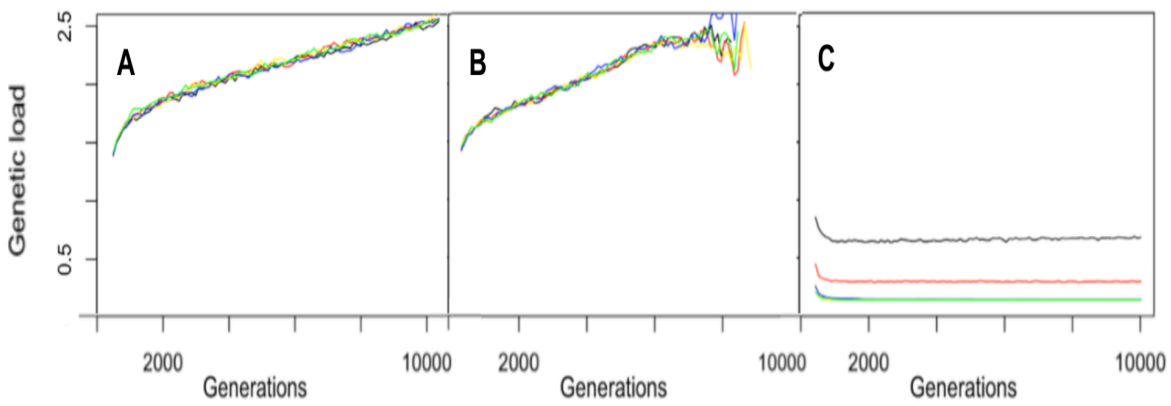


Figure 2.2 - Genetic load accumulation over 10,000 generations in populations with varying fecundities under different selection models. (A) Hard selection, **(B)** Density-dependent hard selection, and **(C)** Soft selection, with $\mu = 2e^{-7}$ and $\rho = 1e^{-6}$. The colour-coded lines represent different model fecundities: black (8), red (32), yellow (128), blue (512), and green (2048). In the hard and density-dependent hard selection models there was no difference in the rate of genetic load accumulation between populations with varying fecundities. Under soft selection, increasing the fecundity results in more effective purging of mutations and thus lower genetic load. The most marked difference in genetic load is observed between populations with a fecundity of 8 and 32. Populations with fecundity of 128, 512 and 2,048 do not differ noticeably in their genetic load.

2.3.2 The effect of fecundity on extinction risk under hard selection

The simulations operationally distinguish r- and K-strategists by offspring number; other correlated life-history traits were not modelled. Figure 2.2 shows that under hard selection, higher fecundity can allow a population to tolerate more load before extinction because more offspring are available, but it does not strengthen discrimination among surviving genotypes.

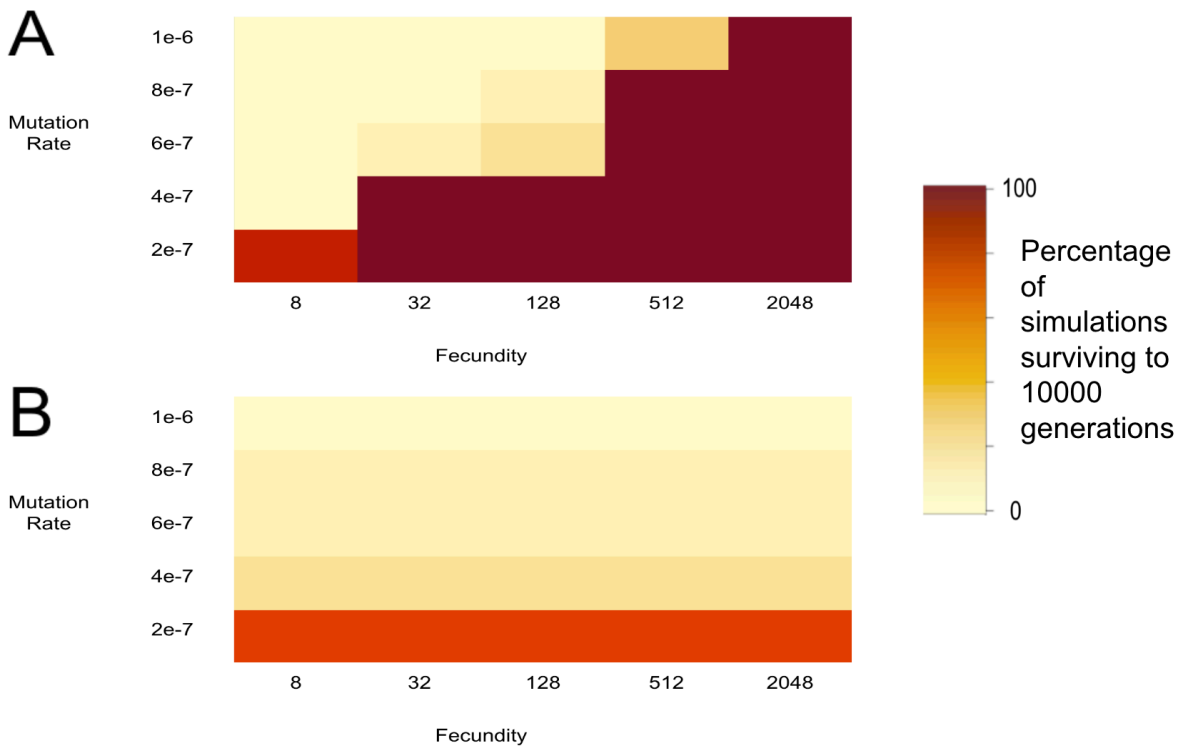


Figure 2.3 - Heatmap illustrating the percentage of simulations that survived up to 10,000 generations across an array of fecundity and mutation rates. Recombination rate = $1e^{-7}$. (A) Hard selection and (B) Density-dependent selection. Under hard selection, r-strategists are able to tolerate the higher genetic load resulting from the higher mutation rate by producing more fit offspring. However, this advantage did not hold true in the density-dependent models, as the negative impact of rescaling survival probability by smaller factors due to higher population densities (i.e., competition) counteracted the benefits of larger populations.

In contrast, under density-dependent hard selection, any advantage gained by increasing offspring numbers is counteracted by rescaling the fitness of the population using the factor *k/N*, wherein *k* is the carrying capacity and *N* the total number of offspring selection is acting on. This scaling factor keeps the population size at carrying capacity, and it causes competition to intensify with increased fecundity. For instance, assume two populations with a carrying capacity of 500, where the first produces two offspring for every adult and the second produces four offspring. The fitness of the first population will be rescaled by a factor of 0.5, while the fitness of the second population will be rescaled by a factor of 0.25, nullifying the advantage of producing twice as many offspring by reducing survival probability. Given that the mortality rate increases linearly and to the same extent as the fecundity, the extinction probability is not affected by the number of offspring in density-dependent models.

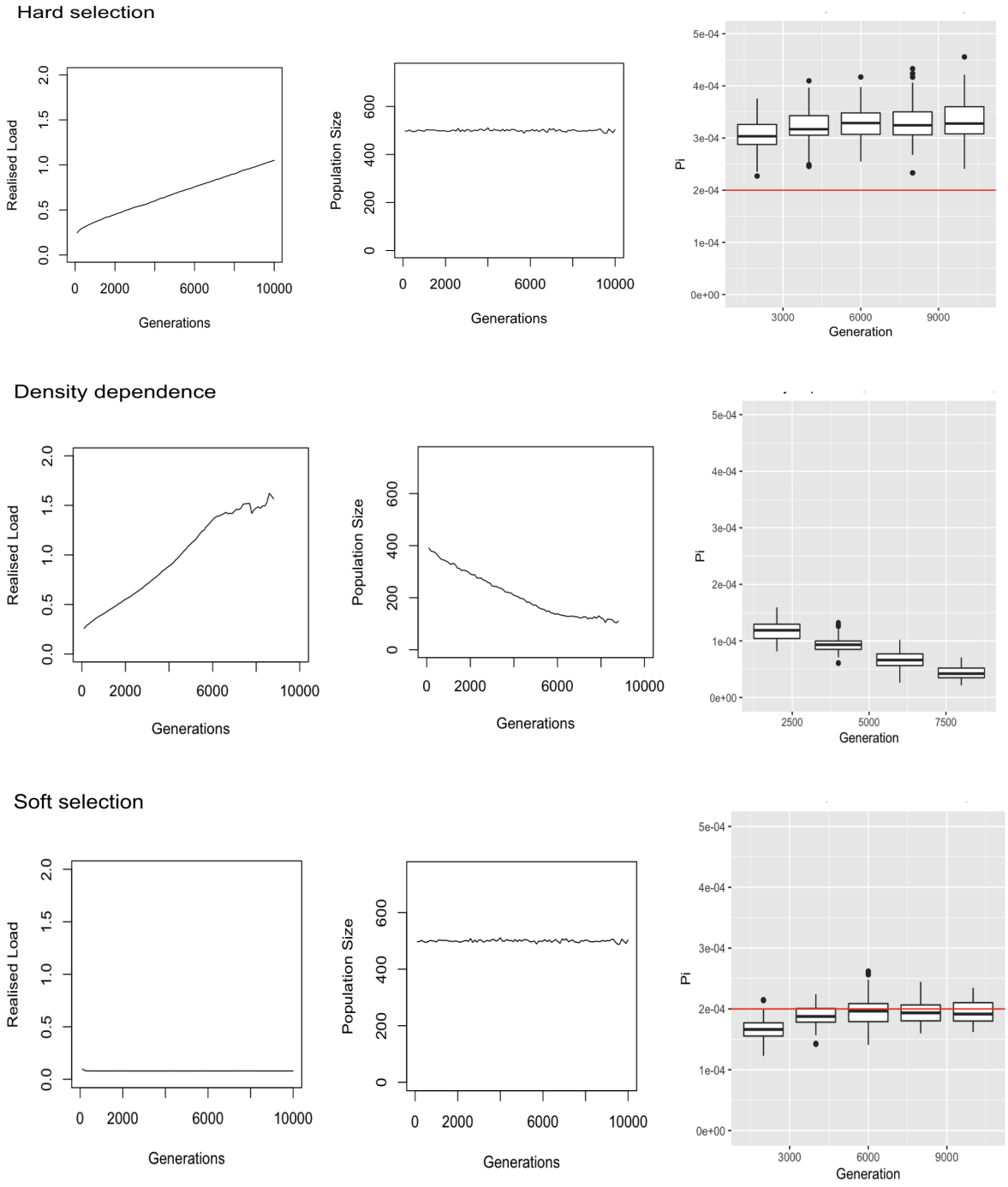


Figure 2.4 - Realised load, census population size (*N*) and neutral nucleotide diversity (π) under hard, density-dependent hard and soft selection. Simulations used fecundity 128, μ = $4e^{-7}$ and ρ = $1e^{-7}$, with 100 replicates. Density-dependent hard-selection replicates ultimately became extinct. *N* is not equivalent to effective population size N_e; the neutral expectation for π depends approximately on $4N_e\mu$ at equilibrium.

2.3.3 Realised load and neutral diversity

The different modes of selection display remarkable variations in their influence on nucleotide diversity and realised load. The following section describes the commonalities and distinctions among these modes concerning realised load, neutral nucleotide diversity, and the risk of extinction.

The realised load determines the fitness of individuals (Bertorelle *et al*., 2022), and as such, it affects the population size and nucleotide diversity. A consistent pattern in the response of realised load was evident in both hard selection and density-dependent hard selection modes, where a continuous increase in realised load over time was observed (see Fig 2.4). However, this increase in realised load was not a prevalent feature of soft selection, except in models with high mutation rates and low fecundity. In most scenarios with varying mutation rates and fecundity levels, realised load generally stabilised at a low equilibrium value (<0.1 *LE*s) after an initial purging process. In the cases of hard and density-dependent hard selection, realised load accumulates because purifying selection struggles to distinguish among individuals, all of which possess sufficient fitness for survival. Consequently, these selection modes are marked by the fixation of slightly deleterious mutations, in a process similar to Muller's ratchet (Govindaraju, Innan and Veitia, 2020).

Realised load can reduce mean fitness and census population size *N*. A reduction in *N* will generally reduce N_e, increasing drift and the probability that deleterious variants fix. *N* and N_e are not assumed to be identical, and π is a genetic outcome expected to scale approximately with $4N_e\mu$ at equilibrium rather than a measure of census size.

Conversely, in the context of soft selection, especially among r-strategists who produce a large number of offspring, there is ample opportunity for purifying selection, effectively preventing the fixation of deleterious mutations and avoiding Muller's ratchet. The competition among offspring enhances the efficiency of selection in distinguishing individuals with slight differences in fitness. As a result, deleterious mutations were far less frequently fixed in these populations.

Under density-dependent hard selection, increasing realised load was associated with declining census size N and neutral nucleotide diversity π. Under hard selection, N sometimes remained near k because high fecundity supplied enough offspring despite increasing load. This does not imply that N, N_e and π are equivalent: reproductive variance and selection can make N_e smaller than N.

Under hard selection, π sometimes exceeded the simple neutral reference. One possible explanation is persistent linkage or haplotype structure generated by selected variation, but this was not tested directly. Analogous sheltered load can occur around loci maintained by balancing selection, including the MHC, although the present simulations do not provide direct evidence for that mechanism. Haplotype and linkage-disequilibrium analyses would be required to distinguish these explanations.

Under soft selection, π was close to the neutral reference in this parameterisation. This is consistent with efficient removal of much selected variation, but it does not demonstrate that the populations evolved neutrally. The simulations did not directly test a nearly neutral model, and the result should be interpreted only as a hypothesis requiring analysis of the site-frequency spectrum and linked selection.

		Hard selection	Density-dependent hard selection	Soft selection
Genetic load	General trend	Accumulates	Accumulates	Is purged and maintained at low level
	K vs r strategists	No difference between K and r-strategists	No difference between K and r-strategists	Reduced genetic load in r-strategists due to more efficient selection
Risk of extinction	General trend	Dependent upon fecundity	Very vulnerable	No extinction possible
	K vs r strategists	K strategists significantly more at risk of extinction than r strategists	No difference between K and r-strategists	Not applicable (no extinctions)
Neutral nucleotide diversity	General trend	May be reduced (due to background selection), or enhanced (due to population substructure)	Reduced	Equal to neutrality or slight reduction due to background selection.
	K vs r strategists	No difference between K and r-strategists	No difference between K and r-strategists	r-strategists purge genetic load fast and are hence more likely to evolve neutrally K-strategists tend to have lower diversity than r-strategists due to more intense background selection

Table 2.1 (above) - **An overview of trends in the genetic load, extinction risk, and neutral nucleotide diversity in populations experiencing hard selection, density-dependent hard selection, and soft selection.** Also shown are the differences between the different forms of selection in K and r strategists.

Genetic Load:

Both hard selection and density-dependent hard selection are less efficient in purging deleterious mutations than soft selection. Both types of hard selection primarily operate against large-effect mutations with a substantial impact on an individual's risk of mortality. Consequently, genetic load accumulates gradually over time in the form of small-effect mutations. Notably, there is no distinction between K and r-strategists under hard selection, as an increased number of offspring does not lead to greater competition, nor to increased purging. As long as individuals are sufficiently fit to survive, they can pass on their deleterious mutations under hard selection. In contrast, soft selection can discern small differences in fitness among individuals. Such competition between individuals results in “survival of the fittest”, which is more efficient than “survival of the sufficiently fit”. The high efficiency of soft selection leads to the purging of genetic load, often maintaining it at a low level with few segregating mutations. This effect is more pronounced in r-strategists, where intense competition among their numerous offspring enhances the efficacy of soft selection. K-strategists tend to accumulate a larger genetic load than r-strategists if mutations are purged by soft selection.

Extinction Risk:

K-strategists under hard selection are vulnerable to extinction due to the accumulation of genetic load. It leads to an increased number of selectively disadvantaged individuals over time. The mortality rate of K-strategists with a high genetic load may exceed their recruitment rate, ultimately resulting in population extinction. In contrast, r-strategists can withstand higher genetic load because their large number of offspring increases the likelihood of producing a sufficient number of viable offspring, i.e., a “sufficient number of sufficiently fit”. This prevents population decline. Under density-dependent hard selection, the risk of extinction is generally high, irrespective of fecundity. The increase in selective deaths reduces the population size, leading to an increased realised genetic load. In turn, this leads to further deaths, reducing the population size and population viability. This creates a positive feedback loop driving the population toward extinction, i.e., an Extinction Vortex (Fagan and Holmes, 2006). Notably, the benefits of having a large number of offspring in r-strategists are counterbalanced by the reduced likelihood of individual offspring survival (due to density dependence). Therefore, there is no difference in extinction risk between K and r-strategists under density-dependent hard selection. In populations solely subject to soft selection, extinction is technically impossible. The potential for soft selection to purge genetic load can safeguard natural populations from extinction. Finally, with soft selection, the absolute fitness and population

viability is highest for r strategists because the large number of offspring increases the efficacy of soft selection.

Neutral Diversity:

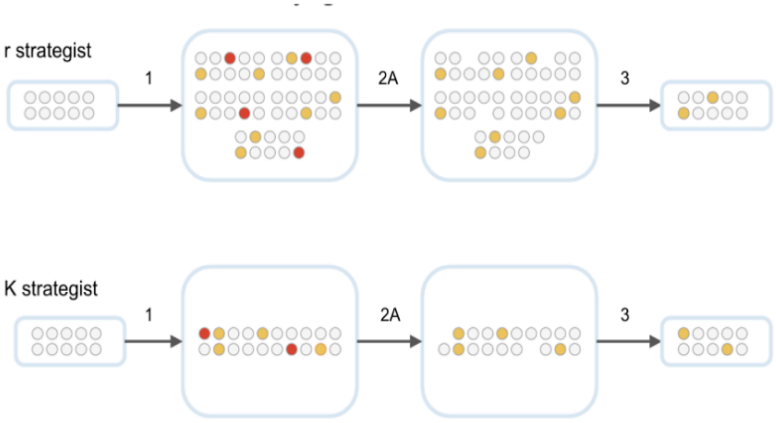
All forms of selection have the potential to reduce neutral nucleotide diversity due to increased linked selection. However, under hard selection, there is another process that can counteract the loss of neutral diversity. If the genetic load is very high, population structure can evolve even in (originally) panmictic populations. The subpopulations consist of incompatible haplotypes that cannot produce homozygotes (i.e., individuals with two copies of the same haplotype are lethal). This increases nucleotide diversity beyond estimates for populations evolving neutrally. In nature, examples of this may be found in the evolution of the major histocompatibility complex (MHC) of vertebrates (van Oosterhout 2009). Given that these immune genes are involved in host parasite coevolution, it is likely that hard selection operates on these genes, i.e., individuals either survive or die from a parasite infection, depending on their MHC genes or haplotype. Both K and r-strategists exhibit similar levels of linked selection because they both efficiently purge deleterious mutations. However, only r-strategists are likely to tolerate the high genetic load that can lead to an increased genetic diversity. Populations undergoing density-dependent hard selection experience a decline in neutral nucleotide diversity due to population contraction resulting from increased selective deaths. Populations under soft selection may also experience reduced neutral nucleotide diversity due to increased linked selection. However, if the majority of segregating deleterious mutations are purged these populations may evolve in a nearly neutral manner. This maintains a level of nucleotide diversity consistent with neutral evolution.

2.4 Discussion

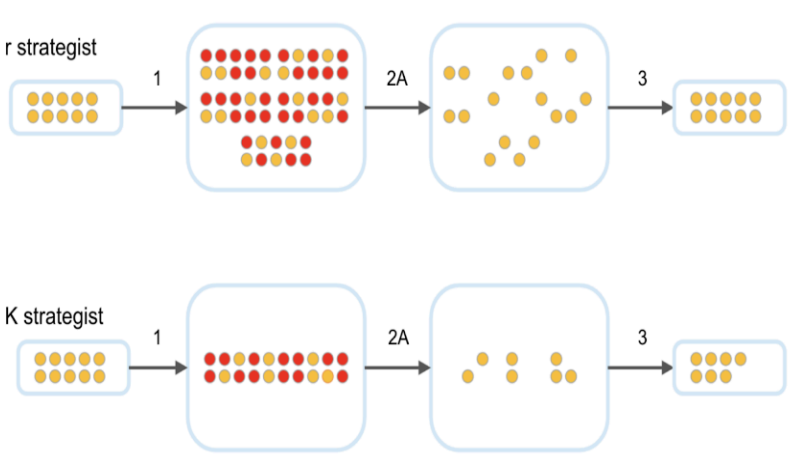
With soft selection, the survival probability of individuals is dependent on the density and frequency of other individuals in the population. Soft selection is therefore sometimes referred to as density- and frequency-dependent selection. Fitness of individuals becomes relative under models of soft selection, and in turn, this promotes the most efficient between-individual competition census “survival of the fittest”. Computer simulations show that soft selection purges the genetic load more efficiently than both hard selection and density-dependent hard selection. Furthermore, increasing fecundity can enhance soft selection, but it does not affect hard or density-dependent hard selection. Therefore, there are both quantitative and qualitative differences between these modes of selection that influence population viability and genetic diversity. Our findings on the effect of fecundity under hard selection are consistent with those of Caballero, Bravo and Wang *et al*., (2019). Fig 2.5 elucidates the rationale behind the

augmentation of soft selection's impact specifically under, but not limited to, hard selection. Furthermore, it illustrates the capacity of r-strategists to endure a greater mutational load than K-strategists in the context of hard selection.

Hard selection, low mutation load



Hard selection, high mutation load



Soft selection

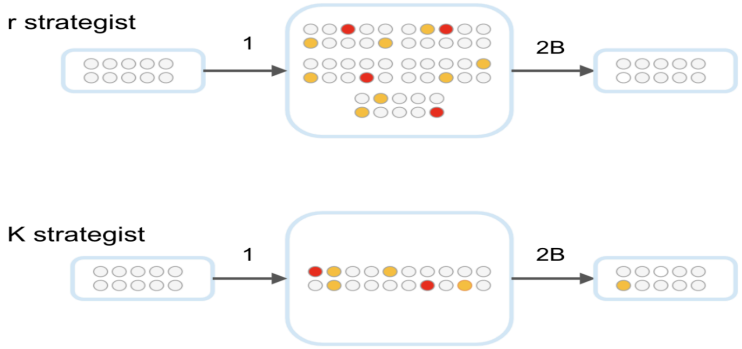


Figure 2.5 - **Conceptual figure illustrating the accumulation of the genetic load in r and K strategist populations under hard and soft selection.** Each dot represents an individual's fitness: white for fully fit individuals, yellow for partially unfit individuals, and red very unfit individuals. Under low mutation load and hard selection, r and K strategist do not differ fundamentally in the rate of genetic load accumulation. However, with a high mutation load, K strategists are more likely to go extinct under hard selection. Under soft selection, K strategists are also accumulating genetic load faster than r strategists.

Numbered Processes:

1. The adult population produces offspring in the subsequent generation.
2. In the offspring populations, selection leads to a reduction in population size. The selection is either hard (A) or soft (B).
3. In populations under hard selection, further reduction in population size occurs due to random death of the offspring.

Genetic Load Accumulation under Hard Selection:

Assuming that variables such as mutation rate (μ), and recombination rate (ρ), are the same for all above populations, they produce offspring with the same distribution of fitness, with the only difference being that r strategists produce more offspring. Under hard selection, very unfit individuals are lost to selective death, while there is no differentiation between maximally fit and partially unfit individuals. Thus, the ratios of maximally fit and partially unfit individuals remain the same between r and K strategists after random death brings the population down to carrying capacity. Consequently, genetic load accumulates at the same rate in both r and K strategists. However, r strategists are capable of enduring more genetic load than K strategists, as depicted in the "Hard selection, high mutational load" section of the diagram. Here, genetic load accumulates at the same rate between both populations, resulting in the production of more very unfit individuals in their next offspring. The loss of very unfit individuals to selective death drives the K strategist population below replacement level due to the smaller number of offspring. In other words, K-strategists may produce insufficient numbers of the “sufficiently fit”, ultimately leading to their extinction.

Genetic Load Accumulation under Soft Selection:

Under soft selection, there is potential for selection to occur between partially fit and maximally fit individuals, effectively purging genetic load from the populations. This process is more efficient among r strategists due to more intense conspecific competition, more efficient “survival of the fittest”. As a result, genetic load is more likely to accumulate in K strategists.

2.4.1 Can life exist without soft selection?

Under hard selection, selection for survival separates individuals that are insufficiently fit to survive from the rest of the population. However, there is a lack of discrimination among those fit enough to survive, even if they exhibit differences in fitness. Using the example detailed in Chapter 1, in the context of salmon ascending waterfalls for spawning, if the selection pressures upon jumping traits are purely binary, there will be no distinction between those effortlessly surmounting the waterfall and those struggling initially but ultimately succeeding. Consequently, genetic load specific to waterfall jumping accumulates. This would lead to fewer individuals capable of performing the task in subsequent generations, resulting in a progressive decline in individual fitness and population viability.

Muller's ratchet clicks when the least-loaded class is lost by chance. Every remaining individual then carries at least one more deleterious mutation than the previous minimum. This process does not require deleterious mutations to be absent from transmission and can occur under either selection mode, although effective purifying selection slows it.

An increase in the intensity of hard selection, exemplified by a higher waterfall, would result in more selective deaths among partially unfit individuals. This heightened selectivity would decelerate the rate of genetic load accumulation. However, the additional deaths resulting from stronger hard selection come at the cost of a population decline. There exists a threshold beyond which the population loss due to hard selection becomes critical, escalating the risk of inbreeding and the onset of an extinction vortex. Consequently, hard selection that is either too weak allows for the accumulation of genetic load, or too strong leads to extinction.

There might be an intermediate level of hard selection strength that sustains the population while purging mutations. However, the influence of environmental stochasticity makes it improbable for hard selection pressures to remain at the optimal level. For instance, if the height of a waterfall permits just enough salmon to leap over it, maintaining the population while purging genetic load, this delicate balance could be disrupted by changes in the river's flow. An increase in flow rate of the river could impede many salmon from clearing the waterfall, posing a risk of an extinction vortex. Conversely, a decrease in flow rate would allow less-fit

salmon to succeed, increasing genetic load. In turn, this would compromise the fitness of their offspring, ultimately hindering future generations from clearing the waterfall. Hard selection alone may be incapable of maintaining populations without an inevitable accumulation of genetic load.

If soft selection were also at play in shaping the phenotypic traits facilitating the ascent of waterfalls by salmon, it could serve as a mechanism to purge genetic load without the associated risk of extinction. Salmon capable of effortlessly ascending waterfalls might gain a competitive advantage over those facing difficulties in this regard. This advantage could occur if clearing the waterfall incurs a less severe depletion of their energy reserves. Consequently, when engaging in competitive interactions for the right to spawn, individuals with superior jumping abilities would have an increased likelihood of success. This process would effectively purge less-fit jumpers from the population, thereby limiting the accumulation of genetic load. Importantly, soft selection purging would not pose a threat of extinction. Assuming a predetermined number of individuals were destined to prevail in reproductive competition, soft selection would merely determine the criteria upon which these individuals are selected. As a result, soft selection has the potential to discern subtle differences in fitness among individuals without introducing additional selective deaths.

Wallace (1975) was the first to recognise the significance of soft selection in purging genetic load. It continues to be considered a possible explanation for how weak purifying selection may act against a larger number of loci preventing unviable genetic load (Charlesworth, 2013). The importance of soft selection has been attributed to varied evolutionary phenomena such as the driver sweeps states success in Atlantic cod (Árnason *et al*., 2023) and selection against Neanderthal introgression in humans (Juric *et al*., 2016). Our results strongly indicate that without soft selection, the accumulation of genetic load becomes inevitable due to a mutational meltdown and an Extinction Vortex. In particular, genetic polymorphisms with small selection coefficients ($s<1/4N_e$) can increase in frequency due to drift (Dussex *et al*.,2023). Over time, they become fixed in the population, thereby increasing the realised load, and reducing fitness. Without compensatory mutations, this will inevitably lead to a reduction in population viability and possible population extinction. However, this is not the case with soft selection which is more efficient at purging load. Although a single mutation with $s<1/(4N_e)$ still may not be detected under soft selection, individuals with multiple of these mutations possess a fitness that is significantly worse than mutation-free individuals. This

enables the purging of those mutations under soft selection. This mode of selection may therefore be essential for maintaining populations without accumulating an unsustainable genetic load.

2.5 Mode of selection in natural populations

Given the quantitative and qualitative differences between soft, hard, and density dependent hard selection it is important to consider the scenarios each may occur in natural populations. For a trait to be under hard selection, the fitness effects should be absolute. The fitness impact of a trait should be independent of its fitness impact on other conspecifics. To survive and reproduce, an individual must possess sufficient fitness to overcome environmental challenges. Therefore, hard selection is referred to here as the “survival of the sufficiently fit”, as opposed to soft selection which is the “survival of the fittest”. In most species, competition for resources among conspecifics is typical, and this intraspecific competition often outweighs interspecific competition (Ehlers *et al*., 2018; Francis, Prager, and Tejada, 1982; Neumann, Werner, and Rauber, 2009; Nottebrock *et al*., 2017).

Invasive species provide a possible, but not general, test of relative competitive fitness. Founder effects can increase drift, inbreeding and load in the invader and may oppose any advantage over native populations. Outcomes will depend on source population size, admixture, propagule pressure and load in both populations. The proposed advantage should therefore be treated as a testable hypothesis rather than an assumption.

2.5.1 Hard selection

Soft selection and the concept of the “survival of the fittest” might be the most important drivers of adaptive evolution and for the purging of the genetic load. On the other hand, the most commonly observed cause of natural deaths in terrestrial vertebrates is predation, not direct competition with conspecifics (Hill, DeVault, and Belant, 2019). As the survival of an individual in a population is generally not influenced directly by conspecifics, it could be concluded that hard selection pressures may be of the greatest consequence. Such conclusion,

however, may be influenced by an observational bias, as predation is easier to observe and record than other likely causes of mortality.

Furthermore, while an animal must be fit enough to evade its predator if preyed upon (i.e., hard selection), the likelihood of it being preyed upon is also influenced by relative fitness with conspecifics (i.e., soft selection). For example, weaker individuals are selectively targeted by predators (Genovart *et al.*, 2010). Furthermore, fitness differences between individuals will affect an individual's ability to effectively gather resources, which subsequently could influence its chance to survive predation (Fussmann, Loreau, and Abrams, 2007; Pelletier, Garant, and Hendry, 2009). This indirect impact of relative fitness on survival has been observed against abiotic selection pressures that may otherwise be considered to only select for absolute fitness. An example of this is fitter individuals more efficiently gathering resources to survive a harsh winter in populations of red deer (Albon *et al*., 2000) and the Natterer's bats (Mordue *et al*., 2023). Considering the likelihood of intraspecific competition for resources within populations, relative fitness becomes a significant factor in determining survival probability.

Traits involved in early development (e.g., during zygote or embryo development) are more likely to be under hard selection, although competition among conspecifics (or siblings) cannot be excluded (see below). Soft selection between siblings may vary among taxa as the fitness of siblings may impact each other if they share the same developmental environment, such as a mammalian womb (which are typical K-strategists), but not if they develop independently in eggs (more typical for r-strategists) (Hudson and Trillmich, 2008).

If early development affects competitive ability later in life, it may also influence soft selection. Maternal stress affects reproductive success in zebrafish (Naguib, Nemitz, and Gil, 2006), and early-life environment affects later fitness in bank voles (Van Cann *et al*., 2019). Such carry-over effects are distinct from antagonistic pleiotropy, which specifically requires an allele to confer an early-life benefit but a later-life cost.

Perhaps the only mutations to be exclusively under hard selection are those that are lethal. If their effect is to kill their carrier, it means that their fitness effects relative to conspecifics are irrelevant. This type of mutation represents a small, though not insignificant, proportion of strongly deleterious mutations, with estimates of 0.5-0.8% in humans (Gao *et al*., 2015). However, other deleterious mutations whose effect is to reduce fitness without necessarily killing

their carrier are likely to experience both hard and soft selection. While the effects of hard selection are not irrelevant, given that an organism must be sufficiently fit to survive within its environment, soft selection has the potential to be more efficient and may, therefore, play a greater role in shaping the genetic composition of populations.

2.5.2 Density-dependent hard selection

Density-dependent hard selection is believed to manifest when a population surpasses its carrying capacity, leading to heightened intraspecific competition for scarce resources (Beichman *et al*., 2020; Dussex *et al*., 2021; Kyriazis, Wayne, and Lohmueller, 2021; Xie *et al*., 2021). This scenario is prevalent, if not ubiquitous, in natural populations where resource scarcity poses a pervasive challenge. However, the concept of density-dependent hard selection suggests a uniform reduction in the survival probability of all individuals by the same factor. This assumption appears unrealistic, as resource scarcity is unlikely to uniformly impact the entire population. Instead, individuals with higher fitness are expected to demonstrate superior capabilities in competing for limited resources, thereby gaining a further fitness advantage and enhancing overall competitiveness. This self-reinforcing selection process amplifies initially subtle differences in genetic quality among individuals (Charlesworth, 2013). In light of this dynamic, it seems that resource scarcity would drive a mode of selection akin to soft selection. Consequently, the occurrence of density-dependent hard selection from resource scarcity in natural populations remains unclear.

When the population size is below the carrying capacity and resources are abundant, intraspecific competition is minimal or non-existent. In such scenarios, individual survival probability remains uninfluenced by the relative fitness of conspecifics, relying solely on the ability to overcome environmental challenges, a capacity heightened by the abundance of resources. This mode of selection is characterised as hard yet relaxed. The phenomenon of relaxed selection is evident during population expansions (Nicolaisen and Desai, 2013; Saxena *et al*., 2019) and colonisation events (Spratt and Lane, 2022), and possibly also during conservation management and captive breeding (Jolly and Philips, 2021). Invasive species, during the colonisation of new territories, can also undergo relaxed density-dependent selection (DeWeber *et al*., 2022). Therefore, while density-dependent hard selection may not be prevalent in instances of resource scarcity, it may manifest when resources are abundant. However, as

populations exceed their carrying capacity, intraspecific competition initiates, intensifying selection in the mode of soft selection. Selection may be further strengthened in low-density populations due to Allee effects, for example because a reduced population size or population density may negatively impact cooperative feeding or defence (Kramer *et al*., 2009). Nevertheless, this is likely to be categorised as soft selection, as survival probability is contingent on the relative fitness of conspecifics.

2.5.3 Soft selection

Soft selection manifests when conspecifics compete for limited resources, and when survival probability depends on the relative fitness of conspecifics, e.g., between prey animals during predation. The near-ubiquitous prevalence of competition in natural populations suggests that many traits superficially considered under hard selection are concurrently also experiencing soft selection. Thus, soft selection is pervasive in the natural world but may manifest in its purest form during sexual selection, where individuals of one sex compete for mates, or when mates are chosen based on specific phenotypes (Klug, Lindström, and Kokko, 2010). In this context, competition is more direct, and selection becomes contingent on relative fitness rather than absolute fitness. Furthermore, mortality due to competition between siblings (juveniles and zygote) may also contribute to soft selection, but the extent of such selection remains unknown in most species as it is more difficult to record and quantify. We also still know very little about possible selection at the gametic level (but see: Immler and Otto, 2018; Tang *et al*., 2013), which due to the large numbers of sperm, could be a great arena for soft selection. Soft selection in early life history stages might play an underappreciated role with respect to purging of the genetic load. In addition, soft selection may play a role in mating system evolution, given that the competition between the gametes of multiple males would give polygamous mating a large advantage over monogamous mating (Scott and Immler, 2023).

The bulb-mite experiment observed a lower genetic load or inbreeding depression in the treatment with stronger male competition (Parrett *et al.*, 2020). Interpreting this difference as purging caused specifically by sexual selection is supported by the experimental design, but the causal genetic steps were not observed directly. Related experiments in flour beetles and studies of polyandry likewise show that stronger sexual competition can improve population fitness (Lumley *et al*., 2015; Parker, 2006).

2.6 Limitations

Our models may not reflect natural populations in several ways, and future models can be improved in several ways. In the present models, the carrying capacity remained relatively stable, whereas natural populations undergo significant population fluctuations due to global environmental changes (e.g., variation between season, years, ice ages). In addition, many species experience bottlenecks, and the incidence of such dramatic demographic events is currently exacerbated by anthropogenic activities. This likely increases the risk of extinction from the fixation of deleterious mutations (Dussex *et al.*, 2023). Our findings suggest that soft selection may protect populations from extinction by purging and preventing the accumulation of genetic load. While soft selection is unlikely to prevent the fixation of alleles during a population bottleneck, it may mitigate this effect by purging genetic load more efficiently pre- and post-bottleneck.

The current model is also limited because it simulated a small chromosome of 1Mb. This was done for computational reasons, and it enabled me to test the effect of very high fecundity within prohibitive computational limits. More work needs to be done investigating the efficiency of different modes of selection on larger, more representative genomes.

The computer models also did not include pre- or post-zygotic selection, and this could increase the efficacy of purifying selection significantly by merit of acting on more genotypes. Again, the computational limitations of the current generation of SLiM models prevented me from examining this in further detail. It would be interesting to examine feedback loops between the impact of the genetic load reducing an individual's fecundity, and the smaller offspring numbers reducing the efficacy of soft selection.

Finally, the models did not examine the impact of epistatic interactions, which could increase the efficacy of purifying selection (Barton, 2017; Charlesworth 2013). In the case of synergistic (negative) epistasis, the fitness impacts of multiple harmful mutations amplify each other, resulting in a larger-than-expected loss in fitness. The removal of the least fit individual by selection thereby also eliminates multiple harmful mutations in a single selection event (i.e., one

death). This increases the efficacy of purifying selection with a modest cost of selection (Charlesworth, 1998).

2.7 Conclusion

In this study, I assessed the effect of fecundity on purging genetic load and population viability under hard selection, density-dependent hard, and soft selection. The most notable finding was that increasing fecundity increased the efficiency of purging genetic load under soft selection, but that it had no effect under forms of hard selection. This illustrates the importance of soft selection in purging mutations and preserving population viability. However, many studies assessing population viability only include hard selection, thereby underestimating the efficacy of purifying selection, and potentially overestimating the impact of the genetic load on the extinction risk. Hard selection tends to be modelled in conservation genetics studies in part due to the fact that soft selection models do not go extinct, making them unsuitable for studying extinction. Individuals in natural populations need to be sufficiently fit to withstand hard selection pressures that are independent of conspecifics and also to compete successfully with conspecifics to be among the fittest. Therefore, both hard and soft selection act upon individuals and influence their genomic evolution. Including both hard and soft selection in assessments of population viability due to genetic load would make conservation genomic computer models that aim to study the process of extinction more realistic.

3 The limitations of soft selections

3.1 Introduction

Wallace (1975) proposed that soft selection has the potential to efficiently select allelic variation at many loci simultaneously. There are two reasons that can explain the greater efficacy of soft selection. Firstly, the efficacy of hard selection is limited by the number of allelic variants that can be under selection because of the resulting selective deaths. If too many severely deleterious mutations are under selection, the number of genetic deaths exceeds the reproductive capacity of the species, resulting in extinction.

Secondly, soft selection is more efficient because it can eliminate mutations with minor impacts on relative fitness. Minor effect mutations are more likely to be tolerated with hard selection, and hence, they are not removed from the gene pool. Small-effect mutations do not noticeably increase in the probability of death as long as the individual is fit enough to survive (cf. ‘survival of the sufficiently fit’; see Chapter 2). In contrast, soft selection removes the least fit individuals, irrespective of the absolute fitness of the individuals. Consequently, soft selection prevents declines in fitness and reduces the risk of population extinction, especially for the genetic load of small-effect mutations. Soft selection is likely to make populations more resilient to extinction, and it has therefore been proposed to explain why genetic load does not rise to intolerably high levels (Charlesworth, 2013). Findings in Chapter 2 corroborate this hypothesis, indicating that within simulated populations, heightened fecundity leads to heightened competition, consequently enhancing the effectiveness of soft selection. Under certain simulated parameters, it is possible for soft selection to eliminate virtually all segregating deleterious mutations from a population and prevent novel mutations from being established.

Many traits crucial for individuals' reproductive success and survival also influence the competitive ability of individuals with conspecifics. Hence, many traits are likely to be affected by soft selection. Despite challenges in quantifying selection coefficients for polymorphisms, evidence suggests a significant prevalence of deleterious mutations across various taxa (Robinson *et al.*, 2023). Early evidence supporting this notion comes from experiments by Charles Darwin, who observed decreased fitness in inbred plants (Álvarez, Ceballos, & Berra,

2015). This phenomenon, termed inbreeding depression, occurs when the progeny of related individuals exhibits lower survival and reproductive success compared to offspring of unrelated individuals (Hedrick and Garcia-Dorado, 2016). Inbreeding depression arises due to increased homozygosity of recessive deleterious mutations.

Debate surrounds the relative contributions of overdominance, where heterozygotes possess a fitness advantage over homozygotes, and the presence of low-frequency recessive deleterious mutations to inbreeding depression (Charlesworth & Willis, 2009). However, due to limited direct evidence of true overdominance, the latter is generally considered the primary factor (Waller, 2021). Among livestock, a 30-year meta-analysis on inbreeding depression indicates that a 1% increase in inbreeding results in a 0.13% decline in phenotypic traits (Doekes, Bijma, & Windig, 2021), suggesting the presence of numerous masked recessive polymorphisms. Despite variations in severity, inbreeding depression affects virtually all taxa, indicating the presence of deleterious mutations that have not been eliminated by selection or genetic drift.

The masked and realised components of load can be expressed in lethal equivalents (*LE*). One diploid lethal equivalent is a set of deleterious alleles whose combined expected effect is equivalent to one lethal allele when made homozygous. It is commonly estimated from the decline in log fitness with increasing inbreeding and is not necessarily a count of lethal mutations (Morton *et al*., 1956).

Lethal equivalents summarise the combined effects of many deleterious alleles. Their estimates depend on the trait, environment, life stage and method used, as well as demographic history, mating system, N_e and the distributions of selection and dominance effects. They should therefore not be interpreted as a direct count of lethal variants or attributed to N_e alone.

Research on 38 captive mammal species reported a mean of 4.6 and a median of 3.4 lethal equivalents (Ralls, Ballou, and Templeton, 1988). Estimates of one to five lethal equivalents have been reported for mammalian diploid genomes (O'Grady *et al*., 2006; Reynolds *et al*., 2021), while Drosophila, killifish and zebrafish show estimates around 1.6 (Wade *et al*., 2023). Severe recessive variants have therefore been documented across multiple populations, although estimates are not always directly comparable.

3.1.1 Potential causes for the inefficiency of soft selection

One limitation on selection is recessivity. A completely recessive deleterious allele has no expressed fitness effect while confined to heterozygotes, so neither hard nor soft selection can distinguish its carriers. It becomes visible to selection only when homozygotes occur. Across studies, new deleterious mutations have a mean dominance coefficient below complete additivity, although estimates vary with effect size (Manna, Martin, and Lenormand, 2011).

Soft selection may also be less efficient due to the low heritability of many traits due to the significant contribution of environmental variation. If the fitness of a phenotype is determined largely by environmental variation, then soft selection cannot effectively distinguish among the genetic differences between individuals. This would reduce the efficacy of soft selection. Empirical data indicates that trait heritability can be as low as h^2=0.05 (Lehmann *et al*., 2006), which implies that 95% of the variation in a fitness trade could be due to environmental variation. In humans, estimates for qualitative traits such as height and body mass index range from 0.48-0.68 and 0.2-0.4 respectively (Wainschtein *et al*., 2022). However, traits closely linked to fitness tend to have a low heritability (Kruuk *et al*., 2000; Merilä, and Sheldon, 1999). It is possible that a preponderance of low trait heritability prevents effective purging by soft selection and contributes to the persistence of genetic load in natural populations. In addition, these qualitative traits are affected by many genetic loci (i.e., polygenic). Consequently, a given variant that reduces the mean of a trait value could be deleterious or beneficial, depending on its genetic background (van Oosterhout *et al.*, 2022). In other words, a given mutation is never unconditionally deleterious. For example, if a mutation would find itself in a genetic background with genetic variants that produce a suboptimal large trait value, this mutation would be beneficial. Conversely, this same mutation would be detrimental in a genetic background that produces a suboptimal small trait value (van Oosterhout *et al*.,2022).

In soft selection models performed in Chapter 2, all individuals compete to be among the surviving population. In extreme cases with fecundity set at 2048, over a million individuals compete to be among 500 survivors. Although this highlights the potential of soft selection, it may not be representative for many species. While there are observations of populations producing hundreds of millions of propagules (Smith *et al.*, 2018), it is unlikely that they all compete directly. Instead, many propagules perish randomly, and spatial structuring confines soft selection to overlapping conspecific ranges. This reduced competition diminishes the

efficacy of soft selection. Spatial variation in the distribution of population likely has a similar effect of reducing competition, because individuals can only compete against their immediate neighbours. This will diminish the efficacy of soft selection in fragmented populations. Environmental pressures that cause habitat fragmentation may undermine the efficacy of soft selection further, potentially reducing fitness and population viability. In addition, occasional migration between otherwise isolated subpopulations can lead to sweepstake success, whereby the genomes of slightly fitter immigrants (and their more heterozygous offspring) can replace the genetic variation in the recipient gene pool.

In addition to habitat fragmentation, it is likely that the fecundity of species and their effective population size (Ne) will also influence the strength of soft selection. Previously, I demonstrated that soft selection is more efficient in populations of r-strategists compared to K-strategists (see Chapter 2). This is due to the increased competition caused by the large number of offspring. Large N_e populations also have large numbers so also enhanced competition, increasing the efficacy of soft selection. However, genetic load (and in particular the masked load) may also be higher in large N_e populations as mutations are less likely to be in a homozygous state and therefore less available to selection (Lohr and Haag, 2015). Thus, recessive deleterious mutations are able to drift until they reach relatively high counts, making them less prone to be lost by genetic drift (Waller, 2021). Therefore, soft selection may be weakened due to an excess of mutations in a heterozygous state. It is therefore unclear how the strength of soft selection is correlated to Ne.

3.1.2 Genetic load arising from soft selection

Due to its high efficacy, soft selection may also contribute to an accumulation of deleterious mutations, particularly with the introduction of a rare, large-effect mutation, and during adaptation to changing environmental conditions. This phenomenon might arise through the scenario where intense selection on one particular trait leads to a relaxation of selection pressure on other traits, ultimately resulting in an increase in genetic load through genetic drift. (This increase of load due to drift is in addition to the more localised effects caused by genetic hitchhiking, see below). For instance, a notable illustration of this phenomenon can be observed in Dutch great tits, wherein the ongoing process of climate change has led to a selection for earlier hatching in the breeding season (Gienapp *et al*., 2013). Individuals that hatch earlier

experience higher survival rates, leading to diminished competition later in life. Consequently, traits crucial for reproductive success during later life stages face weaker soft selective pressures. This scenario may not be unique to Dutch great tits; various unicellular and multicellular organisms with intricate life cycles, encompassing distinct morphological and ecological stages, may also encounter similar challenges (Formery and Lowe, 2023). Intense selection acting on one stage of the life cycle can potentially induce a relaxation of selection in other stages, thereby increasing genetic load through genetic drift.

Another facet of soft selection that warrants consideration is sexual selection. While sexual selection has the potential to purge deleterious alleles effectively (Parret *et al*., 2022), it can also lead to a diminished selection intensity in traits not directly targeted by sexual selection. Illustrative examples of this phenomenon include the selection for larger horns in male Rocky Mountain bighorn sheep, *Ovis canadensis* (Kardos *et al*., 2015), and the prominent head and thorax horns in male Asian rhinoceros beetles, *Trypoxylus dichotomus* (Zinna *et al*., 2018). These sexually dimorphic traits, being selected specifically in males of these species, might inadvertently result in reduced selection pressures acting on other traits, potentially contributing to an increase in genetic load.

Genetic draft can also increase load through linkage (Neher, 2013). A positively selected allele may carry linked mildly deleterious variants to higher frequency by hitchhiking when recombination does not break the association. This effect is strongest in low-recombination regions and during strong selective sweeps.

Alternatively, populations may not be able to achieve their phenotypic optima due to the presence of linked deleterious mutations. When linked allelic variants exhibit similar selection coefficients but with opposing effects (positive and negative), their impacts may neutralise each other. Consequently, selection pressures within that specific genomic region might be neutral, and molecular evolution proceeds primarily through genetic drift (Neher and Shraiman, 2011). Such limit to adaptive evolution and fitness may be particularly significant in small N_e populations, and for traits with loci on sex chromosomes and chromosomal regions with suppressed recombination. In addition, polymorphic genes under balancing selection may also accumulate a significant load of recessive deleterious mutations that reduce population fitness due to a segregation load (van Oosterhout 2009).

Aim

It is the aim of this Chapter to test the hypothesis that low trait heritability, and small effective population size can weaken the effect of soft selection.

3.2 Methods

All models were forward-time non-Wright-Fisher simulations run in SLiM version 3 (Haller and Messer, 2019). Hard and soft selection were implemented using the same definitions and rank-based soft-selection algorithm as in Chapter 2. Chapter 3 varied population size and the contribution of environmental noise; it did not introduce a new form of soft selection.

All populations had a genome of 1 Mb which was divided into 1000 genes of 1000 base pairs. Recombination could occur between these genes but not within them at a rate of 0.1, giving a whole genome recombination rate of $1e^{-7}$. A total of 1000 selection coefficients (s) and dominance coefficients (h) were randomly sampled from 3070 distributions obtained from previously modelled stable populations (van Oosterhout *et a*l., 2023). Genetic load was calculated every 100 generations which included calculations of its component parts of masked load and realised load, which sum to the genetic load. There were 100 replica runs per model. To ensure a mutation-selection-drift equilibrium, 100 burn-in populations were produced. These burn-in populations were run for 40000 generations with only neutral mutations to ensure coalescence and a neutral genetic diversity (π) that is the expected value according to Watterson's estimator ($4N_e\mu$). The models were then run for a further 1000 generations with deleterious mutations under hard selection to generate genetic load. The rate of deleterious mutations was either $1.15e^{-7}$ used in both the heritability and N_e models $1.15e^{-7}$ used in the N_e models only producing populations with either two or one lethal equivalents of masked load respectively.

Under hard selection, each individual's survival probability was its genotype-derived fitness w; if survivors exceeded the target k, k individuals were sampled to reproduce. Under soft selection, w was combined with environmental noise as in Chapter 2, and the k highest

composite scores were selected. *k* is the carrying capacity and *N* is census size; neither is assumed to equal N_e.

For the environmental-noise models, K was drawn each generation from a normal distribution with mean 500 and standard deviation 35. Each mating pair produced a Poisson-distributed number of offspring with mean 16. Genotype-derived fitness was rescaled by a specified genetic contribution and combined with random environmental variation. These settings alter the genotype-phenotype association but do not guarantee that realised h^2 equals the input value; realised heritability would require calculation from variance components.

3.3 Results

3.3.1 The effect of *Ne* variation under hard selection

Under hard selection, all simulations with target population sizes above 800 survived for 10,000 generations, whereas smaller treatments became extinct; 84 of 100 replicates at 800 survived. This transition is specific to the simulated mutation rate, fecundity, genome and selection model and should not be interpreted as a universal N_e threshold. Figure 3.2 includes an additional treatment of 500 in the alternative genome parameterisation, but no additional run was available for Figure 3.1.

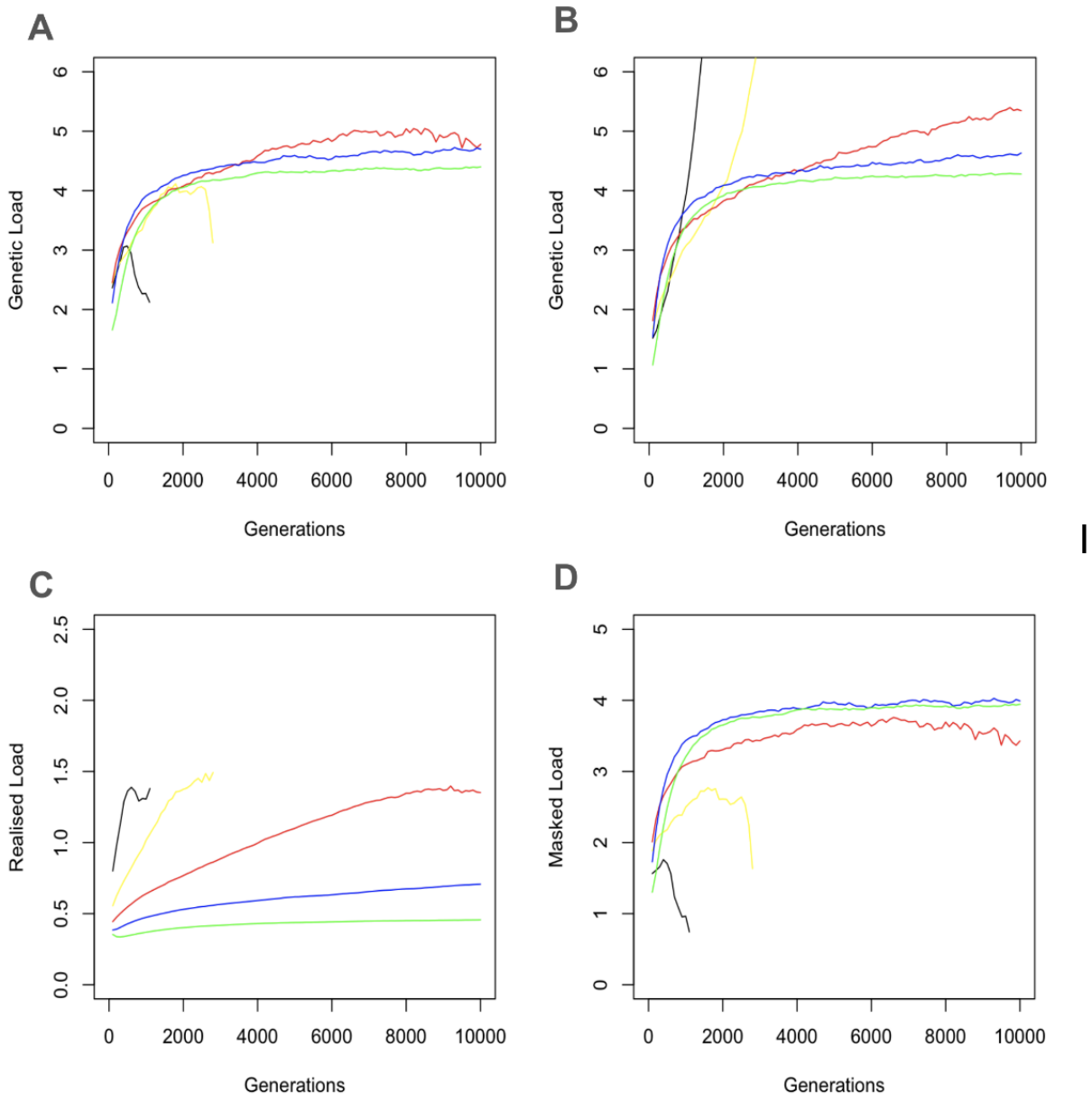


Figure 3.1 - Graphs showing the effect of varying *Ne* on the accumulation of different forms of genetic load under hard selection after 10,000 generations. A The mean total genetic load for K-strategists, **B** The mean total genetic load for r-strategists, **C** The mean realised load for K-strategists, and, **D** The mean masked load for K-strategists. Colours represent the N_e of each group of simulations. **Key:** Black - N_e=50, Yellow - N_e=200, Red N_e=800, Blue - N_e=3,200, and Green - N_e=12,800

Among the surviving treatments, equilibrium loads were more similar than expected. The compact 1-Mb genome and low recombination created extensive linkage, so loci did not behave independently and linked-selection interference obscured the expected population-size pattern.

This result is therefore a property of the parameterisation, not evidence that N_e has little effect on equilibrium load. Low recombination does not impair genetic drift. Instead, it increases linkage among loci and reduces the number of effectively independent genomic regions, strengthening interference and linked-selection effects. The large starting load may also have reduced differences among the surviving population-size treatments.

To test sensitivity to linkage and starting conditions, additional models began without load and used five larger autosomes with recombination within and among genes. This high-recombination treatment was intended to reduce linkage and is not presented as a realistic genome. Direct comparisons are restricted to overlapping population-size treatments.

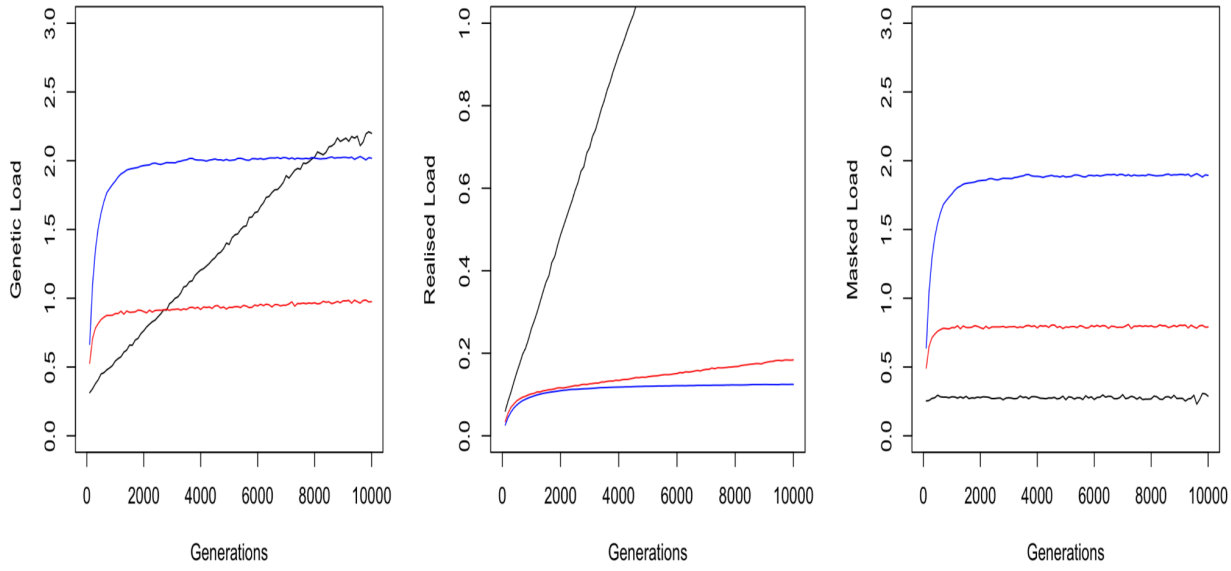


Figure 3.2 - The mean genetic, realised and masked load values in populations of varying *Ne* after 10,000 generations. **Key:** Black - N_e=50, Red - N_e=500, Blue - N_e=5000. These simulations started with no genetic load and had larger exomes that allowed for more recombination than those used in **Figure 3.1**. This is perhaps why they reached mutation-selection-drift balance at differing amounts of masked load correlating with N_e. The realised load for the N_e=50 populations continued to rise throughout as the small population size would increase the likelihood that deleterious mutations would occur in their homozygous state.

3.3.2 The effect of varying *Ne* under soft selection

Under soft selection, larger populations generally purged load more efficiently. When simulations began with only one lethal equivalent, populations of 800 or more converged near the lower attainable load, producing a floor effect. Smaller populations differed more strongly in their ability to avoid mutational meltdown.

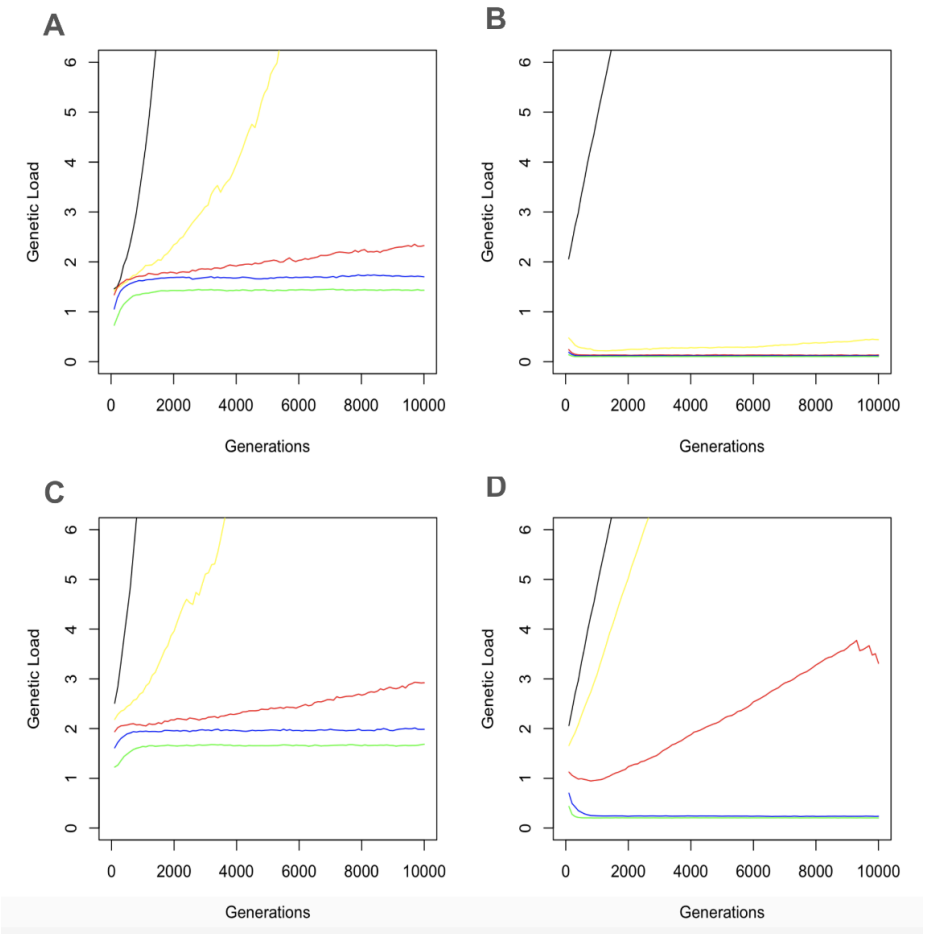


Figure 3.3 - Graphs of mean genetic load under soft selection over 10,000 generations for k and r-strategists with varying initial genetic load. A. K-strategists starting with 1 lethal equivalent, **B.** r-strategists starting with 1 lethal equivalent, **C.** K-strategists starting with 2 lethal equivalents, and **D.** r-strategists starting with 2 lethal equivalents. Colours of the lines represent the *Ne* of each group of simulations. **Key:** Black - N_e=50, Yellow - N_e=200, Red N_e=800, Blue - N_e=3,200, and Green - N_e=12,800.

The r-strategist outcome was partly bimodal: some replicates purged to very low load, whereas others underwent mutational meltdown. K-strategists showed a narrower response but could retain more load than the successfully purged r-strategist replicates. Means should therefore be interpreted alongside replicate survival and variation.

3.3.3 The effect of varying heritability under hard and soft selection

The findings of varying heritability (h^2) under soft selection were consistent with those predicted as increasing trait heritability led to more efficient selection. Under soft selection, only simulations with h^2=0.1 underwent mutational meltdown while those with h^2=0.25 experienced a gradual increase in genetic load over 10,000 generations (Fig 3.4). This would be expected as less genetic variation contributes to phenotypic variation, the less able selection is able to influence genetic evolution.

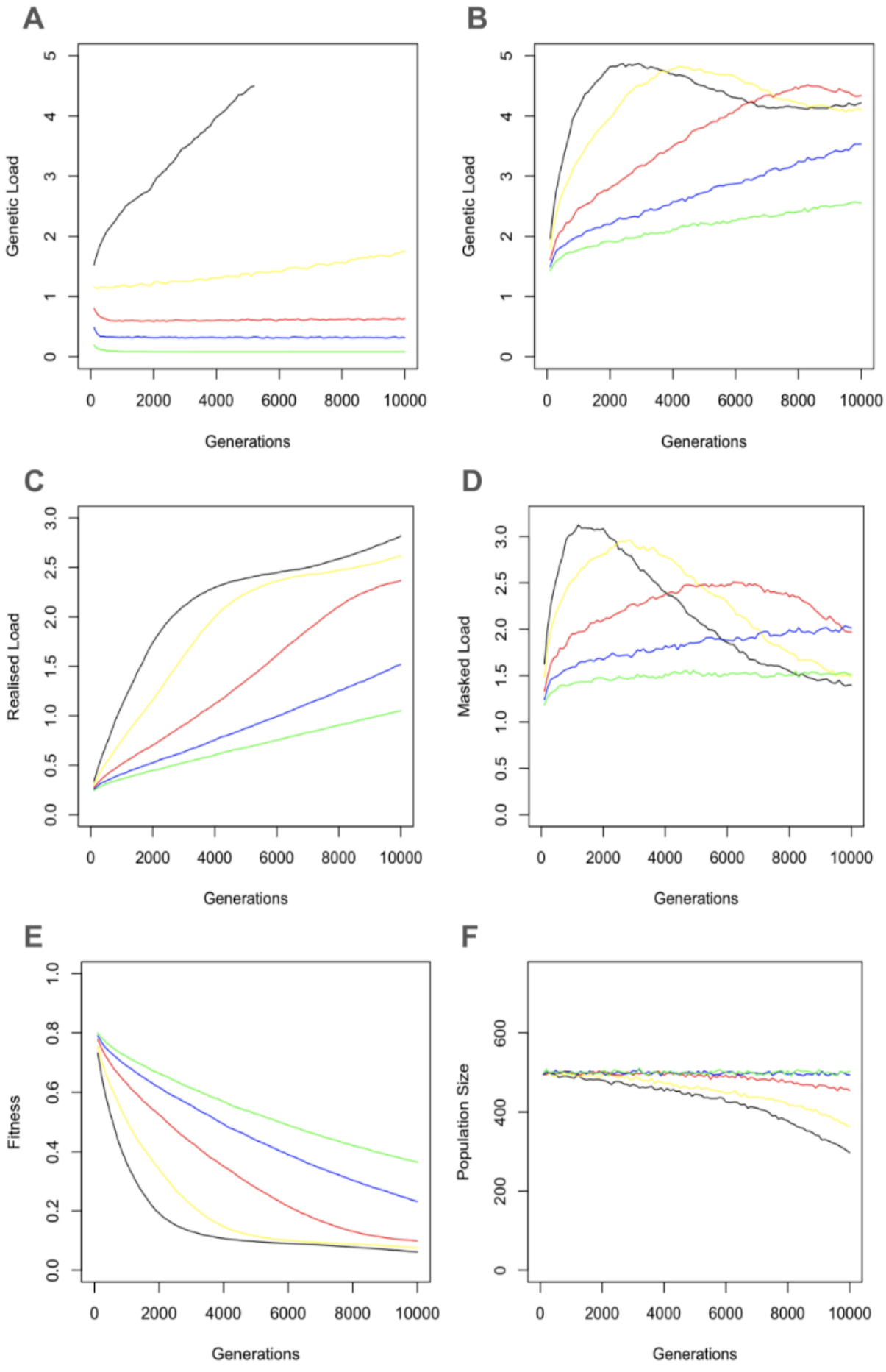

A
Genetic Load
0
1
2
3
4
5
0
2000
4000
6000
8000
10000
Generations
B
Genetic Load
0
1
2
3
4
5
0
2000
4000
6000
8000
10000
Generations
C
Realised Load
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2000
4000
6000
8000
10000
Generations
D
Masked Load
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2000
4000
6000
8000
10000
Generations
E
Fitness
0.0
0.2
0.4
0.6
0.8
1.0
0
2000
4000
6000
8000
10000
Generations
F
Population Size
0
200
400
600
0
2000
4000
6000
8000
10000
Generations

Figure 3.4 - The effect of varying heritability under hard and soft selection over 10,000 generations. **A** Mean genetic load under soft selection, **B** Mean genetic load under hard selection, **C** Mean realised load under hard selection, **D** Mean masked load under hard selection, **E** Mean fitness under hard selection, **F** Population size post hard selection. **Key:** Black - h^2=0.1, Yellow - h^2=0.25, Red - h^2=0.5, Blue - h^2=0.75, Green - h^2=1

Under hard selection, low h^2 also resulted in weaker selection but with some unusual outcomes. Hard selection is relatively weak, thus when weakened further due to low h^2 the result is a very fast initial accumulation of genetic load (Fig 3.4) This led to a rapid deterioration in fitness and caused the number of selection survivors to fall below the carrying capacity. From approximately 4000 offspring, less than 500 would survive. However, because their survival is largely due to environmental variation, which is random, they are very resilient to extinction. They therefore slowly undergo a population contraction as a consequence purge masked load. This resulted in the low h^2 populations possessing less masked load at the end of the simulations than the high h^2 populations. Therefore, modelling with hard selection has practical limitations in assessing the effect of heritability on genetic load. Using hard selection is essentially to determine whether the individuals are fit enough to survive their environment, but when trait heritability is low their survival is determined by environment rather than their fitness against it.

3.4 Discussion

The effective population size and trait heritability impact the effectiveness of soft selection. Soft selection is limited by weaker competition in small N_e populations. Furthermore, as with fecundity, varying N_e results in qualitative difference in the accumulation of genetic load. The impact of N_e on genetic load may be even more significant than the impact of fecundity. Firstly, increasing N_e under hard selection has the potential to increase the level at which the masked load becomes stable, i.e., reaches an equilibrium. Similarly, under soft selection, increasing N_e results in a more efficient purging of genetic load. The N_e is arguably more important than fecundity in which increasing fecundity reduces load under soft selection but has no impact under hard selection. Moreover, while fecundity is a biological factor that is not easily altered, Ne is largely influenced by the environment and can potentially be managed.

We are currently facing the risk of a sixth mass extinction (Cafaro, 2015). Various anthropogenic activities are driving the decline of wild populations, increasing their risk of extinction. Even if anthropogenic impacts are successfully mitigated and populations are restored to seemingly stable levels, a genetic threat persists. This threat arises from “drift-debt”, i.e., the slowness of genetic drift and evolutionary genetic change (Pinto *et al*., 2024). Previously rare deleterious mutations, which were masked in the heterozygous state, become homozygous in generations after recovery from a bottleneck (Dussex *et al*., 2023; Jackson *et al*., 2022). This process can be exacerbated by inbreeding (Ochoa *et al*., 2020). As populations expand, these mutations, once rare, become more common, thereby reducing the overall fitness of the population (Pinto *et al*., 2024). An illustrative case is the pink pigeon (*Nesoenas mayeri*) of Mauritius, which, despite recovering from a severe population decline in the late 20th century, now harbours a genetic load of approximately 15 lethal equivalents (Jackson *et al*., 2022).

If soft selection significantly contributes to purging deleterious mutations, the decline in wild populations may have a more profound impact on their fitness than previously thought. This is because soft selection suppresses the segregation of deleterious mutations, but its effectiveness depends on maintaining sufficiently large populations to generate strong competition, under little environmental perturbation. A decline in N_e not only increases the risk of converting masked load to realised load through higher homozygosity and inbreeding but also reduces selection efficiency. With relaxed soft selection due to population decline, deleterious mutations that were previously suppressed might segregate freely. Additionally, even a relatively small reduction in N_e could lead to this outcome, given the dynamic nature of soft selection. Estimating the minimum viable population size remains a significant challenge (Chaudhary and Oli, 2020). The fact that the strength of selection can be influenced by population size, which in turn affects fitness and viability, adds a layer of complexity to this issue. Codon-usage bias consistent with stronger translational selection has been reported in large-N_e taxa in the comparative analysis of Galtier *et al*. (2018), including Caenorhabditis brenneri and the common vole, but was weaker or absent in large mammals, birds and reptiles. This taxonomic variation cautions against treating the relationship between N_e and selection efficacy as universal.

If soft selection is prevalent in natural populations, it may offer some optimism for preserving populations that have experienced drift-debt. This is because soft selection might be enhanced by the loss of diversity that occurs during population bottlenecks. With fewer segregating mutations, the genetic variation contributing to phenotypic variation arises from a

relatively small number of mutations, which then come under intense selective pressure. Consequently, any remaining or new deleterious mutations are likely to be purged, especially in populations that have recovered in size. Although soft selection cannot initially eliminate the genetic load from mutations that became fixed during the bottleneck, once back or compensatory mutations arise to counteract their negative fitness effects, these can be strongly selected for by soft selection (Robinson *et al.*, 2023). Therefore, increasing N_e may further enhance the viability of such populations by both raising the likelihood of beneficial mutations and increasing the efficiency of their selection.

The preceding assumptions rest on the assumption that soft selection is prevalent in natural populations. In Chapter 2, I argued this point, suggesting that any phenotype influencing an individual's survival or reproductive success will almost certainly affect its ability to compete with conspecifics, and hard selection is too inefficient to prevent the accumulation of genetic load that could drive a population to extinction. However, if these assumptions are incorrect and soft selection is uncommon, the implications of N_e for species viability are substantial. Under hard selection, the efficiency of selection does not weaken as N_e declines; however when N_e becomes sufficiently low, mutations may become fixed due to genetic drift (Martin *et al.*, 2023). Hard selection primarily acts against mutations when they are homozygous, as it is too weak to be effective against non-dominant mutations. Therefore, as populations contract, selection under hard selection will likely become more stringent, suppressing segregating mutations but at the cost of further population decline. However this enhanced purging is likely to be less important than the increased fixation due to genetic drift (Dussex *et al.*, 2023). After recovering from a population bottleneck, any benefits from the suppression of genetic load through hard selection would be offset by the reduced population size. Thus, hard selection may provide limited benefits in fully restoring population fitness. Understanding the relative importance of hard and soft selection in natural populations is crucial, as it significantly influences the viability of threatened populations and impacts conservation strategies.

3.5 Soft selection and Lewontin's paradox

At equilibrium, neutral nucleotide diversity is expected to scale approximately with $\theta = 4N_e\mu$ (Watterson, 1978). N_e is effective population size rather than census size N or carrying capacity k. Across taxa, diversity increases much more slowly than simple differences in population abundance would predict, a pattern known as Lewontin's paradox.

Several explanations have been proposed for this phenomenon. These include mutational bias and a negative correlation between mutation rate and N_e (Charlesworth and Jensen, 2022). The accuracy of measuring past N_e has also been questioned. If a population has expanded from a bottleneck, it would exhibit lower-than-expected diversity because its current population has fewer ancestors (Grundler *et al*., 2019). This situation could arise from a historically smaller ancestral population size (*Na*) due to past environmental constraints or skewed offspring numbers. Taxa with large N_e often consist of short-lived individuals that produce numerous offspring, making them more susceptible to sweepstakes reproductive behaviour (Eldan and Stephan, 2024). However, many studies use Kingman coalescence to estimate historical Ne, which does not account for sweepstakes success (Tellier and Lamaire, 2014). There is evidence against historically low Ne, such as the site frequency spectrum (SFS) signal in the marine algae *Emiliania huxleyi* (Filatov, 2019). If a population has expanded from a smaller past, it will show a high number of low-frequency polymorphisms and a low number of high-frequency polymorphisms due to recent mutations occurring in the larger population, resulting in a distinctive SFS. This abundant microbe has a far lower nucleotide diversity than expected, but its SFS suggests it has not undergone a recent population expansion. Therefore a small ancestral population size is unlikely to be the cause of the low genetic diversity observed in this marine microbe.

Enhanced selection is another possible explanation. This can manifest as background selection, where nucleotide diversity is reduced due to the negative selection of deleterious mutations; selective sweeps, where positive selection for beneficial mutations causes the fixation of their linked neutral mutations; or selection on nearly neutral loci (Charlesworth and Jensen, 2022). Each of these mechanisms would result in lower than expected nucleotide diversity. The results of this study suggest that soft selection is stronger in populations with large

N_e. In more abundant populations, relatively unfit individuals are less likely to be evolutionarily successful. Therefore, soft selection could underlie and drive the other forms of selection. If soft selection is prevalent in nature, it may tentatively explain Lewontin's paradox.

4 Compound selection

4.1 Introduction

The results from Chapter 2 and 3 offer a robust demonstration that genetic load inevitably accumulates in populations under hard or density-dependent selection. Ultimately, this accumulation of genetic load is likely to render populations inviable. If hard selection is very strong, too many selective deaths are likely to occur to sustain the population, especially in K-strategists. Alternatively, when it is weak, not enough moderately unfit individuals are removed, leading to the accumulation of load in the population. There is only a limited parameter space (of mutation, drift, and selection) under which hard selection or density-dependent selection can restrain the genetic load. This inefficiency of hard selection has been a longstanding challenge for evolutionary genetics (Cook and Turner, 2020). Our results also provide evidence in support of Wallace's solution, suggesting that soft selection has the potential to efficiently purge load without additional selective deaths (Wallace, 1975). Soft selection may, therefore, be the solution to how species prevent an unviable accumulation of genetic load (Charlesworth, 2013). If this is true, it must be prevalent throughout nature.

This poses a challenge for research relying on simulations to estimate the extinction risk of species, as there is a tendency to incorporate hard or density-dependent hard selection in these models (Beichman *et al*., 2020; Caballero *et al.*, 2017; Dussex *et al.*, 2021; Grossen *et al*., 2020; Kyriazis, Wayne, and Lohmueller, 2021; Perez-Pereira *et al*., 2022; Pinto *et al*., 2023; Xie *et al*., 2021). This may result in a significant underestimation of a population's capacity to purge genetic load. It remains unclear whether this tendency leads to an under or overestimation of extinction risk. It is likely that the purging of mutations results in fitter populations when maintained at a stable effective population size (N_e). However, population bottlenecks may drive the population to extinction because of excessive mortality during purging of the realised load.

Conversely, soft selection can be extremely efficient, purging an unrealistic amount of load, and creating populations that never go extinct. Under soft selection, the extent to which population bottlenecks (e.g., due to environmental fluctuations) may reduce the efficiency of purging remains unclear (Gilbert, Peischl, and Excoffier, 2018). Although extinction is avoided

under soft selection, bottlenecks may also lead to an abrupt relaxation of purging, causing sharp accumulation of genetic load. The parameter space (of mutation, drift, and selection) under which soft selection can result in long-term viable populations may be broader than under hard- and density dependent selection, but soft selection can lead to a run-away process of genetic load accumulation, which does not reflect the evolution of load in natural populations.

Standard Wright-Fisher models have fixed N and therefore cannot represent extinction caused by declining fitness. Some modified models use mean fitness to determine next-generation population size (Kardos and Luikart, 2021; Sachdeva, Olusanya, and Barton, 2022). Neither the mean nor the median alone is a sufficient demographic criterion: extinction depends on the number and distribution of individuals that survive and reproduce. Models linking fitness to population size should therefore retain this individual-level variation rather than assume a universal shape for the fitness distribution.

It is, therefore, necessary to develop models that incorporate both hard and soft selection so that simulated populations have the potential capacity to purge genetic load effectively while also going extinct if fitness declines to a sufficiently low level. The most obvious solution would be to model populations that employ hard and soft selection separately. This may be appropriate when there is known competition within a population for a discrete resource such as mates or territory. This competition could be modelled, generating a soft selection that reasonably reflects the biology of the real population. However, competition between individuals is often cryptic, and hence, difficult to model such soft selection. Yet, from the previous chapters and the summary above we know soft selection is often necessary to prevent an unviable accumulation of genetic load. Such soft selection may be realised by competition for discontinuous resources such as nutrients (Svanbäck and Bolnick, 2007). Without an obvious competitive dynamic, such as males competing for females or individuals competing for a specified territory, it is very difficult to know how to calibrate soft selection to accurately represent its efficacy in real populations. Too little soft selection leads to a mutational meltdown (without extinction), and overly efficient soft selection purges virtually all the genetic load. In this chapter, frequency dependence means dependence on fitness relative to conspecifics, following Wallace (1975); it is distinct from the common modern use in which the fitness of a discrete phenotype changes with its frequency.

4.2 The concept of compound selection

Here, I propose to modify absolute fitness so that it becomes both frequency and density-dependent. This adjustment results in a selection mechanism where an individual's survival or reproductive success is influenced by both its own absolute fitness and its relative fitness within the population. To transition from hard selection to density-dependent hard selection, each individual's fitness can be rescaled by the factor k/N, where k represents the carrying capacity and N is the current population size. This rescaling accounts for resource scarcity or abundance, applying a uniform factor to adjust the fitness of each individual and thus determining their survival probability.

The second adjustment I propose involves the impact of resource scarcity. It is very likely that resource scarcity does not affect all individuals equally. Rather, less fit individuals experience the effects of resource scarcity more acutely than their fitter counterparts, who can outcompete them. Consequently, less fit individuals are more vulnerable to mortality due to predation or disease, in addition to the direct impact of nutrient deficiency. Therefore, their survival could be disproportionately reduced compared to fitter individuals, even if the initial differences in fitness are small. By adjusting the density-dependent factor according to each individual’s relative fitness, we can achieve a survival probability that reflects both frequency and density-dependence. This method creates a novel form of selection that incorporates aspects of soft selection, as described in Wallace's theory (1975, Table 4.1), while not fixing the number of survivors. This approach could address the inefficiencies of hard selection and the challenges of modelling extinction in soft selection. I will refer to this novel mode of selection as compound selection.

	Density independent	Density-dependent
Frequency Independent	Hard selection	Density-dependence
Frequency-dependent	Frequency-dependence	Soft selection

Table 4.1 - Wallace's (1975) classification of selection according to dependence on density and relative fitness. Rescaling fitness by a term that includes both components can approach the efficiency of soft selection without fixing the number of survivors.

Compound selection combines a uniform density term with a relative-fitness term. Multiplying all individuals by K/N applies the same density scaling. In the compound model, this density factor is raised to a power based on relative fitness, so the same crowding level reduces survival more strongly for individuals farther below the fittest genotype.

E.g.

For example, with k = 100 and N = 1,000, k/N = 0.1. An individual with w = 0.9 relative to w_{MAX} = 1 therefore receives a substantially lower compound survival probability than the fittest individual. The prose, equation and worked values use these same k and N values.

$\frac{0.9*100}{100}0^{\frac{0.9}{0.9}}$ = 0.09

An individual with a fitness of 0.8 in the same population would be:

$\frac{0.8*100}{100}0^{\frac{0.9}{0.8}}$ = 0.05999153674

And an individual with a fitness of 0.7:

$\frac{0.7*100}{100}0^{\frac{0.9}{0.7}}$ = 0.03625632275

If the same individuals were rescaled only by k/N, their survival probabilities would be 0.09, 0.08 and 0.07. Compound rescaling therefore lowers the survival of relatively unfit individuals more strongly and may improve purging, while still allowing population size to fall.

Aim

The objective of this chapter is to test whether compound selection can purge genetic load while retaining extinction as a possible outcome. I compare the effects of fecundity and population size on load, nucleotide diversity and viability under compound, hard, density-dependent hard and soft selection.

4.3 Methods

All models were forward genetic nonWF models run using SLiM version 3 software (Haller and Messer, 2019). Four different methods of selection were tested: hard selection, density-dependence, frequency-dependence and soft selection (see below). Non- overlapping generations with hermaphroditic and purely sexually reproducing diploid individuals were simulated. Individuals mated randomly and monogamously. Each generation a carrying capacity (k) was randomly selected from a normal distribution with a mean of 500 and a standard deviation of 35.

The mutation rate was $\mu = 1.17e^{-7}$, and the recombination rate was $\rho = 1e^{-7}$ unless otherwise stated. These values were used consistently in the chapter's methods and figure legends.

One hundred replicates were run for each treatment. Burn-in populations were simulated for 40,000 generations with neutral mutations to establish genealogies and π near the neutral expectation. Deleterious mutations were then introduced for 1,000 generations at $\mu = 1.17e^{-7}$ to produce a mean masked load of approximately one lethal equivalent. The expression $4N_e\mu$ uses effective, not census, population size.

4.3.1 Modes of selection

As per Chapter 2, for the strict hard selection models the survival probability is equal to fitness (w). So the equation for hard selection:

$$S_h = w$$

[Equation 4.1]

For frequency-dependent selection, the recalculateFitness() callback was used to return the w of each individual of the offspring population. The distribution of all individual fitness values was used to determine the maximum fitness (w_{MAX}) at generation t. The fitness of each individual was rescaled by dividing by w_max of that generation. The equation for survival probability under frequency-dependency (S_f) is:

$$S_f = w / w_{MAX}$$

[Equation 4.2]

For both the hard selection and frequency-dependent selection, if the numbers surviving selection exceeded the carrying capacity (k), a number of k individuals were randomly selected from the S_h or S_f survivors to contribute to the next generation. The remaining excess number of individuals were removed. In order to achieve this, each generation of reproduction occurred over two phases in SLiM. In the first phase, offspring are reproduced and undergo hard or frequency-dependent selection. In the second phase survivors were randomly chosen to reproduce the next generation.

For density-dependent w was rescaled by density-dependent factor k/N, thus the equation for the survival probability is as follows:

$$S_d = w * k/N$$

[Equation 4.3]

I explored two methods of selection that both meet the definition of soft selection by Wallace (1975), in that they are both frequency and density-dependent. The first method is what I refer to as compound selection. It determines the survival probability under compound selection (S_c) by multiplying w by the density-dependent factor (k/N) raised to the power of w_{MAX} / w, giving the equation:

$$S_c = w \times (k/N)^{wMAX/w}$$

[Equation 4.4]

The exponent w_{MAX}/w makes the density penalty stronger for individuals below the maximum fitness. Thus, crowding reduces survival disproportionately for less-fit individuals. Under density-dependent hard selection, all individuals receive the same k/N scaling. Under compound selection, the exponent varies with relative fitness, so less-fit individuals are penalised more strongly. Equation 4.2 gives the frequency-dependent component; Equation 4.4 combines it with density dependence.When $N \leq k$, the density factor is capped at 1 so that survival probabilities cannot exceed 1, and the frequency-dependent competition term is not applied. This represents weak or absent competition when the population is below carrying capacity.

		Density	
		Independent	Dependent
Frequency	Independent	Hard selection $S_h = w$	Density-dependence $S_d = w * k/N$
	Dependent	Frequency-dependence $S_f = w / w_{MAX}$	Soft selection $S_c = w * k/N \wedge w_{MAX} / w$

Table 4.2 - Reproduction of the schematic produced by Wallace (1975) containing the equations to calculate survival probabilities under hard, frequency-dependent hard, density-dependent hard, and compound selection. Key: S - survival probability of an individual, w - fitness of an individual, w_{MAX} - maximum fitness within the offspring population, k - carrying capacity, N - number of offspring.

Compound selection is density- and frequency-dependent in Wallace's sense because outcomes depend on population density and relative fitness. This use of frequency dependence differs from the common modern meaning in which the fitness of a discrete phenotype changes with its frequency. Under compound selection, absolute survival remains possible, so population size is an outcome rather than fixed at k.

The soft selection models produced in Chapter 2 will also be included in order to make comparisons between compound selection and true soft selection. In the soft selection models the *w* of each individual is then divided by two and added to a random number from 0.00 to 0.50 selected from each individual. The individuals are ranked according to this combined score and the top k are selected to survive to the next generation where they reproduce. Those not in the top k die.

In addition to investigating the effect of varying fecundity under different selection models, the effect of varying N_e under density-dependent hard selection and compound selection was also investigated. These populations had larger exomes of 5 autosomes comprising 1000 genes of 1500 base pairs. Recombination within genes occurred at a rate of $1e^{-9}$ and between genes at a rate of $1e^{-6}$. Free recombination occurred between genes. 1000 deleterious mutants of the same profile as those used above were added at a rate of $1e^{-8}$. The simulations ran with no existing genetic load, under either density-dependent hard selection or compound selection as described above.

4.4 Results

4.4.1 Genetic load under different modes of selection

Compound and frequency-dependent hard selection were more efficient than hard and density-dependent hard selection under matched parameters (Figure 4.1). Increasing fecundity enhanced selection only under compound and soft selection. Frequency-dependent rescaling alone did not create this fecundity response.

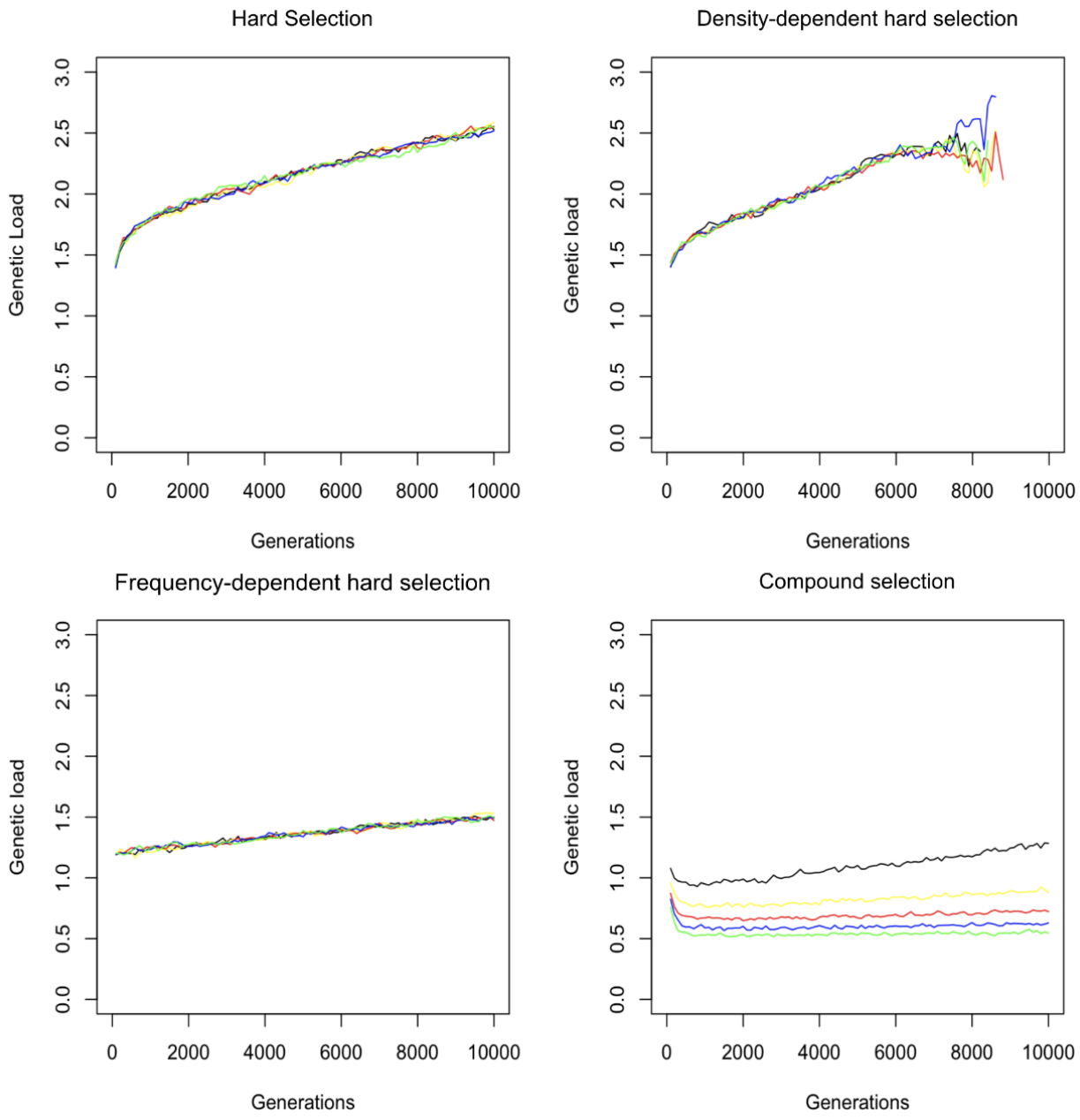


Figure 4.1 - The effect of varying fecundity under hard, density-dependent hard, and compound selection on genetic load. The mutation rate was $1e^{-7}$. **Key:** Black - fecundity=8, Yellow - fecundity=32, Red - fecundity=128, Blue - fecundity=512, Green - fecundity=2,048. The impact of varying selection pressures only leads to significantly different rates of genetic load accumulation under compound selection. This is due to the intensified competition resulting from a higher number of offspring.

Soft selection was expected to be more efficient than compound selection because pure rank-based selection always fills K places using relative performance, whereas compound

selection retains absolute survival and stochastic mortality. The biological interest is therefore the trade-off between efficient purging and extinction risk.

To understand why increased fecundity does not necessarily increase the rate of purging under soft selection, one needs to consider the variation in fitness of the selected individuals. In soft selection, these fittest k individuals are chosen for the next generation. Beyond a certain threshold, these k individuals become equally matched. For example, selecting the top 10 numbers (i.e., the 10 fittest offspring) from a uniform distribution would yield similar results whether you pick from 10,000 or 100,000 numbers (of offspring). Both the fittest 0.1% and the fittest 0.01% individuals would likely be close to the maximum number in the uniform distribution. Hence, the efficacy of soft selection does not continue to increase with increasing fecundity.

In compound selection, absolute fitness is rescaled by density-dependence *k/N* raised to the power of frequency-dependence w_{MAX}/w. Differences in frequency-dependence diminish with increasing frequency; the fittest individual in a population with fecundity of 128 is likely the same as in a population with fecundity of 512. However, k/N consistently decreases with higher fecundity, leading to a greater reduction in the survival probabilities of less fit individuals, thus enhancing selection efficiency.

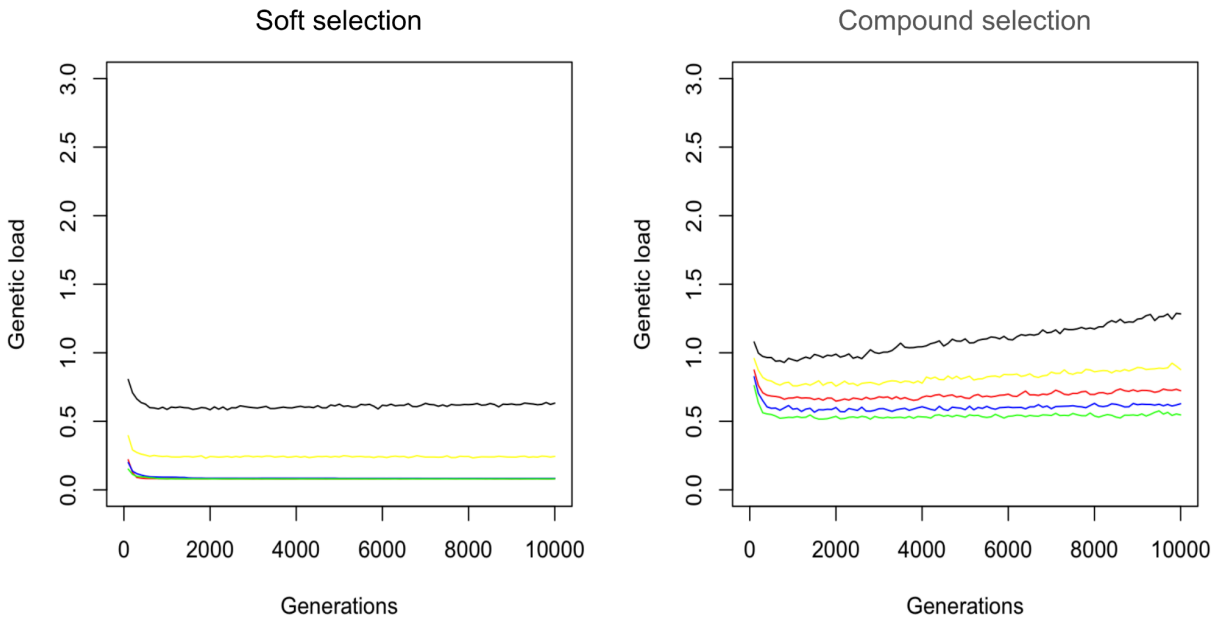


Figure 4.2 - The effect of varying fecundity on genetic load under soft and compound selection. The mutation rate was $2e^{-7}$. **Key:** Black - fecundity=8, Yellow - fecundity=32, Red - fecundity=128, Blue - fecundity=512, Green - fecundity=2,048. Under the same conditions, soft selection is demonstrated to be more efficient than compound selection. While increasing fecundity typically enhances selection efficiency, soft selection can be so effective that its efficiency cannot be further improved even with higher fecundity.

4.4.2 Density-dependent hard selection versus compound selection

Density-dependent hard selection and compound selection are comparable modes of selection as both use the density-dependent factor *k/N* to regulate the population size. This makes the number of survivors of selection a dependent outcome, rather than a predetermined one as in soft selection. Moreover, the factor *k/N* ensures the number of survivors does not significantly exceed k, thus controlling the population without randomly selecting individuals as in hard selection. The dependent and variable number of survivors affects the impact of selection differently in future generations for density-dependent hard selection and compound selection. In both types, fewer survivors result as genetic load increases because selection is at least partially hard in both. This leads to population decline, although it may occur primarily in less fecund populations under compound selection. Under compound selection, the selection efficiency in r-strategists may be high enough to prevent the accumulation of genetic load (Fig 4.5).

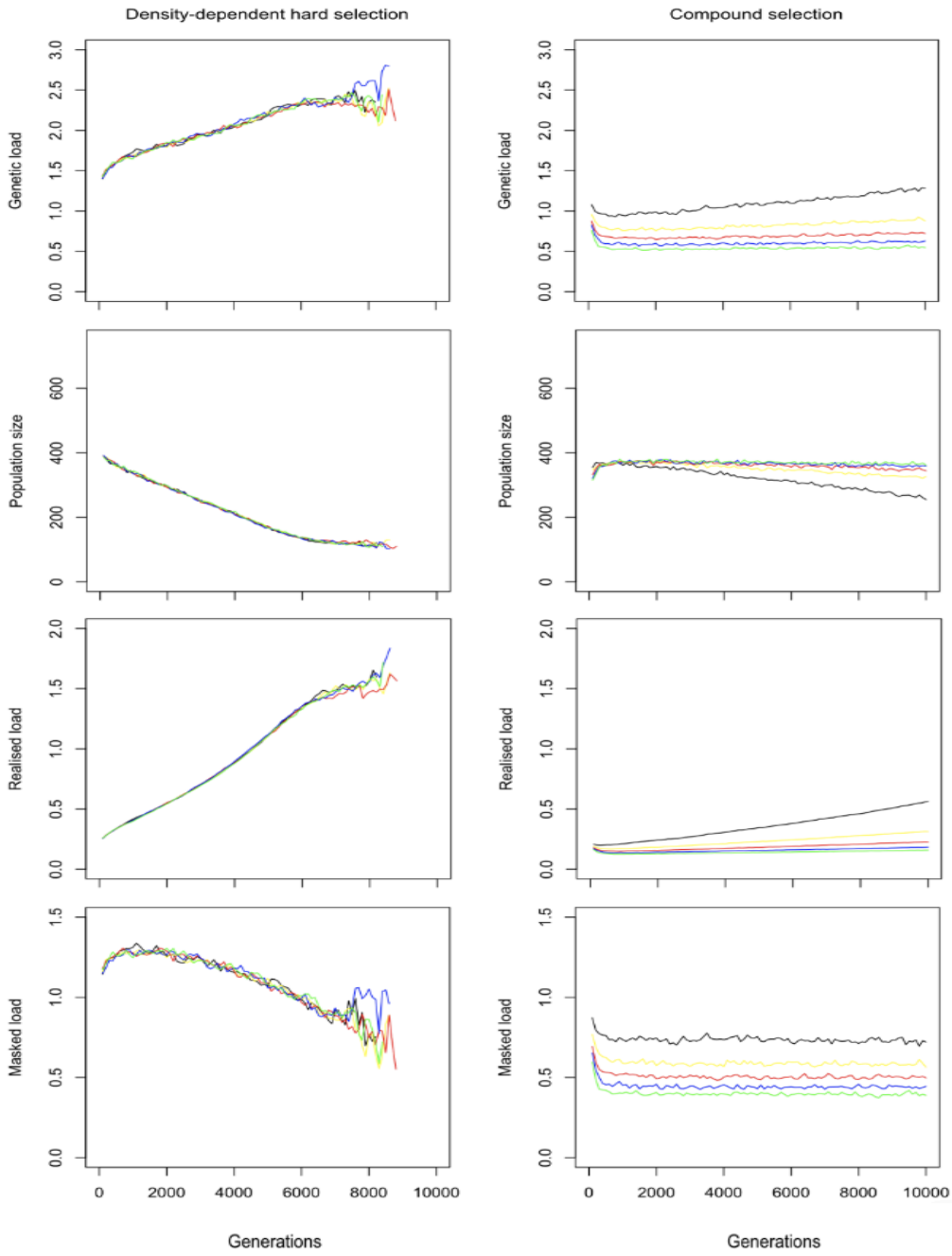


Figure 4.3 - The effect of varying fecundity under density-dependent hard selection and compound selection on genetic load, population size, realised load and masked load. The mutation rate was $2e^{-7}$. **Key:** Black - fecundity=8, Yellow - fecundity=32, Red - fecundity=128, Blue - fecundity=512, Green - fecundity=2,048. Under density-dependent hard selection, selection efficiency remains unaffected by varying fecundity. This form of selection is relatively weak, leading to the accumulation of genetic load, increased selective deaths, and population decline. The declining population likely exacerbates the rate of realised load accumulation due

to increased homozygosity in smaller populations. Additionally, population decline reduces the masked load, as smaller populations have fewer segregating mutations. Compound selection is significantly more efficient than density-dependent hard selection, especially with higher fecundity. Compared to density-dependent hard selection, under compound selection, populations are able to cope with a considerably higher genetic load and realised load, particularly r-strategy species.

Under both density-dependent hard selection and compound selection, population contraction likely accelerates the accumulation of realised load, as smaller populations have a higher probability of mutations existing in a homozygous state (Fig 4.3). Crucially, the realised load continues to increase under density-dependent hard selection, resulting in a progressive loss of fitness and ultimate population extinction. Such a pattern is not observed under compound selection. The population decline under density-dependent hard selection decreases the masked load because smaller populations carry fewer segregating mutations. Conversely, in compound selection, population decline reduces competition, weakening selection efficiency. Consequently, although smaller populations have fewer segregating mutations, selection against deleterious mutations becomes less effective, and hence, they are purged less efficiently. Depending on the model parameters, the loss of deleterious mutations due to population contraction can be offset by the reduction in purging, causing masked load to remain constant (Fig 4.4 and Fig 4.5). However, under less favourable conditions, the reduction in purging may exceed the loss due to population contraction, leading to an increase in masked load (Fig 4.6).

Under compound selection with $\mu = 2e^{-7}$, less-fecund populations accumulated genetic load and declined without extinction (Figure 4.3). At $\mu = 3e^{-7}$, only the highest-fecundity treatment survived for 10,000 generations (Figure 4.4). The Isle Royale wolf population is not evidence for this mechanism: it was already severely inbred and affected by very small population size, disease and demographic stochasticity before genetic rescue. It is used only as a cautious analogy for extreme reproductive skew.

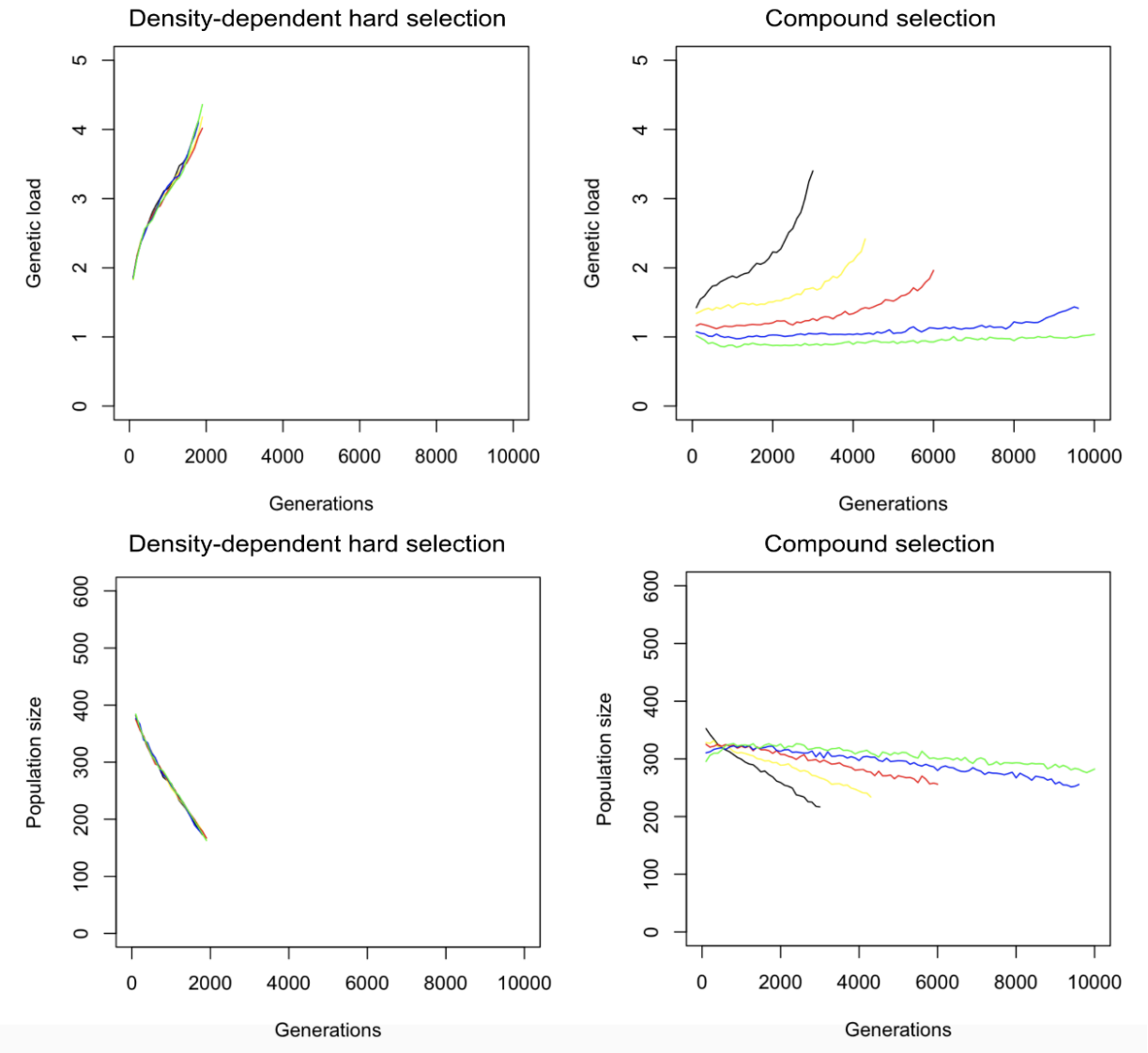


Figure 4.4 - The effect of varying fecundity under density-dependent hard selection and compound selection on genetic load and population size, with a mutation rate of $3e^{-7}$. **Key:** Black - fecundity=8, Yellow - fecundity=32, Red - fecundity=128, Blue - fecundity=512, Green - fecundity=2048. Compound selection was more efficient, allowing populations to survive longer. However, in high-fecundity populations under compound selection, despite more efficient selection and a slower accumulation of genetic load, extinction occurred at lower levels of genetic load and larger population sizes. This was due to the presence of highly fit individuals, which negatively impacted the survival probabilities of less fit individuals.

4.4.3 Neutral diversity during extinction

Increasing realised load lowers mean fitness and was therefore associated with declining census population size N in these models. Under compound selection, mild population decline also reduced segregating-site density and π, although the two diversity measures need not respond identically.

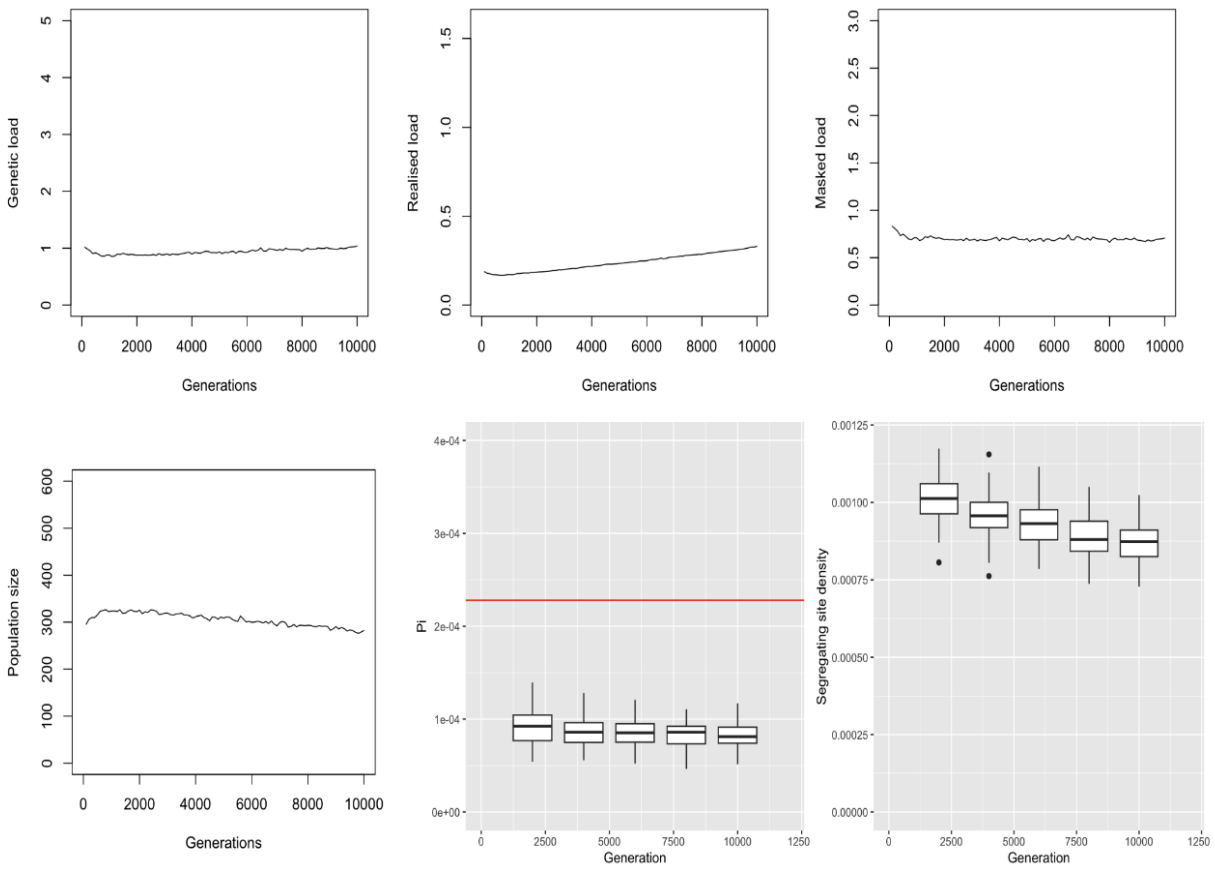


Figure 4.5 - Mean genetic load, realised load, masked load, population size, neutral nucleotide diversity (π), and segregating site density under compound selection in populations with a fecundity of 2048 and mutation rate of 3e^{-7}. The red line indicates the estimated π according to Watterson's estimator ($4N_e\mu$). Shown are the medians, the interquartile range (boxes), the range (whiskers), and the outliers.

During mutational meltdown, replicate trajectories diverged as stochastic loss, changing survivor numbers and differential extinction increased between-replicate variation. Tajima's D was not

calculated, so differences between π and segregating-site density cannot be attributed to expansion, positive selection or any specific site-frequency-spectrum process without further analysis.

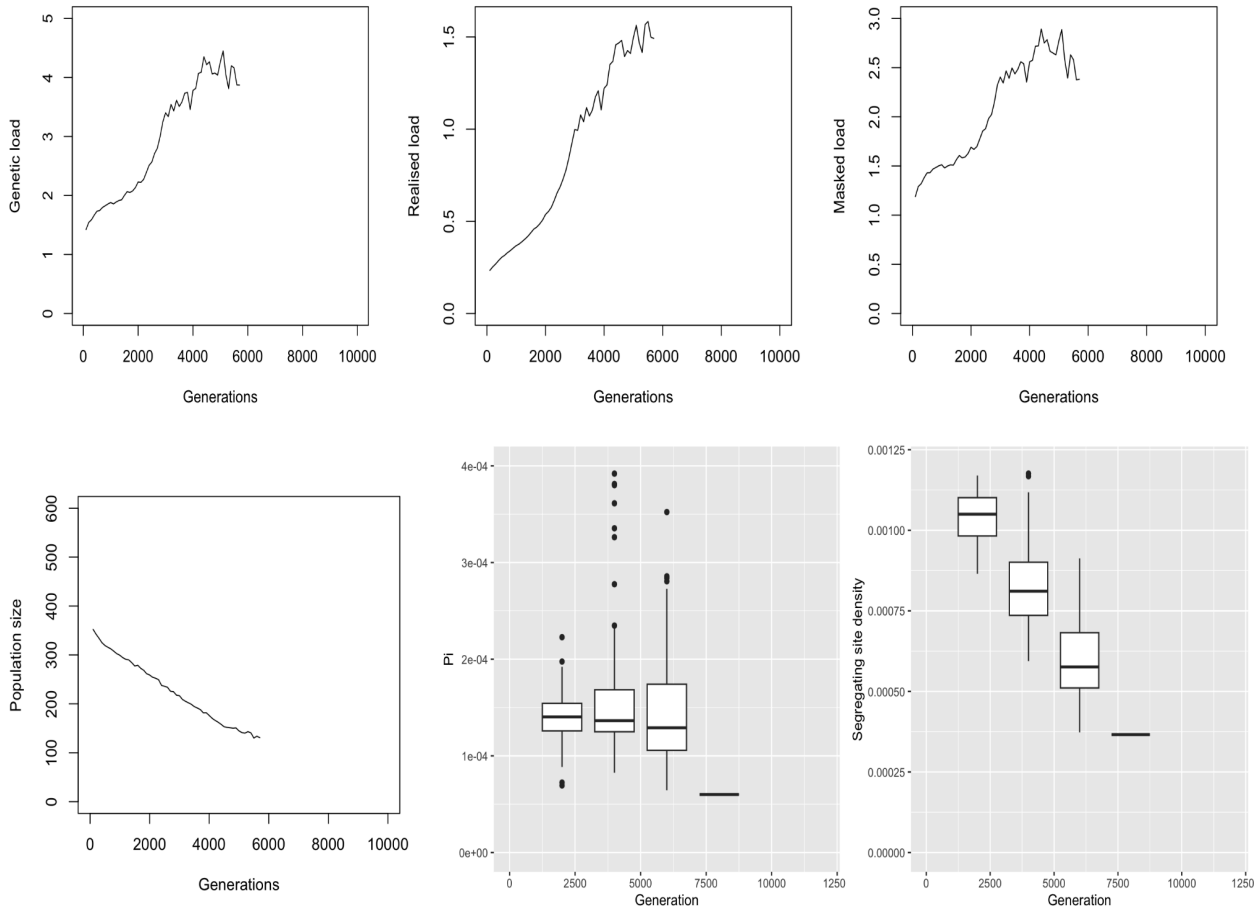


Figure 4.6 - Mean genetic load, realised load, masked load, population size, neutral nucleotide diversity (*π*), and segregating site density under compound selection in populations with a fecundity of 8 and mutation rate of 3e^{-7}. The data for genetic load components and population size are only displayed if at least 75 of 100 replicates are extant to avoid the stochastic processes during extinction that can inflate the variance in population genetic summary statistics. Shown are the medians, the interquartile range (boxes), the range (whiskers), and the outliers.

Under density-dependent hard selection, declining segregating-site density was accompanied by declining π as populations contracted (Figure 4.7). Neutral diversity records

demographic history and may inform inbreeding and adaptive potential, but it is an imperfect proxy for realised load or current extinction risk. High diversity does not guarantee low load, and low diversity alone does not demonstrate imminent extinction; functional and fitness data are also required.

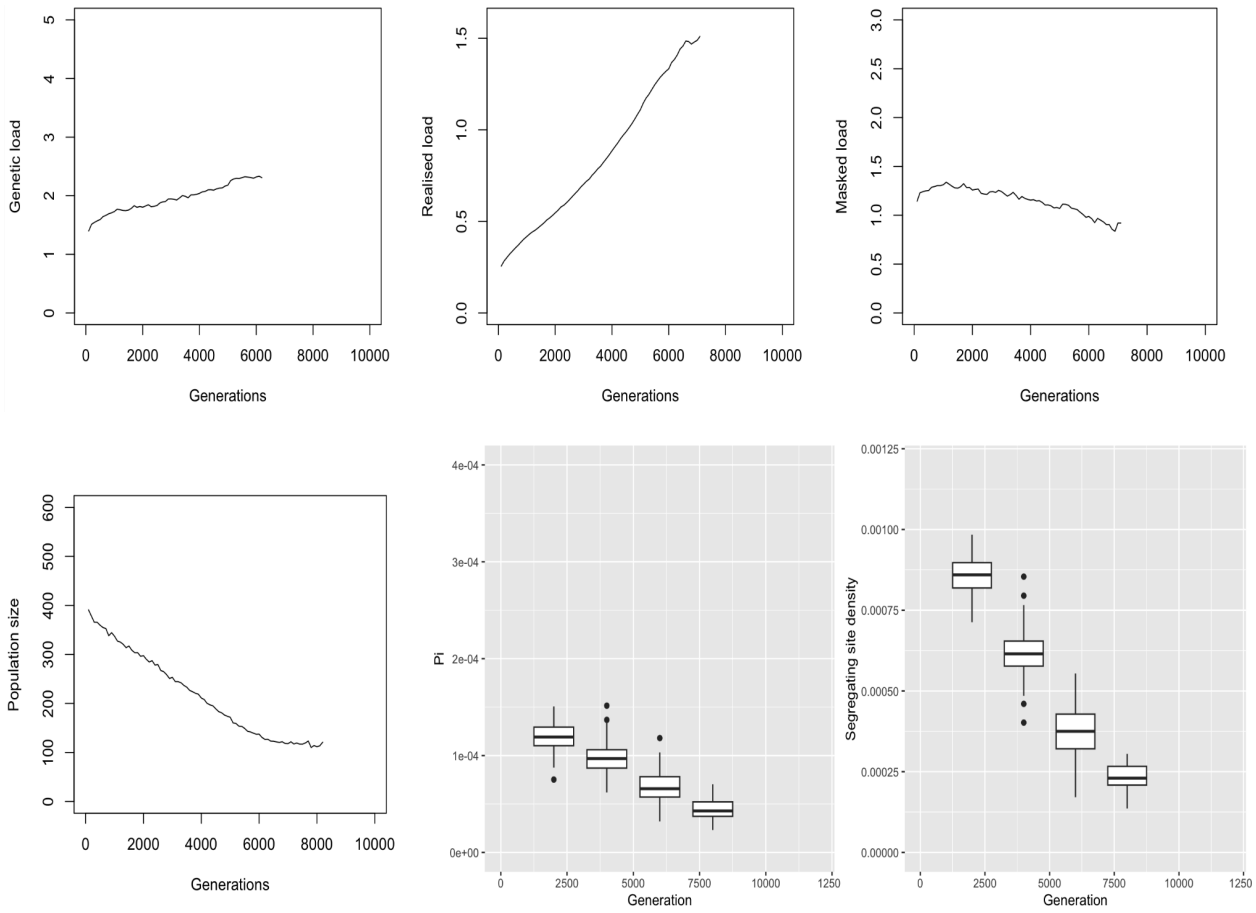


Figure 4.7 - Mean genetic load, realised load, masked load, population size, neutral nucleotide diversity (π), and segregating site density under density-dependent hard selection in populations with a fecundity of 8 and mutation rate of $2e^{-7}$. Shown are the medians, the interquartile range (boxes), the range (whiskers), and the outliers.

4.4.4 Varying *Ne* under compound and density-dependent hard selection

Under compound selection, larger target population sizes reached a higher equilibrium masked load (Figure 4.8), as also observed under hard and density-dependent hard selection. This pattern contrasts with rank-based soft selection, where larger populations strengthened competition. The result is a pattern of this parameterisation rather than a general consequence of N_e.

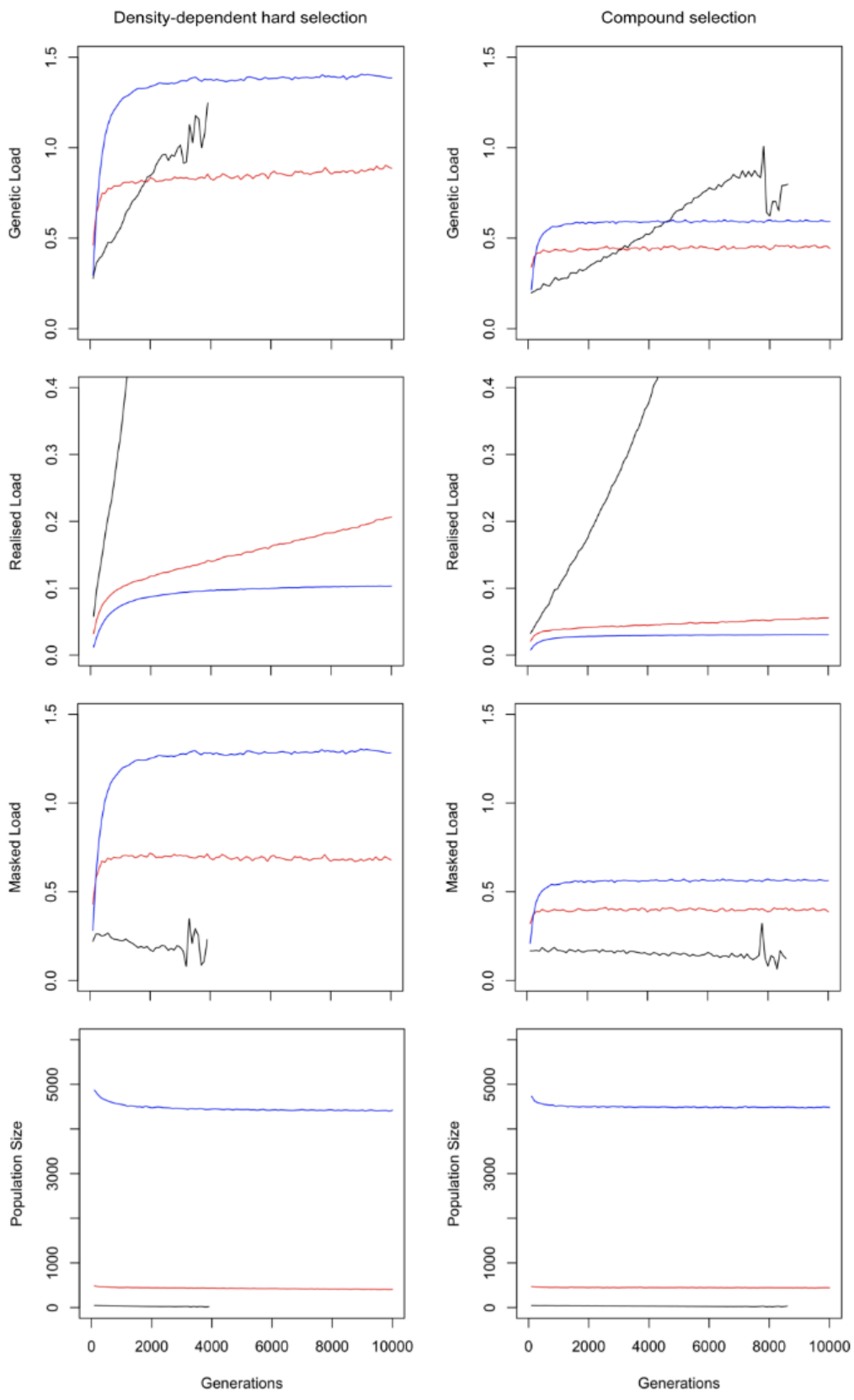

Density-dependent hard selection
Compound selection
Genetic Load
Realised Load
Masked Load
Population Size
Generations
0.0
0.5
1.0
1.5
0.1
0.2
0.3
0.4
0
1000
3000
5000
0
2000
4000
6000
8000
10000

Figure 4.8 - The effect of varying N_e on the mean genetic load, realised load, masked load and population size under density-dependent hard and compound selection. **Key:** Black - N_e=50, Red - N_e=500, Blue-N_e=5000. Higher N_e populations stabilise with larger amounts of genetic load under both density-dependent hard and compound selection.

4.5 Discussion

As modelled here, both frequency-dependent hard and compound selection caused the survival probabilities of less fit individuals to be rescaled disproportionately lower than those of the fittest individuals. This results in populations containing relatively fewer unfit individuals after selection (Table 4.3). Consequently, these modes of selection are more efficient than hard and density-dependent hard selection. This indicates that selection is more effective when it is based, at least partially, on being among the fittest within the population, rather than merely being sufficiently fit. In simple terms, in terms of purging, selection based on relative fitness is more efficient than selection based on absolute fitness. However, only compound and soft selection showed increased fecundity enhancing selection efficiency.

Increased fecundity does not enhance purging under frequency-dependent hard selection. A detailed breakdown of the effect each mode of selection has on the survival of individuals based on their fitness in K- and r-strategist populations is provided in Table 4.3. It shows that under compound selection, r-strategists experience enhanced selection because less fit individuals are rescaled to a greater extent relative to fitter individuals than in K-strategists. This does not occur under the other modes of hard selection. As a result, under compound selection, r-strategists have fewer selection survivors than K-strategists when matched for mean pre-selection fitness. However, the mean fitness of r-strategists is improved, leading to a fitter subsequent generation and greater long-term viability of the population. The downside of compound selection is that due to mortality, less fecund populations may go extinct if the mutation rate becomes too high. Such extinctions are not observed under traditional models of soft selection, making such models unsuitable for the analysis of extinction risk (see below).

Table 4.3 The mathematics explaining how fitness is rescaled under various selection modes: hard, frequency-dependent hard, density-dependent hard, compound, and soft selection, in populations of K- and r-strategists that are matched in fitness.

The second column shows the composition of the population before selection. Each population comprises individuals with fitness values of 0.9, 0.8, 0.7, and 0.5. The number of individuals is 500 for K-strategists and 5000 for r-strategists, maintaining the same relative distribution of fitness values.

For K-strategists, the populations have 100 individuals with a fitness value of 0.9, denoted as 100(0.9i), while r-strategists have 1000 individuals, denoted as 1000(0.9i). Similarly, K-strategists have 100 individuals with a fitness of 0.8, represented as 100(0.8i), while r-strategists have 1000 such individuals, represented as 1000(0.8i), and so on. Thus, the mean fitness for both K- and r-strategists before selection is 0.7.

The third column explains how the rescaling factor for individuals of each fitness value is calculated depending on the selection mode. The survival probabilities are determined by multiplying the rescaling factor by the fitness value.

The final column shows the number of individuals of each fitness class that survive each mode of selection, calculated by multiplying the number of individuals in each class by their survival probability. It also provides the total number of selection survivors and their mean fitness.

Key:
N - number of individuals within each population
w - fitness of a given individual
w_{MAX} - fitness of the fittest individual within a population
k - carrying capacity

Selection type	K- or r-strategist	Rescale factor	Survival probabilities	Selection survivors
Hard selection	K	Unrescaled		
	100(0.9i)		0.9	100(0.9i) * 0.9 = 90(0.9i)
	100(0.8i)		0.8	100(0.8i) * 0.8 = 80(0.8i)
	100(0.7i)		0.7	100(0.7i) * 0.7 = 70(0.7i)
	100(0.6i)		0.6	100(0.6i) * 0.6 = 60(0.6i)
	100(0.5i)		0.5	100(0.5i) * 0.5 = 50(0.5i)
	N=500 Mean w = 0.7			N = 350 Mean w = 0.729
	r			
	1000(0.9i)		0.9	1000(0.9i) * 0.9 = 900(0.9i)
	1000(0.8i)		0.8	1000(0.8i) * 0.8 = 800(0.8i)
	1000(0.7i)		0.7	1000(0.7i) * 0.7 = 700(0.7i)
	1000(0.6i)		0.6	1000(0.6i) * 0.6 = 600(0.6i)
	1000(0.5i)		0.5	1000*0.5=500
	N=5000 Mean w = 0.7			N = 3500 Mean w = 0.729
Frequency-dependent hard selection	K	w/w_{MAX}		
	100(0.9i)	0.9/0.9 = 1	0.9*1= 0.9	100(0.9i) * 0.9 = 90(0.9i)
	100(0.8i)	0.8/0.9 = 0.889	0.8*0.889=0.711	100(0.8i) * 0.711 = 71(0.8i)
	100(0.7i)	0.7/0.9 = 0.778	0.7*0.778=0.54	100(0.7i) * 0.54 = 54(0.7i)
	100(0.6i)	0.6/0.9 = 0.667	0.6*0.667=0.4	100(0.6i) * 0.4 = 40(0.6i)
	100(0.5i)	0.5/0.9 = 0.556	0.5*0.556=0.278	100(0.5i) * 0.278 = 28(0.5i)
	N=500 Mean w = 0.7			N = 238 Mean w = 0.897

	r	w/w_{MAX}		
	1000(0.9i)	0.9/0.9=1	0.9*1=0.9	1000(0.9i) * 0.9 = 900(0.9i)
	1000(0.8i)	0.8/0.9=0.889	0.8*0.889=0.711	1000(0.8i) * 0.711 = 710(0.8i)
	1000(0.7i)	0.7/0.9=0.778	0.7*0.778 = 0.54	1000(0.7i) * 0.54 = 540(0.7i)
	1000(0.6i)	0.6/0.9=0.667	0.6*0.667=0.4	1000(0.6i) * 0.4 = 400(0.6i)
	1000(0.5i)	0.5/0.9=0.556	0.5*0.556=0.278	1000(0.5i) * 0.278 = 280(0.5i)
	N=5000 Mean w = 0.7			N=2380 Mean w = 0.897
Density-dependent hard selection	K	k/N		
	100(0.9i)	250/500=0.5	0.9*0.5=0.45	100(0.9i) * 0.45 = 45(0.9i)
	100(0.8i)	250/500=0.5	0.8*0.5=0.4	100(0.8i) * 0.4 = 40(0.8i)
	100(0.7i)	250/500=0.5	0.7*0.5=0.35	100(0.7i) * 0.35 = 35(0.7i)
	100(0.6i)	250/500=0.5	0.6*0.5=0.3	100(0.6i) * 0.3 = 30(0.6i)
	100(0.5i)	250/500=0.5	0.5*0.5=0.25	100(0.5i) * 0.25 = 25(0.5i)
	N=500 Mean w = 0.7			N = 175 Mean w = 0.729
	r	k/N		
	1000(0.9i)	250/5000=0.05	0.9*0.05=0.045	1000(0.9i) * 0.045 = 45(0.9i)
	1000(0.8i)	250/5000=0.05	0.8*0.05=0.04	1000(0.8i) * 0.04 = 40(0.8i)
	1000(0.7i)	250/5000=0.05	0.7*0.05=0.035	1000(0.7i) * 0.035 = 35(0.7i)
	1000(0.6i)	250/5000=0.05	0.6*0.05=0.03	1000(0.6i) * 0.03 = 30(0.6i)
	1000(0.5i)	250/5000=0.05	0.5*0.05=0.025	1000(0.5i) * 0.025 = 25(0.5i)
	N=5000 Mean w = 0.7			N = 175 Mean w = 0.729

Compound selection	K	$(k/N)^{wMAX/w}$		
	100(0.9i)	$(250/500)^{(0.9/0.9)}=0.5$	0.9*0.5=0.45	100(0.9i) * 0.45 = 45(0.9i)
	100(0.8i)	$(250/500)^{(0.9/0.8)}=0.459$	0.8*0.459=0.367	100(0.8i) * 0.367 = 37(0.8i)
	100(0.7i)	$(250/500)^{(0.9/0.7)}=0.410$	0.7*0.410=0.287	100(0.7i) * 0.287 = 28(0.7i)
	100(0.6i)	$(250/500)^{(0.9/0.6)}=0.354$	0.6*0.354=0.212	100(0.6i) * 0.212 = 21(0.6i)
	100(0.5i)	$(250/500)^{(0.9/0.5)}=0.287$	0.5*0.287=0.144	100(0.5i) * 0.144 = 14(0.5i)
	N=500 Mean w = 0.7			N = 145 Mean w = 0.754
	r	$(k/N)^{wMAX/w}$		
	1000(0.9i)	$(250/5000)^{(0.9/0.9)}=0.05$	0.9*0.05=0.045	1000(0.9i) * 0.045 = 45(0.9i)
	1000(0.8i)	$(250/5000)^{(0.9/0.8)}=0.03$	0.8*0.034=0.027	1000(0.8i) * 0.027 = 27(0.8i)
	1000(0.7i)	4	0.7*0.021=0.015	1000(0.7i) * 0.015 = 15(0.7i)
	1000(0.6i)	$(250/5000)^{(0.9/0.7)}=0.02$	0.6*0.011=0.006	1000(0.6i) * 0.006 = 6(0.6i)
	1000(0.5i)	1	0.5*0.005=0.002	1000(0.5i) * 0.002 = 2(0.5i)
		$(250/5000)^{(0.9/0.6)}=0.011$		
	N=5000	$(250/5000)^{(0.9/0.5)}=0.00$		N = 95
	Mean w = 0.7	5		Mean w = 0.813

Soft selection	K	Not applicable		
	100(0.9i)	Top k (250) individuals		100(0.9i)
	100(0.8i)	selected		100(0.8i)
	100(0.7i)			50(0.7i)
	100(0.6i)			
	100(0.5i)			N=250
				Mean w = 0.82
	N=500			
	Mean w = 0.7			

r	Not applicable	
1000(0.9i) 1000(0.8i) 1000(0.7i) 1000(0.6i) 1000(0.5i) N=5000 Mean w = 0.7	Top k (250) individuals selected	250(0.9i) N=250 Mean w = 0.9

4.5.1 Frequency-dependent hard selection

Recent studies on the impact of genetic load on population viability and extinction risk have predominantly used density-dependent hard selection (Beichman *et al*., 2020; Dussex *et al*., 2021; Kyriazis, Wayne, & Lohmueller, 2021; Pinto *et al*., 2023; Xie *et al*., 2021). This mode is considered suitable because it allows population size post-selection to be a dependent factor, while rescaling by k/N maintains population size without randomly selecting individuals when there is a surplus of selection survivors, as required in simple hard selection (Caballero *et al*., 2017; Grossen *et al*., 2020; Perez-Pereira *et al*., 2022). However, because selection is hard, it is relatively inefficient in purging deleterious mutations. As such, such models of hard selection are likely to underestimate population viability, and potentially overestimate extinction risk.

This chapter introduces two novel forms of selection modelling that enhance selection by making the probability of survival dependent on the relative fitness of conspecifics. I argue that this is a more biologically realistic approach to modelling fitness and selection. Consequently, selection was more effective compared to models that did not incorporate this dependency. Of these, frequency-dependent hard selection encountered the same practical issue as simple hard selection: there is a high probability that the number of selection survivors will exceed the carrying capacity. This necessitates randomly selecting individuals to form the next generation, making the model less realistic. True, individuals die of stochastic processes, but this is not necessarily a function of the excess in their numbers. Even in cases where this is so, the probability that an individual dies in such overcrowded conditions is unlikely to be purely

stochastic. Rather, fitter individuals are likely to have a better chance to survive. This feature is only captured by models of compound - and soft selection. In other words, because compound selection is density-independent, the survival of an individual is influenced by its fitness relative to the fittest individual, but not by the number of individuals competing relative to the carrying capacity.

Under density-dependent hard selection, survival is determined by the number of individuals competing relative to the carrying capacity, but not by their relative fitness. Hence, survival probability is independent of the relative fitness of the excess of individuals that survive selection, and which need to be culled down to the carrying capacity of the environment.

Frequency-dependent hard selection posits that survival is dependent on relative fitness, but not on the number of individuals competing relative to how many can exist in a given area. Consequently, frequency-dependence (as modelled here) is unlikely to occur in nature, given that population sizes are constrained by the carrying capacity of the environment.

Here, frequency dependence means that survival depends on an individual's fitness relative to conspecifics. This differs from the common modern use, in which the fitness of a discrete phenotype changes as that phenotype becomes rare or common (Herdegen-Radwan *et al*., 2021; Svensson and Connallon, 2019).

4.5.2 Compound selection

By incorporating both density-dependence and frequency-dependence, compound selection overcomes the issues associated with modelling frequency-dependence alone. This mode of selection addresses many limitations present in other selection modes. It is more efficient than hard and density-dependent hard selection, thereby avoiding the inevitable buildup of genetic load that can undermine population viability. However, it is not as efficient as soft selection, meaning it avoids the unrealistic scenario of purging an excessive amount of genetic load. In compound selection, population size is a dependent outcome rather than a fixed one as in soft selection, again making it more realistic. The number of survivors under compound selection does not tend to exceed k, eliminating the need to randomly choose survivors. While density-dependent hard selection also prevents this, it is very inefficient, which severely

compromises population viability due to a continuous decline in population size coupled with an increase in genetic load. Under compound selection, a smaller number of selected survivors is typically associated with a greater increase in mean fitness, as there is a relatively high number of selective deaths among less fit individuals. This leads to improved population viability.

However, the enhanced selection under compound selection presents a significant drawback. As shown in Fig 4.4, when genetic load starts to increase (e.g., because of an increase in mutation rate), it can lead to sudden extinction, even with only a slight increase in genetic load and a modest population decline. Surprisingly, this effect is more pronounced in r-strategists. Although selection efficiency is higher among r-strategists—similar to soft selection—they are less capable of tolerating high levels of genetic load compared to under hard selection. This vulnerability arises because, while rescaling by w_max/w, the presence of a very fit individual can adversely impact the survival of the rest of the population. Such exceptionally fit individuals are more likely to occur among r-strategists due to their larger number of offspring.

Compound selection combines efficient relative competition with absolute mortality, so strong reproductive skew can have demographic costs. The Isle Royale wolf population should be treated only as a cautious analogy: it was already very small and severely inbred, and disease and demographic stochasticity also contributed to its decline before and after the arrival of a migrant male (Hervey *et al*., 2017; Orning *et al*., 2020). The case therefore does not demonstrate compound selection, but illustrates that extreme reproductive skew can reduce effective population size. The alternative formulation using an adjustable parameter α was developed after examining the original model and is therefore presented as a refinement rather than retrofitted into the primary analysis. Testing α is an important sensitivity analysis, and the main conclusions are restricted to the implemented formulation.

4.5.3 Neutral diversity as an indicator of population fitness

Neutral nucleotide diversity has important but limited value in conservation. It records demographic history and contributes to inbreeding and adaptive potential, but it is not a direct measure of realised load or current viability. Functional prediction also has errors, including false-positive and false-negative classifications, incomplete regulatory annotation, transfer across species, and uncertain mapping from scores to *s* and *h*. Empirical calibration against

fitness is therefore required. The simulations in this chapter suggest that depending on the mode of selection, neutral nucleotide diversity may be affected by changes in the strength of selection during population contractions. If selection is partially soft, as in compound selection where it is both density- and frequency-dependent, it will be influenced by fluctuations in population size and the relative fitness of individuals within the population. Thus, it is likely to have a more pronounced impact on population dynamics compared to strict hard selection.

Changes in selection efficiency could consequently alter the composition and size of future generations, potentially exacerbating perturbations in natural populations through a feedback relationship with selection. Therefore, understanding the degree to which selection is hard or soft is crucial. Monitoring declining populations for consistent loss of whole-genome nucleotide diversity, correlating with the expected reduction in segregating sites, could indicate primarily hard selection. Conversely, if whole-genome nucleotide diversity remains uncoupled from the loss of segregating sites, it may suggest that selection is at least partially soft. However, this decoupling might only occur if population contraction results from an unsustainable buildup of genetic load rather than environmental changes reducing carrying capacity. Furthermore, the type of selection will have implications on Tajima's D, and this is a subject that requires further investigations.

4.6 Conclusion

While acknowledging the limitations of compound selection as modelled here, I argue that it more accurately reflects the dynamics of selection in natural populations. It is likely that the relative fitness of conspecifics within a population and the population size relative to the carrying capacity of the environment both influence the survival probability of individuals. Compound selection encapsulates this mechanism in a way that allows for realistic population dynamics. Consequently, it warrants further exploration as a mode of selection in future research.

5 Traits that enhance soft selection in K-strategists

5.1 Introduction

These simulations operationally represent r- and K-strategists by fecundity, but extinction risk covaries with many other traits, including generation time, adult survival, body size, trophic level, mating system and parental investment (Pearson *et al*., 2014). Broad taxonomic contrasts should therefore not be interpreted as a universal causal effect of fecundity. Given that low fecundity is a trait generally associated with high extinction risk, it remains unclear how K-strategists have managed to persist over long evolutionary timescales. One possible explanation is that low fecundity covaries with other traits that enhance selection, thus ensuring their survival over r-strategists. One such trait could be their ability to reproduce across multiple generations. K-strategists, often being larger and longer-lived than r-strategists, may benefit from this longevity. This allows relatively fit individual K-strategists to contribute to the gene pool over several generations, whereas relatively fit r-strategists contribute only within a single generation. This greater reproductive contribution by fit individuals across generations may lead to more efficient selection, particularly if the selection mode is predominantly soft.

Another potential trait is parental investment. K-strategists, particularly birds and mammals, often produce altricial young that require significant parental care, a strategy less common in r-strategists except for colonial insects like hymenoptera. Although this parental care incurs a cost to the parents, it increases the likelihood of offspring surviving to adulthood, thereby enhancing the recruitment rate of sexually mature individuals. Consequently, while K-strategists produce fewer offspring, there is a greater probability of these offspring reaching sexual maturity. The impact of this on selection may vary depending on whether it is predominantly hard or soft. Under hard selection, increased parental investment might weaken selection and lead to a greater accumulation of genetic load, as unfit individuals are more likely to survive. Conversely, if selection is predominantly soft, competition among adults for resources would lead to less fit individuals being outcompeted by fitter ones, reducing their chances of reproduction and contribution to genetic load. In this case, parental investment would likely enhance selection efficiency by increasing the likelihood that fit individuals survive to adulthood, maintaining robust competition. This dynamic could be mirrored in conservation strategies that

increase the recruitment rate of adults within a population. Thus, the effectiveness of conservation measures aimed at ensuring juvenile survival to adulthood may vary in its impact on genetic load, depending on the prevailing mode of selection.

Aim

To test whether parental investment and overlapping generations can modify selection in low-fecundity populations, these traits were varied under hard, density-dependent hard, soft and compound selection. Conservation investment was examined in parallel.

5.2 Methods

For the models investigating parental investment and generational overlap, populations were constructed using SLiM version 3 (Haller and Messer, 2019) with non-Wright-Fisher (non-WF) modelling. The populations, with an N_e of 500, were subjected to a burn-in period of 10,000 generations under hard selection, during which deleterious mutations were introduced at a rate of $1.17e^{-7}$ to achieve a stable masked load of approximately 1 lethal equivalent. These mutations were sampled from previously modelled stable populations (van Oosterhout *et al*., 2022) with a realistic distribution of selection coefficients (*hs*). Each individual in the populations possessed an exome consisting of 1,000 genes, each 1,000 base pairs in length. Recombination was set to occur between genes at a rate of 0.1 but did not occur within the genes. All populations were hermaphroditic. Mating was random and monogamous during both the burn-in period and the subsequent simulations.

Hard, density-dependent hard, soft and compound selection were modelled as described in Chapter 4. Under hard selection, survival probability equalled genotype-derived fitness *w*. If survivors exceeded *k*, *k* individuals were sampled. Density-dependent hard selection used *k/N*. Compound selection used the chapter's density- and relative-fitness formulation, and soft selection selected the *k* highest ranked individuals.

In the parental investment models, all populations underwent two rounds of selection per generation, with the first round always being hard selection. The extent of parental investment was modelled by adding 0, 0.1, 0.2, 0.3, or 0.4 to the offspring's fitness during this initial

selection stage, simulating the contribution of parental investment to their fitness during development. This addition was removed for the second stage of selection, which could be hard, density-dependent hard, soft, or compound selection. Simulations ran for 10,000 generations.

The conservation investment models followed a similar setup, but the contribution of investment was varied at larger intervals (0, 0.2, 0.4, 0.6, 0.8), representing different conservation strategies such as additional food supplements, extra shelter, or captive breeding programs. These simulations ran for 200 generations to reflect a more realistic timeframe for observing potential benefits. Populations were burned in with a higher mutation rate of $1.5e^{-7}$ to produce a masked load of approximately 2 lethal equivalents, reflecting the higher genetic load expected in vulnerable populations. Both the parental investment models had non-overlapping generations.

For the models investigating the effect of generational overlap, populations underwent one round of either hard, density-dependent hard, soft, or compound selection each generation. Individuals could live for 1, 2, 3, 4, or 5 years, provided they survived selection. In models incorporating parental investment, soft selection, and compound selection, new mutations were introduced at a rate of $1e^{-7}$ throughout. Recombination occurred as it did in the burn-in populations.

5.3 Results

5.3.1 Parental investment

At fecundity 8, all models accumulated load, although soft and compound selection initially purged more efficiently. Their advantage was modest because relatively few offspring competed. Under hard and density-dependent hard selection, lower parental investment strengthened the first round of selection and slowed load accumulation (Figure 5.1).

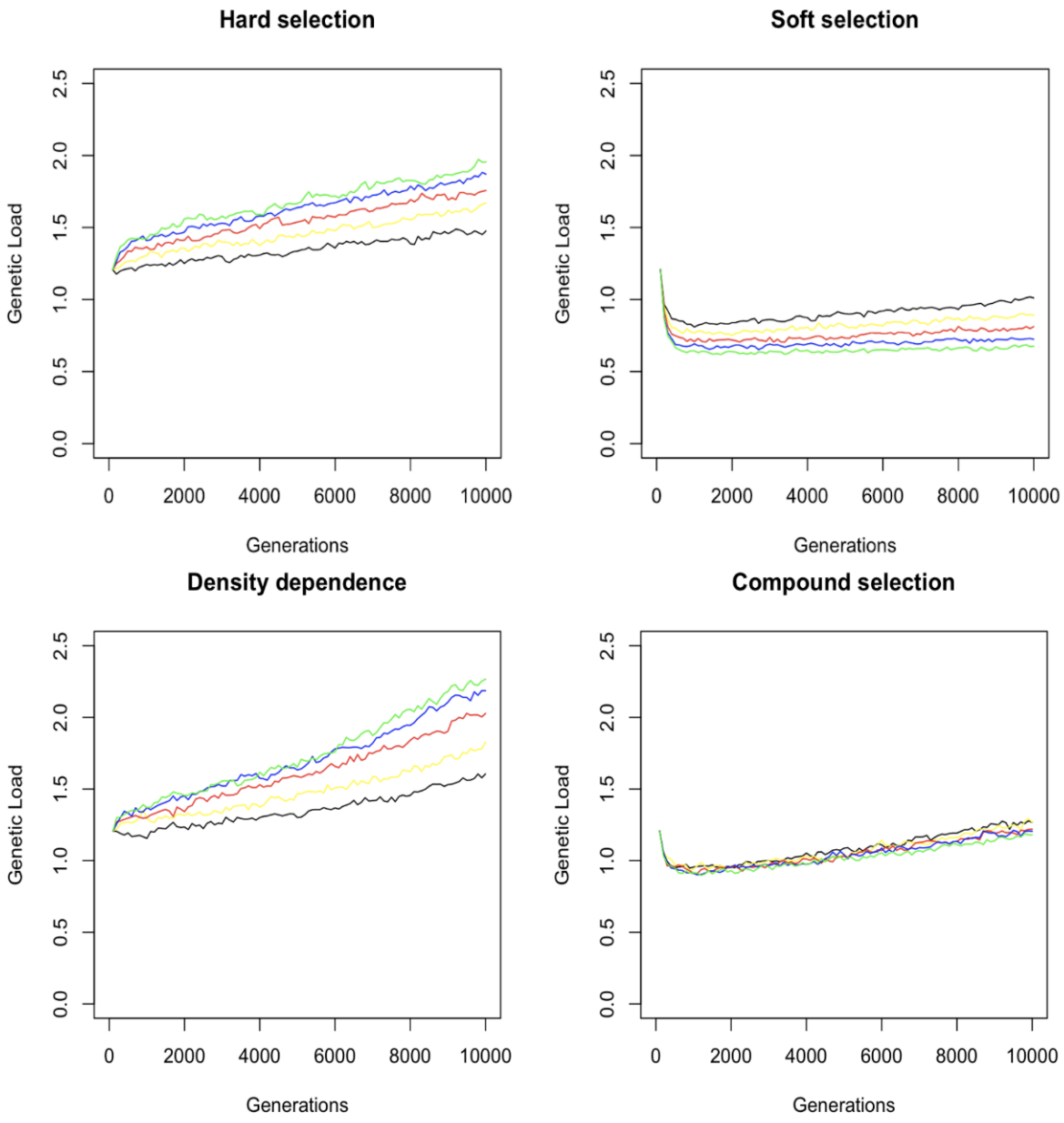


Figure 5.1 - The Effect of Varying Parental Investment on Genetic Load Over 10,000 Generations Under Different Modes of Selection in Populations with a Fecundity of 8.

Each population undergoes one round of hard selection per generation, followed by one round of either hard, soft, density-dependent hard, or compound selection. Parental investment is reflected as an addition to the fitness of offspring during the first round of hard selection, quantified by the parental investment value (PI). The PI value indicates the magnitude of the fitness increase provided by parental investment.

Legend: Black - PI = 0, Yellow - PI = 0.1, Red - PI = 0.2, Blue - PI = 0.3, Green - PI = 0.4

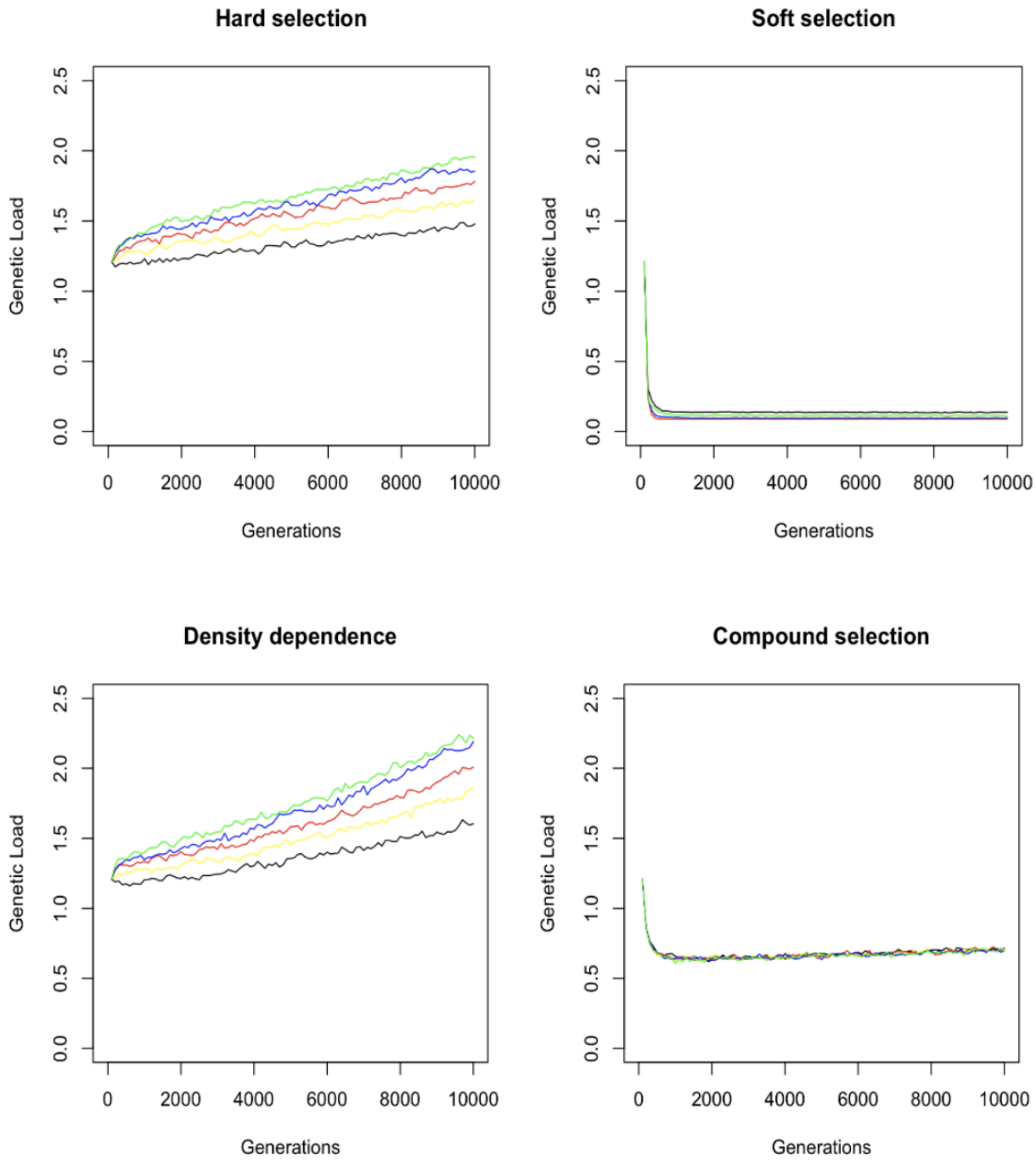


Figure 5.2 - The Effect of Varying Parental Investment on Genetic Load Over 10,000 Generations Under Different Modes of Selection in Populations with a Fecundity of 128.

Each population undergoes one round of hard selection per generation, followed by one round of either hard, soft, density-dependent hard, or compound selection. Parental investment is reflected as an addition to the fitness of offspring during the first round of hard selection,

quantified by the parental investment value (PI). The PI value indicates the magnitude of the fitness increase provided by parental investment.

Legend: Black - PI = 0, Yellow - PI = 0.1, Red - PI = 0.2, Blue - PI = 0.3, Green - PI = 0.4

At fecundity 128, hard and density-dependent hard selection changed little, whereas soft and compound selection purged substantially more efficiently than at low fecundity, especially under pure soft selection. The larger offspring pool intensified relative competition; parental investment consequently had little additional effect under the strongest soft selection (Figure 5.2).

5.3.2 Conservation investment

The results from the conservation investment simulations mirrored those from the parental investment scenarios. Both hard and density-dependent hard selection models benefitted from lower conservation investment due to the stronger initial round of selection, which effectively eliminated less fit individuals (Fig. 5.3 and Fig. 5.4). Varying fecundity did not influence these modes of selection. Under soft selection with a fecundity of 8, greater conservation investment was advantageous as it increased competition by allowing more individuals to survive to the soft selection stage (Fig. 5.3). With a fecundity of 128, there was no significant difference in the elimination of genetic load due to varying conservation investment, except that populations with no conservation investment continued to purge load at a faster rate after other populations' rates began to slow (Fig. 5.4). The reason for this anomaly is unclear. Varying conservation investment did not impact the rate of genetic load purging in with either fecundity, although r-strategists purged load slightly faster overall (Fig. 5.3 and Fig. 5.4).

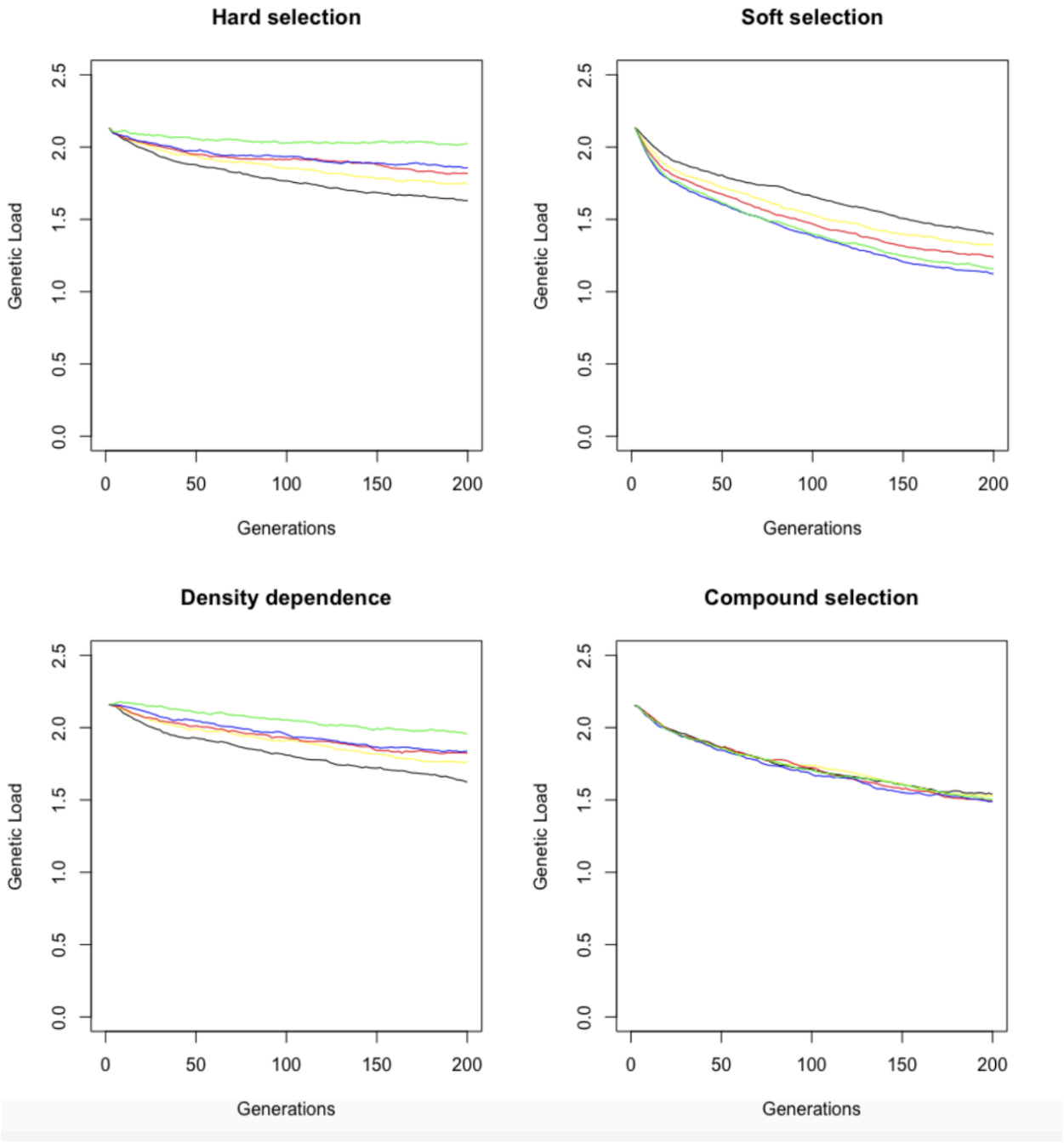


Figure 5.3 - The Effect of Varying Conservation Investment on Genetic Load Over 10,000 Generations Under Different Modes of Selection in Populations with a Fecundity of 8.

Each population undergoes one round of hard selection per generation, followed by one round of either hard, soft, density-dependent hard, or compound selection. Conservation investment is reflected as an addition to the fitness of offspring during the first round of hard selection, quantified by the conservation investment value (CI). The PI value indicates the magnitude of the fitness increase provided by parental investment.
Legend: Black - CI = 0, Yellow - CI = 0.2, Red - CI = 0.4, Blue - CI = 0.6, Green - PI = 0.8

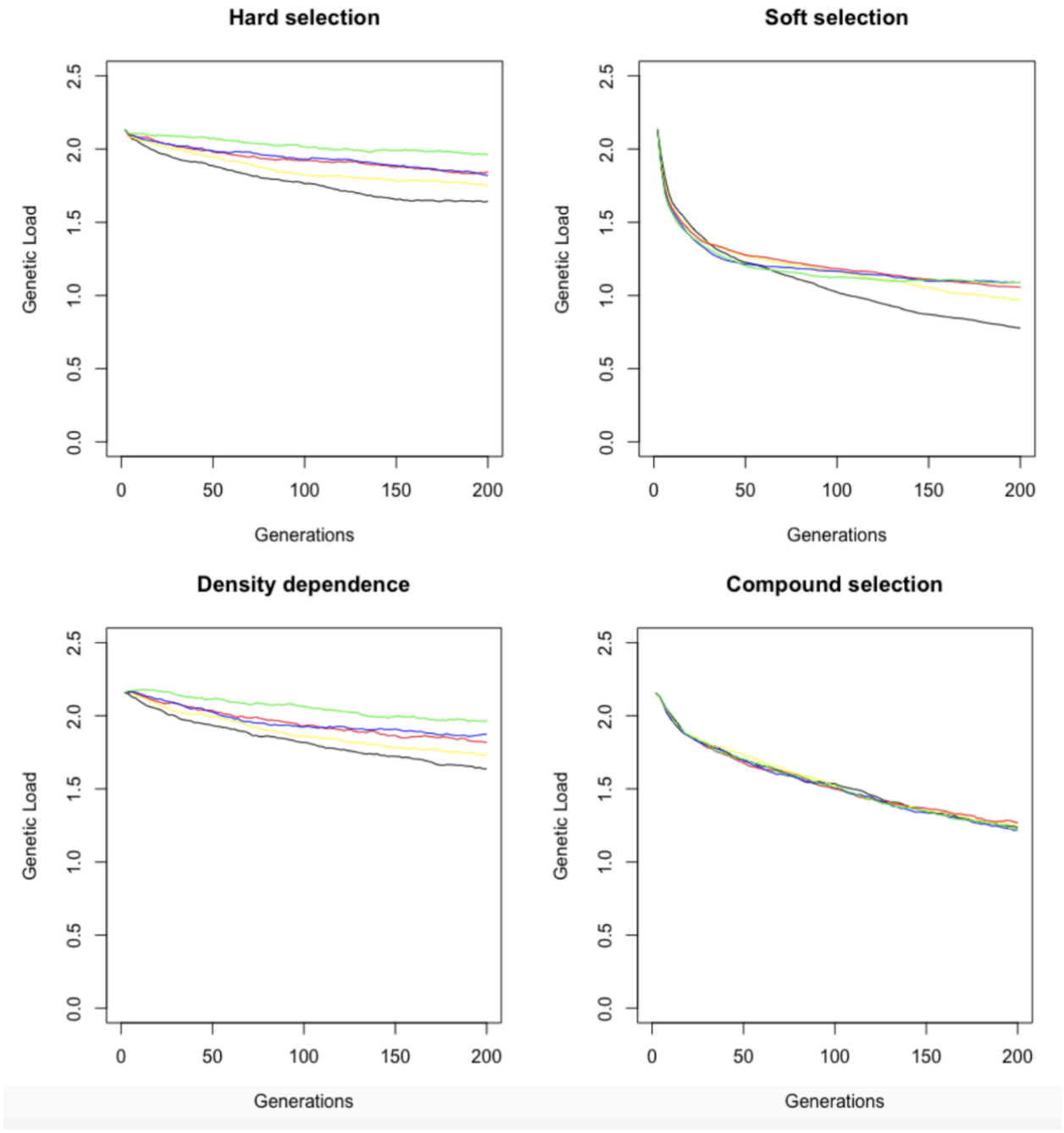


Figure 5.4 - The Effect of Varying Conservation Investment on Genetic Load Over 10,000 Generations Under Different Modes of Selection in Populations with a Fecundity of 128.

Each population undergoes one round of hard selection per generation, followed by one round of either hard, soft, density-dependent hard, or compound selection. Conservation investment is reflected as an addition to the fitness of offspring during the first round of hard selection,

quantified by the conservation investment value (CI). The PI value indicates the magnitude of the fitness increase provided by parental investment.

Legend: Black - CI = 0, Yellow - CI = 0.2, Red - CI = 0.4, Blue - CI = 0.6, Green - PI = 0.8

5.3.3 Generational overlap

In the generation overlap models, the benefit of allowing for more overlapping generations was most pronounced under hard selection. Increasing the number of generations for which individuals could live consistently resulted in a lower rate of accumulated genetic load for both K- and r-strategists (Fig. 5.5 and Fig. 5.6). In populations with a fecundity of 8, under density-dependent hard, compound, and soft selection, varying the number of overlapping generations made no difference except in scenarios where individuals could not live for more than one year in which the rate of accumulation of genetic load was slightly greater (Fig. 5.5). While longer lifespans of fit individuals led to fitter individuals overall, this effect was diminished by density-dependence; only in the density-independent hard selection model did the benefit persist when individuals could live beyond one year. This suggests that incorporating density-dependence into the selection model reduced the likelihood of any individual living beyond one year to such an extent that extending generational overlap had no additional effect. Consequently, varying generation overlap had no significant impact on r-strategists under density-dependent hard, soft, or compound selection. In these scenarios, surviving parents were so outnumbered that their contribution to the next generation was minimal, rendering multiple generation survival of fit individuals ineffective in influencing genetic load accumulation.

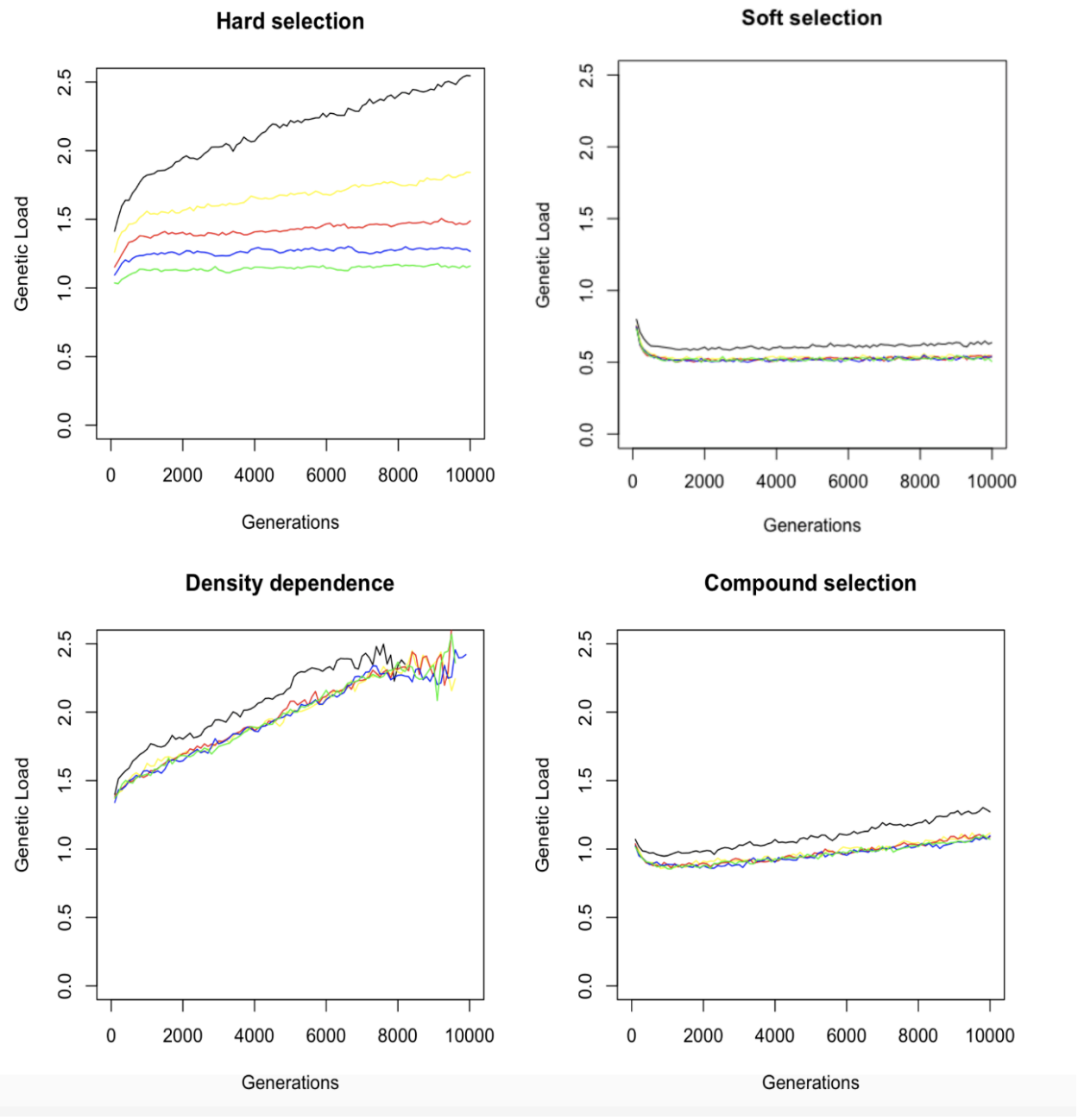


Figure 5.5 - The Effect of Varying Generational Overlap on Genetic Load Over 10,000 Generations Under Different Modes of Selection in Populations with a Fecundity of 8

Populations are subject to one of the following modes of selection: hard, soft, density-dependent hard, or compound selection. The lifespan of individuals, measured in generations, is varied for each mode of selection to study the impact on genetic load.

Legend: Black - 1 generation, Yellow - 2 generations, Red - 3 generations, Blue - 4 generations, Green - 5 generations

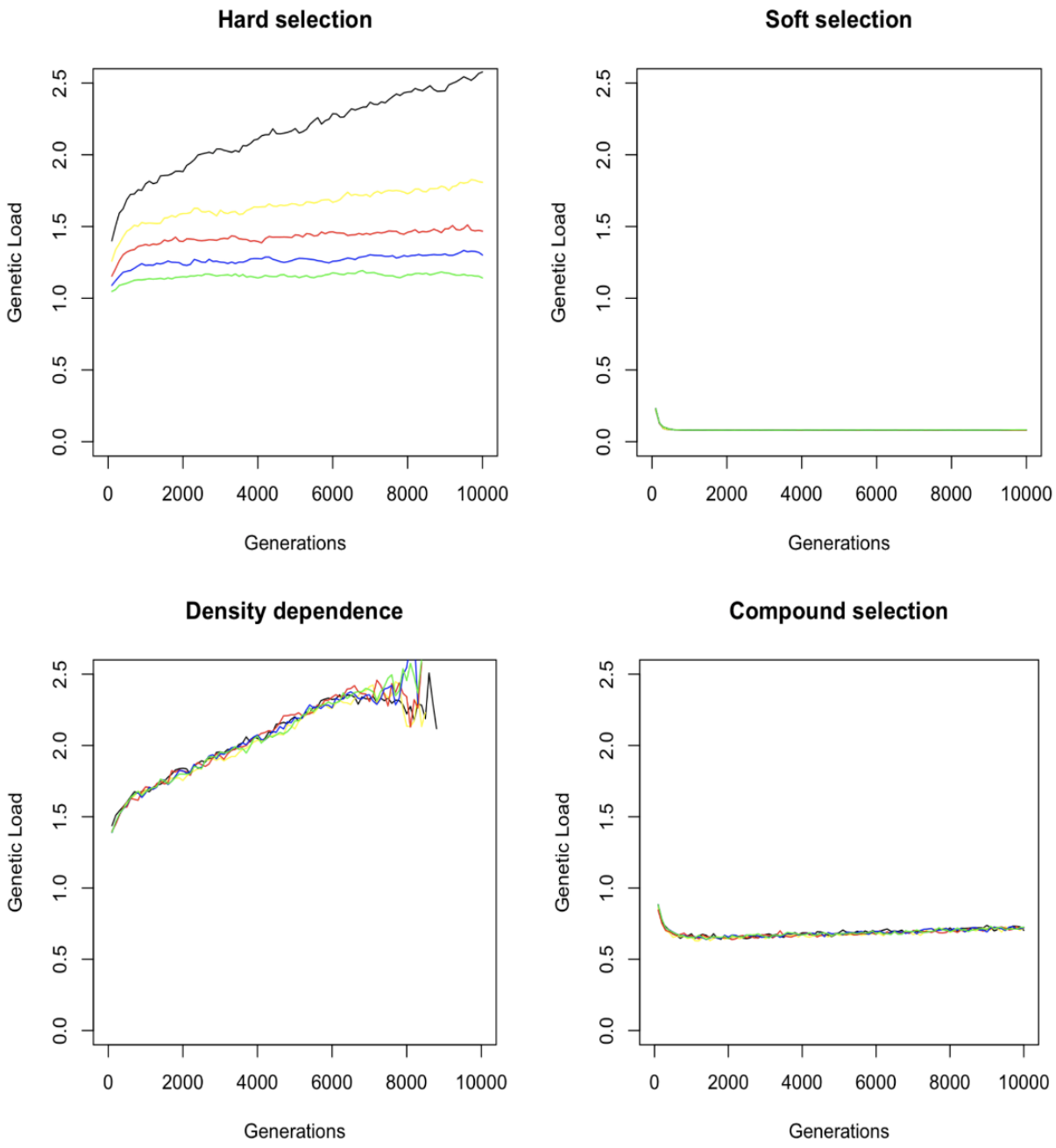


Figure 5.6 - The Effect of Varying Generational Overlap on Genetic Load Over 10,000 Generations Under Different Modes of Selection in Populations with a Fecundity of 128

Populations are subject to one of the following modes of selection: hard, soft, density-dependent hard, or compound selection. The lifespan of individuals, measured in generations, is varied for each mode of selection to study the impact on genetic load.

Legend: Black - 1 generation, Yellow - 2 generations, Red - 3 generations, Blue - 4 generations, Green - 5 generations

5.4 Discussion

These results support the hypothesis that the impact of parental investment varies with the predominant mode of selection. In hard selection models, lower parental investment leads to a reduced rate of genetic load accumulation, as unfit individuals are less likely to survive and contribute to the next generation. Conversely, in soft selection models, lower parental investment results in a higher likelihood of fitter individuals dying prematurely due to luck, thereby reducing competition. This effect is less pronounced in r-strategists, where high competition due to large numbers of offspring diminishes the impact of parental investment. This suggests that in natural populations where soft selection is prominent, enhanced parental investment might serve as an adaptation to maintain genetic load at a manageable level. A limitation of this study is the absence of parental costs in investing in the next generation, which may reduce realism. However, it is plausible that such dynamics would favour fitter parents who can bear these costs and contribute more to their offspring, leading to their overrepresentation in the next generation. This would amplify the benefits of parental investment under all selection modes. This aligns with studies on the evolution of parental investment, which show that small initial differences in investment tend to increase over time due to positive feedback (Fromhage and Jennions, 2016). The burying-beetle experiment tested the immediate consequences of providing or withholding parental care (Pascoal *et al*., 2023). The present simulations likewise do not model the evolution or loss of parental care; that would require heritable variation in care and explicit costs and benefits to parents and offspring.

5.4.1 Mode of selection and conservation management

The results from conservation investment simulations tentatively suggest that certain taxa may benefit more from conservation strategies aimed at improving the recruitment rate of adult individuals compared to those focused solely on increasing population size. For instance, enhancing the quality of bird boxes to better protect hatchlings from predators might be more effective than simply increasing the number of boxes. Such strategies could be particularly advantageous for K-strategists, especially those engaging in competitive behaviours such as territorial fighting or elaborate mating displays. These behaviours indicate a likely prevalence of soft selection, which is also supported by studies showing that sexual selection enhances fitness and reduces genetic load (Grieshop *et al*., 2021; Łukasiewicz, Niśkiewicz, and Radwan, 2020; Lumley *et al*., 2015; Whitlock and Agrawal, 2009). Furthermore, these strategies may

reduce environmental variation, making phenotypic differences, which conspecifics use for competition, more contingent on genetic variation. This reduction in environmental variation can enhance soft selection, as evidenced by studies on heritability discussed in Chapter 3.

These findings should be interpreted cautiously and balanced against the benefits of strategies that increase the effective population size (N_e). As demonstrated in Chapter 3, a larger N_e enhances competition and promotes stronger soft selection. Moreover, maintaining N_e at a level that prevents inbreeding depression is crucial. Additionally, in taxa where there is intense competition for mates, extreme sex ratios can increase demographic stochasticity, and polygyny can accelerate time to extinction (Lee, Sæther, and Engen, 2011). These considerations are vital when developing conservation strategies for such species. However, increasing N_e may only marginally boost offspring numbers, potentially limiting the benefits of enhanced competition. Thus, improving adult recruitment rates to achieve better soft selection gains might be a more effective approach.

5.4.2 Limited impact of varying generational overlap

Varying the number of overlapping generations did not produce significant differences in genetic load, except under hard selection and between allowing individuals to live for one year (no generational overlap) and two years in K-strategists across other selection modes. Although a slight benefit from generational overlap was observed in K-strategists compared to r-strategists, the models did not fully meet expectations that soft selection would favour longevity. The detrimental impact of density-dependence likely inhibited the benefits of generational overlap, and the efficiency of soft and compound selection might lead to generally fitter offspring, making it difficult for parents to compete against their more numerous and potentially fitter offspring.

It is possible that large, long-lived organisms are less impacted by density-dependence, as population density is negatively correlated with animal body mass on a global scale (White *et al*., 2009). Therefore, the hard selection model, which excludes density-dependence, may suggest some advantage to generational overlap. However, this advantage does not seem to confer additional benefits to K-strategists as fecundity is not a factor influencing hard selection. Larger organisms are typically less densely distributed due to their high resource demands. Although the relationship between energy requirements and body mass is complex and varies due to differences in metabolic activity (Wilson *et al*., 2020), larger organisms generally require

more energy. Consequently, while their abundance in a given area may be lower, the carrying capacity is also reduced, reflecting the limitations on available energy and essential nutritional resources. Hence, density-dependence is unlikely to be trivial.

These simulations could be enhanced by incorporating age structuring. This would involve simulating individuals as less fit during their early generations, representing the lower fitness of juveniles compared to adults, achieving peak fitness upon maturity, and potentially declining in fitness with age to represent senescence. This approach aligns with observations in long-lived taxa, where mature adults typically exhibit the highest fitness. It is hypothesised that long-lived species show low variation in adult survival because adult survival is more crucial to their overall fitness than in species with shorter generation times (Péron *et al*., 2016; Warret Rodrigues, Angin, and Besnard, 2021). This phenomenon can be explained by the canalization hypothesis. According to this hypothesis, traits of significant importance are buffered against environmental or genetic disturbances to ensure a stable phenotype. This buffering can be achieved through mechanisms such as genetic redundancy, where multiple genes produce the same phenotype, allowing one gene to compensate if another mutates, and through complex regulatory pathways with feedback loops that correct deviations (Flatt, 2005).

The first evidence supporting the canalization hypothesis came from capture-recapture studies on mammals and birds (Péron *et al*., 2016). These studies demonstrated lower individual heterogeneity in adult survival among taxa with long generation times (more than three years) compared to those with short generation times. The canalization hypothesis has also been applied to conservation management, as seen in the long-lived Lesser Antillean iguana, *Iguana delicatissima* (Warret Rodrigues, Angin, and Besnard, 2021). This species exhibited a 4% annual decline over an eight-year period, with little variation in adult survival, indicating that conservation efforts focused on adult survival may be ineffective. However, significant variation in recruitment rates suggested that eggs and juveniles were more vulnerable to demographic disturbances. The researchers estimated that conservation strategies such as protecting eggs and juveniles from predators could help arrest the population decline. Therefore, modelling populations with enhanced adult fitness to reflect canalised adult survival may provide more accurate insights into the effects of longevity for both K-strategists and r-strategists under different selection modes.

5.4.3 Potential vulnerability of r-strategists

Although simulations in this thesis and most evidence from natural populations suggest that K-strategists are more susceptible to extinction, the susceptibility of r-strategists to extinction should also be considered. Insect populations have experienced global declines due to various anthropogenic effects, such as climate change (Harvey *et al*., 2023), pesticide use (Tosi *et al*., 2022), and urban car use, which harms terrestrial insect populations (Fenoglio *et al*., 2021). Assessing the extent of insect decline is challenging because insects are less frequently documented than more charismatic taxa, such as birds. For instance, out of 44,000 known bird species, 1,500 extinctions have been recorded, compared to only 70 known insect extinctions (Chowdhury *et al.*, 2023). Despite being less studied, insects play crucial ecological roles, with over 80% of flowering plants dependent on them for pollination (Wagner *et al*., 2021). Another ecologically and commercially important group of r-strategists is corals. A 50% loss of global coral coverage was recorded between 1957 and 2007, primarily due to ocean acidification and climate change (Eddy *et al*., 2021). The severe population contractions experienced by corals and insects demonstrate that r-strategists, too, are susceptible to extinction.

The results in this thesis indicate that increased fecundity enhances soft selection, suggesting that producing large numbers of offspring is essential for r-strategists to manage genetic load. Furthermore, r-strategists often exist in larger populations with higher effective population sizes (N_e). As shown in Chapter 3, larger N_e can lead to stronger soft selection. Although larger N_e populations typically harbour more genetic load, if soft selection is occurring, it helps suppress the genetic load from being higher than it might otherwise be. In the face of substantial demographic perturbations, the increased inefficiency of soft selection due to the dual effects of small N_e and reduced offspring numbers could be more pronounced in r-strategists, leading to an increase in genetic load. K-strategists may be partially protected against such perturbations by adaptations like parental investment. In other words, r-strategists may be dependent on their large numbers to suppress genetic load through soft selection Therefore, relative population contractions may result in more severe drift-debt in r-strategists than in K-strategists. Effective conservation management should be grounded in a thorough understanding of the ecology and biology of the threatened taxa, as well as the environmental factors contributing to their decline. Recognizing the differences in how r- and K-strategists are

adapted to suppress genetic load can lead to the application of more targeted conservation strategies that improve fitness, thereby enhancing the success of conservation efforts.

5.5 Conclusion

Reproductive strategy is already central to conservation management. The additional implication of these simulations is that mode of selection may alter how parental or conservation investment affects competition and purging. This inference remains conditional on how closely the modelled selection modes represent natural populations.

6 Optimisation of the breeding regimes of captive populations

6.1 Introduction

Captive breeding of endangered animals represents a potential strategy to mitigate the extinction risk of many different taxa . This approach involves the maintenance and breeding of endangered species in controlled environments such as zoos, aquariums, and specialised facilities. Its primary goal is to bolster populations that face severe threats in the wild, thereby preventing their extinction and promoting their eventual recovery.

One of the key reasons captive breeding is essential is its role in population recovery. Many endangered species have experienced significant declines in their wild populations due to factors such as habitat loss, poaching, climate change, and disease. These populations often become fragmented and genetically isolated, leading to reduced reproductive success and increased vulnerability to extinction. Captive breeding programs provide a critical lifeline by increasing the overall population size beyond what wild populations can sustain alone. For instance, species like the Arabian oryx, *Oryx leucoryx*, and the California condor, *Gymnogyps californianus*, have been successfully bred in captivity to the point where they could be reintroduced into their natural habitats, bolstering wild populations and reducing the risk of extinction (Seddon *et al*., 2014; Snyder *et al*., 1996).

Genetic diversity can also be preserved through the application of captive breeding programs. Small population sizes in the wild often result in genetic bottlenecks, where individuals are closely related and genetic diversity is limited. This genetic uniformity increases the susceptibility of populations to diseases and reduces their ability to adapt to changing environmental conditions. Captive breeding helps mitigate these risks by maintaining and increasing genetic diversity within captive populations. By carefully managing breeding pairs and minimising inbreeding, conservationists can preserve the species' genetic health and resilience (Frankham, 2008). This genetic management is vital for the long-term viability of species in both captivity and, eventually, in the wild through reintroduction efforts.

Captive breeding programs may also serve as valuable platforms for scientific research and education. Zoological institutions not only provide a safe haven for endangered species but also facilitate research on their biology, behaviour, reproductive physiology, and genetics. These studies yield critical insights into the species' ecological needs and conservation requirements, which inform management strategies both within captivity and in their natural habitats. For example, studies on the reproductive biology of endangered species like the black-footed ferret, *Mustela nigripes*, have helped refine breeding techniques and improve the success of captive breeding programs (Santymire *et al*., 2014).

The success of captive breeding programs is often measured by their ability to reintroduce captive-bred individuals back into their natural environments. Reintroduction programs aim to restore self-sustaining populations in areas where threats have been mitigated or habitats restored. Successful reintroductions require careful planning, including habitat assessments, predator control, and monitoring of released individuals. For instance, the reintroduction of the Red Wolf, *Canis rufus*, in the southeastern United States has relied on captive breeding to supplement wild populations and expand their range, contributing to the species' recovery despite ongoing challenges (Phillips, Henry and Kelly., 2003). However, inbreeding, hybridisation with coyotes and human activities continue to hamper its recovery (Hinton, Chamberlain, and Rabon, 2013). Other captive breeding programs have also shown mixed results. An 11-year captive breeding program of the endangered orange-bellied parrot, *Neophema chrysogaster*, found that after the number of eggs laid declined after one year post breeding although the number of surviving fledglings increased after five years (Bussolini *et al*., 2023).

Captive-bred individuals can be less fit after release for several reasons. The cited comparative evidence found that translocated individuals were often outperformed by residents (Gross, Wilson, and Wolak, 2024), but available pedigree data do not exclude inbreeding in every case. Other causes include adaptation to captivity, relaxed or altered selection, drift, husbandry, disease, maternal effects and genotype-by-environment interactions.

Conservation genomics could prioritise variants using evolutionary conservation, variant-effect annotation, known disease alleles, genomic prediction and empirical genotype-fitness associations. These tools do not identify selection coefficients without calibration, but they may help breeding programmes avoid combinations predicted to increase realised load.

6.2 Methods

The aim of the investigation was to determine whether selecting mating pairs to minimise realised load could lead to fitter populations with lower genetic load in simulated captive breeding scenarios. The impact of reducing population relatedness by ensuring a more diverse representation of offspring in the next generation was also assessed, both independently and in conjunction with minimising realised load.

To assess the effectiveness of various mate selection regimes concerning genetic load and nucleotide diversity, four sets of modified non-Wright-Fisher simulations using SLiM 3.0 were conducted (Haller and Messer, 2019). For the simulations, a two-stage "burn-in" process was implemented. Initially, populations evolved without mutations for 140,000 generations to ensure coalescence. Each population comprised separate sexes, with 1,000 individuals of each sex engaging in monogamous mating, producing exactly 64 offspring per pair. Generations were non-overlapping. The genome used in the simulations was a 15 Mb single chromosome with a uniform recombination rate (ρ) of $4e^{-8}$, based on chromosome 14 of the chicken genome (Elferink *et al*., 2010). Subsequently, neutral mutations were overlain on the coalesced populations at a rate of $1.8e^{-7}$ to verify the attainment of nucleotide diversity equilibrium at approximately $4N_e\mu$.

In the second stage of the burn-in process, deleterious mutations at a rate of μ $2e^{-8}$ were introduced. This μ rate was carefully chosen to maintain a stable level of genetic load without causing mutational meltdown. From a pool of 3,070 previously sampled mutations in stable populations, we randomly selected 1,000 deleterious mutations. The selection from stable populations aimed to maintain consistency with the genetic profile of viable populations (van Oosterhout *et al.*, 2022). This second stage ran for 10,000 generations to establish a stable level of genetic load within the population, resulting in a final mean genetic load of 6.6, of which 1.1 was realised load.

For the experimental comparison, each of 20 monogamous pairs produced exactly 64 offspring per generation. This controlled assumption removes stochastic fecundity differences so that the effect of mate choice can be isolated; it is not intended to represent natural reproduction.

In the “Null model,” a male and female were randomly selected as a mating pair. They were rejected if they shared a grandparent. If they were unrelated, they were chosen as a mating pair for producing the next generation. If they were related, a different unrelated male was selected until an unrelated mating pair was found. This process was repeated to select 20 pairs.

In the "Minimise relatedness" regime, one male and one female were randomly selected from each brood and subsequently paired to guarantee that all chosen males and females mated only once with unrelated partners.

In the "Minimise load" regime, a Mendelian segregation was performed on every mutation for every possible unrelated male and female pair to predict the realised load in their offspring. The 20 pairs with the lowest predicted realised load were selected as parents, without the same individual being picked more than once.

In the "Minimise load and relatedness" regime, the predicted realised load was calculated for each possible unrelated female-male pair. The best 13 pairs were selected without more than one female or male being selected from the same brood. For the remaining 7 matching pairs, broods from which females had not been selected were paired with broods from which males had not been selected, ensuring that one male and one female from each brood would mate. Then the male and female with the least predicted load were chosen from each matched brood pair.

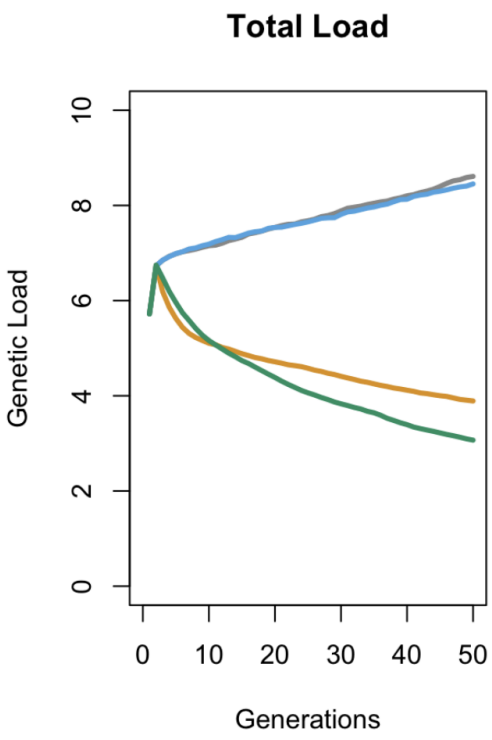
All four mating regimes used the same μ and ρ values as the burn-in models and ran for 50 generations. Genetic load, realised load, and fitness were measured every generation. Tree-sequence data was output every generation and used to calculate π by overlaying neutral mutations at a μ of $2e^{-8}$.

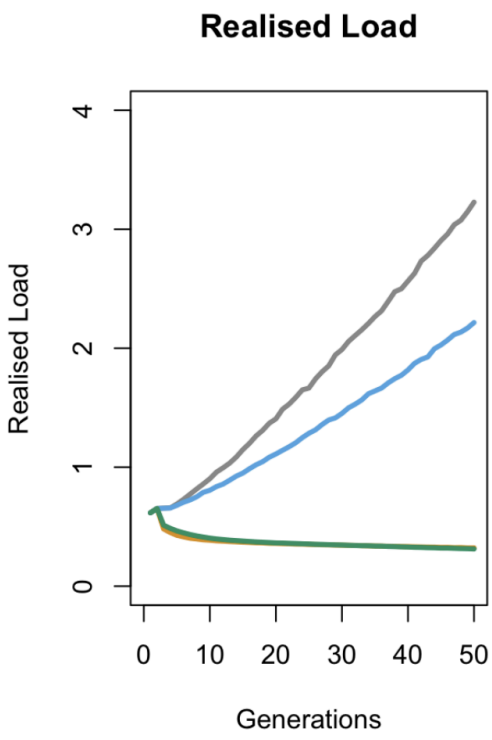
6.3 Results

All 100 replicates for each regime successfully completed 50 generations, with the exception of three replicates from the "Minimise load" regime. This was due to the selected

mating pairs originating from too few different broods, leading to insufficient unrelated offspring for the next generation.

The "Null model" and "Minimise relatedness" regimes showed a steady increase in genetic load over time (Figure 6.1). In contrast, the "Minimise load" and "Minimise load and relatedness" regimes reduced genetic load by selecting pairs with the lowest predicted realised load. The combined regime produced the steepest decline.


Total Load
Genetic Load
0
2
4
6
8
10
0
10
20
30
40
50
Generations


Realised Load
Realised Load
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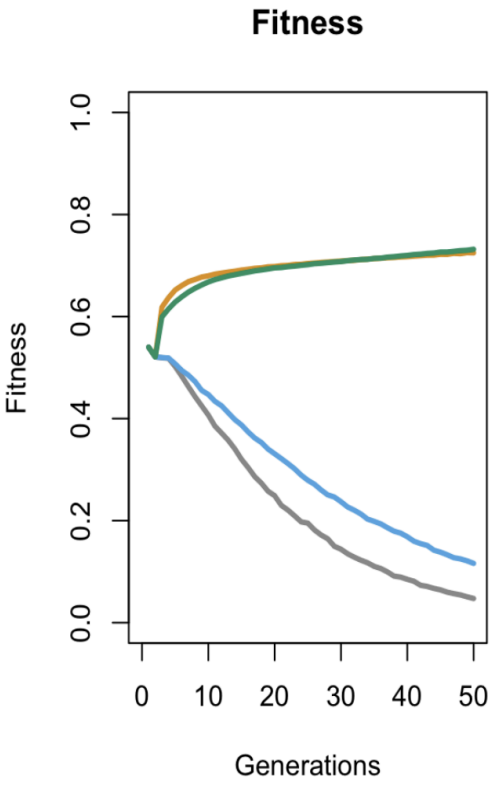
Generations


Fitness
Fitness
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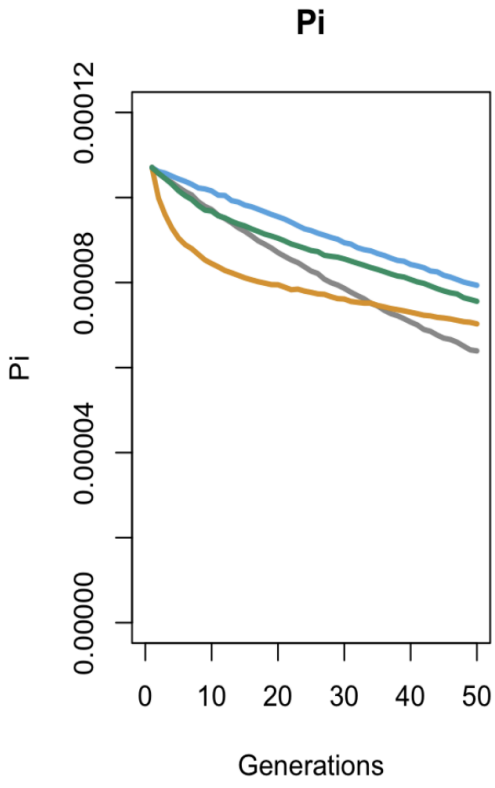
Generations


Pi
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0
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20
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Generations

Figure 6.1 - Impact of the Four Mating Regimes on Genetic Load, Realised Load, Fitness, and Neutral Nucleotide Diversity (π). Each coloured line corresponds to a specific mating regime: "Null model" (grey), "Minimise relatedness" (blue), "Minimise load" (orange), and "Minimise load and relatedness" (green). The values presented in the figure represent the mean results obtained from 100 replicates, with the exception of the "Minimise load" regime, where 3 replicates did not complete 50 generations.

Realised load rose in the "Null model" and, more slowly, in the "Minimise relatedness" regime (Figure 6.1). The "Minimise load" and combined regimes initially reduced realised load and then stabilised, with corresponding differences in mean fitness. All regimes lost neutral nucleotide diversity (π) after the bottleneck from the burn-in population to 40 breeding adults. The two regimes that distributed representation among broods retained diversity best, whereas selecting only for low predicted load caused the largest initial loss.

The initial decrease in diversity observed in the "Minimise load" models may account for the less effective continual purging of genetic load compared to the "Minimise load and relatedness" regime. In the "Minimise load" regime, since individuals are selected from the same broods, certain mutations that were not purged can increase in frequency, making it challenging to eliminate them. The "Minimise load" regime then segregates these high-frequency mutations but does not effectively purge them. Conversely, the "Minimise load and relatedness" regime benefits from more diverse mating pairs, allowing mutations to remain at lower frequencies and facilitating their continuous purging.

Overall, the "Minimise load and relatedness" regime outperformed other strategies by effectively purging genetic load while maintaining genetic diversity better than random mating. These findings support the use of a combined approach, minimising predicted realised load along with minimising relatedness, to achieve the best outcomes for captive breeding populations.

6.3 Discussion

The "Minimise load" regime closely resembles a model of soft selection, similar to those employed in other chapters, where the fittest k individuals are chosen to parent the next generation. Initially, this regime proved highly effective in purging genetic load, due to its focus

on selecting the fittest individuals. However, its efficiency diminished over time, likely because it selected many related individuals, thereby increasing the frequency of shared deleterious variants. It was less adept at eliminating these intermediate frequency mutations, resulting in a slower rate of purging genetic load as the regime progressed. Towards the later stages, mating pairs were likely chosen to avoid homozygosity in offspring rather than solely based on fitness, leading to selection from a more diverse set of individuals. Additionally, populations under this regime experienced the most rapid loss of neutral nucleotide diversity early in the simulations, a probable outcome of selecting from a relatively limited number of broods. As selection expanded to more diverse broods, the decline in neutral diversity decelerated.

The “Minimise load and relatedness” regime shares features with other soft selection models, yet it was designed to enhance both fitness and genetic diversity, leading it to outperform the other regimes overall. This approach offers two key advantages over natural selection. First, selection focuses on offspring rather than parents. In natural populations, even the fittest parents might carry recessive mutations that compromise the fitness of their offspring. This risk can be mitigated in controlled breeding. Second, this regime considers the fitness of the entire population rather than just individual fitness. Mating pairs are selected based on how their offspring will contribute to the overall fitness of the population, rather than merely their own individual fitness. This approach contrasts with the “Minimise load” regime, which simply selects the fittest individuals without considering relatedness. By balancing fitness with diversity, the “Minimise load and relatedness” regime ensures a healthier population over the long term.

An AI-supported breeding model could combine genomic variants and haplotypes, pedigrees or realised relatedness, reproductive and survival records, demography, recombination, environmental covariates and estimated distributions of s and h. Predictions should be validated against withheld generations or independent populations. In practice, uncertainty in variant effects remains a major constraint.

Combined Annotation Dependent Depletion (CADD) integrates multiple annotations into a PHRED-scaled prioritisation score for variants (Kircher *et al*., 2014). It is not a direct selection coefficient. Scores such as 10 and 20 correspond approximately to the top 10% and 1% of predicted deleterious variants in the reference set.

Human-trained CADD scores cannot be assumed to transfer directly to macaws. Conservation applications should use species-specific annotation where possible, comparative conservation metrics and empirical calibration against avian or species-specific fitness data.

6.4 Conclusion

The results of these simulations provide tentative evidence that it is possible to improve captive breeding by developing regimes that reduce genetic load in addition to maximising diversity. This provides scope for producing fitter captive animals which may improve outcomes of reintroduction to preserve endangered species.

7 Modelling genetic rescue of the endangered Arabian leopard

7.1 Introduction

In addition to utilising functional genomics and in silico simulations to optimise breeding regimes in captive populations as demonstrated in Chapter 6, these methods can also guide best practices in genetic rescue. Genetic rescue is a promising yet debated strategy aimed at mitigating the negative effects of inbreeding depression and genetic drift in endangered populations. This approach involves introducing new genetic material into populations suffering from reduced genetic diversity, with the goal of enhancing genetic health and overall fitness. While it has gained attention due to escalating threats to biodiversity, it also raises debates among conservationists due to its complexities and potential risks.

The introduction of novel alleles can counteract the detrimental effects of inbreeding depression, which occurs when closely related individuals mate and produce offspring with reduced fitness due to the expression of deleterious recessive alleles (Hedrick & Fredrickson, 2010). By increasing genetic diversity, genetic rescue can improve a population's ability to adapt to environmental changes and resist diseases, thus enhancing its survival prospects. Historically, the concept is based on the understanding that isolated populations, especially those with small effective population sizes, accumulate harmful genetic variants over time. These variants can become more prevalent due to genetic drift, a stochastic process more pronounced in small populations (Frankham, 2015). Additionally, if the population has contracted from a historically larger one that had contained harmful variants at low frequency, it may suffer from “drift-debt” as these rare deleterious variants become common (Gilroy *et al*., 2017). Genetic rescue introduces genetic variation that can mask these deleterious alleles through heterosis or hybrid vigour, thus enhancing individual and population fitness (Bell *et al*., 2019).

7.1.1 The challenges of genetic rescue

Several challenges must be addressed for genetic rescue to be effective. One major challenge is identifying suitable donor populations. Genetic compatibility is crucial to ensure that introduced individuals can successfully reproduce with the target population and that offspring benefit from hybrid vigour rather than suffer from outbreeding depression. Outbreeding

depression occurs when individuals from genetically distant populations mate, potentially disrupting locally adapted gene complexes and leading to reduced fitness (Tallmon *et al.*, 2004). While the goal of genetic rescue is to enhance genetic diversity, introducing genes from distantly related populations can sometimes result in outbreeding depression. This phenomenon can occur when offspring of such crosses display lower fitness due to the breakdown of coadapted gene complexes or maladaptation to the local environment (Frankham *et al*., 2011). Thus, the risk of outbreeding depression necessitates careful consideration of the genetic and ecological compatibility of donor and recipient populations.

Concerns also exist regarding the potential for genetic interventions to cause unintended ecological consequences or to alter the evolutionary trajectory of the target population in undesirable ways (Weeks *et al*., 2017). Introducing individuals from other populations can potentially disrupt local ecological interactions, such as predator-prey dynamics, competition, or symbiotic relationships. Practical challenges in implementing genetic rescue include logistics of capturing and translocating individuals, monitoring the success of the intervention, and managing the genetic health of both donor and recipient populations. Additionally, financial constraints are significant, as genetic rescue can be expensive, requiring substantial investment in research, monitoring, and management efforts (Fischer & Lindenmayer, 2000).

One major point of contention is the predictability of genetic rescue outcomes. While some researchers advocate for its broad application based on successful case studies, others caution against its use without comprehensive understanding and monitoring (Tallmon *et al*., 2004). Critics argue that the success of genetic rescue is not guaranteed and that the risks of outbreeding depression or ecological disruptions might outweigh the potential benefits (Frankham *et al*., 2011). There is also debate over the appropriate scale and scope of genetic rescue. Some experts argue for its use in critically endangered populations where immediate intervention is necessary to prevent extinction (Hedrick and Fredrickson, 2010). Others suggest a more cautious approach, recommending its use only when the genetic and ecological contexts are well understood, and where less invasive conservation strategies have proven insufficient (Ralls *et al*., 2020). The long-term implications of genetic rescue remain a subject of debate. Proponents highlight the potential for enhancing population resilience and adaptive potential, while opponents warn about the possible negative consequences for evolutionary processes and local adaptations (Weeks *et al*., 2017). There is concern that reliance on genetic rescue might overshadow efforts to address underlying causes of population decline, such as habitat destruction or climate change.

7.1.2 Genetic rescue in practice

The Florida panther, a regional population of Puma concolor, is a frequently cited genetic-rescue case. By the early 1990s, approximately 30 animals remained and showed severe inbreeding-associated abnormalities. Eight female Texas cougars, also Puma concolor, were introduced in 1995, after which genetic diversity and several fitness measures improved (Johnson *et al.*, 2010; Pimm *et al*., 2006). The term Florida panther is used below for the recipient population.

Other examples of success include the mountain pygmy possum *Burramys parvus* (Weeks *et al*., 2017), and the arctic fox *Vulpes lagopus* (Hasselgren *et al*., 2018). These cases highlight the benefits of genetic rescue in boosting genetic diversity and population health. However, not all genetic rescue efforts yield lasting success. A case in point is the small, inbred Illinois population of the greater prairie chicken, *Tympanuchus cupido pinnatus*. Initially, this population was thought to have been successfully rescued in the 1990s after the introduction of genetically diverse individuals from Kansas, leading to a short-term increase in genetic diversity (Mussmann *et al*., 2017). Unfortunately, this initial success was undermined by inadequate habitat conservation, resulting in a population decline and loss of genetic diversity two decades later. These examples illustrate the complexities and challenges of genetic rescue. While it can provide immediate benefits in terms of genetic diversity and fitness, the long-term success of such interventions depends heavily on addressing the underlying habitat issues and ensuring sustainable management practices.

7.1.3 Modelling genetic rescue in the Arabian leopard

The Arabian leopard case provides a relevant captive-to-wild supplementation scenario because the remaining wild population is very small and isolated, while a managed captive population exists. The simulations represent the available wild and captive numbers and their separation, but demographic and genetic parameters without direct Arabian-leopard estimates are illustrative. The objective is to compare supplementation strategies rather than predict a precise outcome.

7.2 Methods

Aim: To determine the optimum number of Arabian leopards (*Panthera pardus nimr*) introduced from a captive population into a wild population to maximally reduce genetic load.

This was performed using Wright-Fisher models in SLiM 3.0 (Haller and Messer, 2019). Fitness, genetic load (including masked and realised load components), and neutral nucleotide diversity were assessed. The populations consisted of separate sexes in a 1:1 ratio. Their genomes comprised an exome composed of 18 pairs of autosomes, each with 1000 genes of 1500 base pairs. The recombination rate within genes was $1e^{-9}$, and between genes $1e^{-3}$ while it was 0.5 between chromosomes. This exome composition was based on a previous study on sea otters that have undergone population bottlenecks (Beichman *et al*., 2023). The populations were created using a two-stage burn-in process. The first stage was a neutral burn-in, with a mutation rate (μ) of 0, for 990,000 generations. This was performed to ensure the population had coalesced, which was necessary for accurately overlaying neutral mutations.

The second stage of the burn-in was run with μ at a level that produced a masked load that was at an equilibrium of approximately 6. The μ that produced this level of masked load was used throughout the rest of the simulations. The population size for both stages of the burn-in was 21,000, believed to be their ancestral population size. The population underwent a linear decline from 21,000 to 1000 over 2,000 generations, simulating their wild population decline over the past 10,000 years up to the start of the 20th century (1 generation = 5 years).

Sixty-four individuals (32 male, 32 female) were then separated from the wild population to form the captive population. The wild and captive populations were isolated from each other for a further 24 generations from the start of the 20th century to the present day. During this period, the captive population size remained at 64, while the wild population declined further to 110.

There was then a 20-generation period in which the effect of different numbers of individuals introduced from the captive population to the wild population was tested. These were either 0, 2, 4, 6, or 8 individuals (with a 1:1 sex ratio). This simulated different genetic rescue strategies over the next 100 years. The captive population size remained at 64. The size of the wild population was randomly determined each generation from a uniform distribution ranging between 100 and 120.

Data (mean fitness, genetic load including realised and masked load, and neutral nucleotide diversity) were output every 100 generations during the population decline from 10,000 years ago to the start of the 20th century and every generation thereafter. One hundred replicates of each variant of introduced individuals were run to ensure robustness of the results.

7.3 Results

All populations where some individuals were introduced experienced benefits in terms of genetic load, fitness, and realised load compared to those where no individuals were introduced (Fig 7.1). In terms of genetic load, each increase in the number of introduced individuals resulted in slightly reduced genetic load. Consequently, the population with eight introduced individuals exhibited the least genetic load after 20 generations. However, the populations with two introduced individuals displayed the highest fitness and the least realised load.

Regarding these outcomes, while introducing any number of individuals showed a substantial benefit compared to none, every increase in the number of individuals beyond two led to slightly lower fitness values and slightly higher realised load. In terms of neutral nucleotide diversity (π), the populations with two introduced individuals performed the best. Although their neutral nucleotide diversity was significantly higher than those with no individuals introduced, this benefit diminished more sharply with each subsequent increase in the number of introduced individuals. After 20 generations, the population with eight introduced individuals exhibited less diversity than those with none.

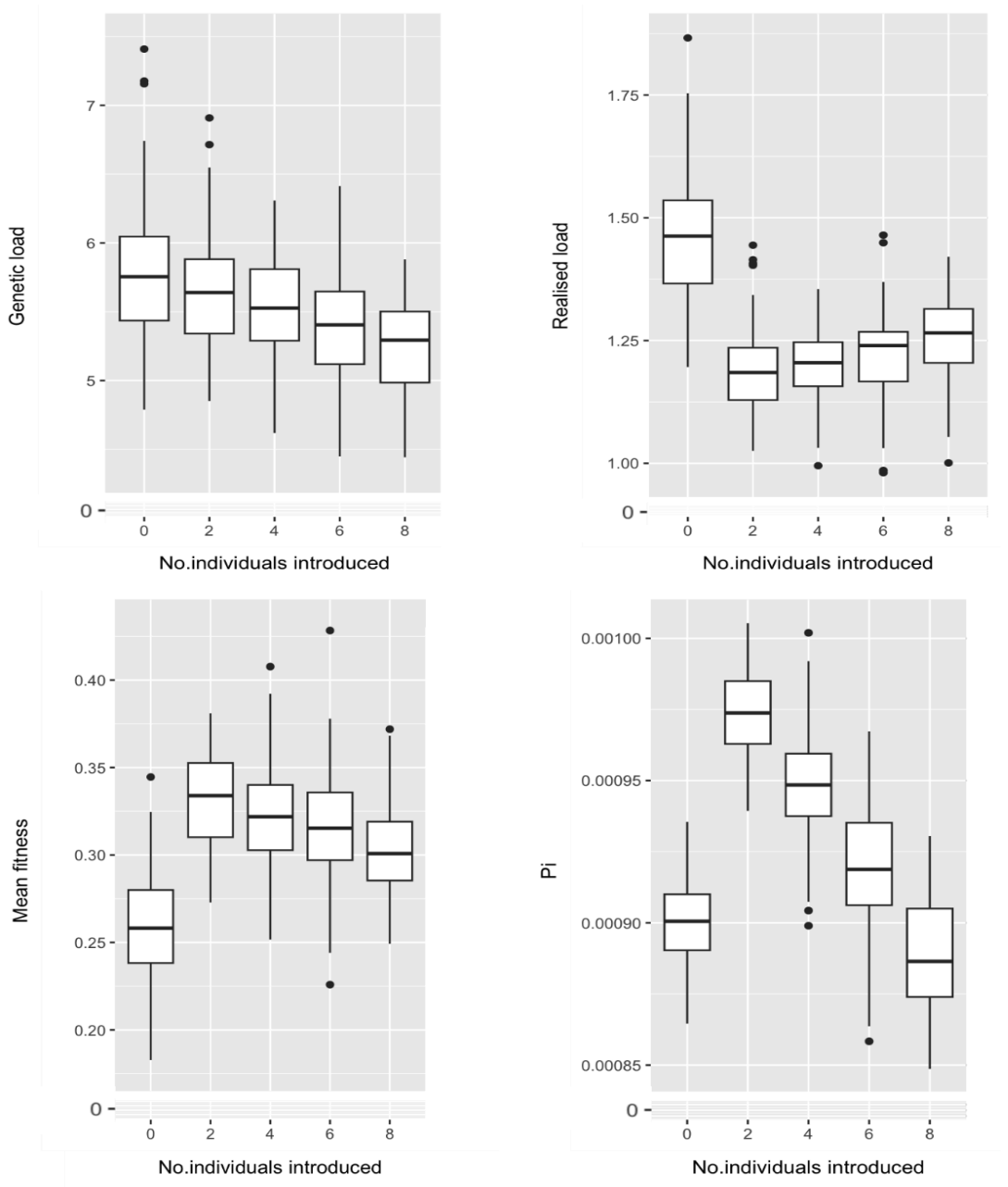

Figure 7.1: The effect of varying the number of captive individuals introduced into simulated wild leopard populations on genetic load, realised load, mean fitness and neutral nucleotide diversity (π) after 20 generations. Either 0, 2, 4, 6, or 8 captive individuals were introduced every generation. Shown are the medians, the interquartile range (boxes), the range (whiskers), and the outliers.

7.4 Discussion

The results highlight the critical importance of developing a well-considered strategy, as even a relatively small increase in the number of introduced individuals significantly altered the outcomes. All populations into which individuals were introduced exhibited notable improvements in realised load and fitness, despite only slight improvements in genetic load. This suggests that these benefits primarily arose through hybrid vigour. The non-deleterious, wild-type allelic variants still present in the captive population appear to have masked the effects of deleterious mutations fixed in the wild populations. Consequently, increased fitness was gained through heterozygosity rather than purging.

However, because the captive population was isolated from the wild population earlier, it had a greater fixation of its own deleterious mutations. As more individuals were introduced from the captive population, the fitness benefits diminished, as more deleterious mutations segregated through the population, increasing the likelihood of their homozygosity. This reduced the benefit to fitness despite the heterozygosity of the deleterious mutations already present in the wild population. Additionally, introducing more individuals from the smaller, less diverse captive population reduced nucleotide diversity, as more individuals descended from the less diverse captive population. This same effect, however, seems to have provided a slight benefit regarding genetic load. The captive population would have lost a considerable amount of genetic load when it became isolated. Thus, introducing more individuals also lowered the genetic load in the wild population.

The primary simulations used the same subspecies to minimise taxonomic and local-adaptation risks. Introducing another leopard subspecies could generate stronger heterosis, as in the Florida panther case, but it could also cause outbreeding depression, disrupt local adaptation and conflict with management constraints. Such a scenario would require prior genomic and ecological assessment.

7.5 Conclusion

Across chapters, the consistent result is not that one mode is universally correct, but that model choice changes both the strength and demographic cost of selection. Rank-based soft

selection can remove small fitness differences efficiently but fixes the number of opportunities; hard selection permits extinction but can allow load to accumulate; compound selection connects relative competition to demographic decline. Conservation forecasts should therefore report the implemented mapping from genotype-derived fitness to survival and reproduction, and test sensitivity to that mapping.

The conclusions remain conditional on simplified genomes, assumed distributions of s and h, idealised mating and limited environmental structure. Empirical tests should combine temporal genomes with survival, reproduction and population growth, estimate N_e rather than equating it with N or K, and validate variant-effect predictions against observed fitness. These data are needed before the relative contribution of hard, soft and compound selection can be inferred for a managed population.

Genetic rescue presents numerous challenges in conservation efforts. By conducting computer simulations and advancing our knowledge of functional genomics, we can improve the accuracy of predicting the outcomes of genetic rescue strategies. Simple models, such as those assessing the impact of varying the number of introduced individuals, provide initial insights. However, more sophisticated models hold promise for evaluating success probabilities across diverse scenarios. These advancements offer crucial tools for refining genetic rescue strategies in conservation practice.

8 Conclusion and scope for further research

8.1 Summary and implications of the main research outcomes

The findings in this thesis validate Wallace's distinction between hard and soft selection. They demonstrate qualitatively different responses to key biological and ecological variables, whether these are largely genetically determined traits such as fecundity, or partially environmentally determined conditions such as effective population size. Beyond their response to these factors, hard and soft selection differ in that hard selection is relatively weak in its ability to purge mutations, providing little protection against the accumulation of genetic load. This weakness is due to two main effects: increased hard selection leads to more selection deaths and thus population decline, and it lacks the power to distinguish between individuals with small differences in absolute fitness. In contrast, soft selection can differentiate between small differences in fitness without causing additional deaths, making it potentially very efficient in purging genetic load. Moreover, while hard selection acts upon absolute fitness and is therefore relatively predictable, soft selection acts on relative fitness, which is influenced by frequency and density. Consequently, the efficiency of soft selection can be highly variable, depending on population size, carrying capacity, and the distribution of fitness within the population. Thus, soft selection can be unpredictable in its efficiency.

The differences between hard and soft selection are highly consequential. Whether hard or soft selection predominates in natural populations will likely be highly deterministic in the outcomes of drift debt caused by environmental damage from anthropogenic activities. This encompasses both the extent of drift debt itself, reflecting the masked load prior to population contraction, and the capacity to purge genetic load afterward, thereby returning to a viable level of fitness. Whether selection is predominantly hard or soft may also significantly influence the effectiveness of conservation management strategies and the applications of functional genetics in these efforts.

Hard and soft components need not operate continuously or with constant strength. They may act at different life stages or simultaneously, and their relative contribution changes with density, environment, competition and trait expression. The central empirical question is

therefore not which mode is universally predominant, but how their contributions vary among populations and through time.

I have attempted to reconcile the dual nature of selection by developing the concept of compound selection. This mode of selection essentially makes hard selection density- and frequency-dependent. It improves the efficiency of selection by more severely punishing the less fit individuals within the population. Consequently, this results in more selective deaths, but since these additional deaths are mostly from the least fit individuals, this trade-off leads to a substantially improved overall fitness.

Competition can also alter exposure to nominally hard causes of mortality. For example, competition for food or territory can reduce condition and thereby increase vulnerability to predation, disease or reproductive failure. These documented links are consistent with a compound effect, although attributing the complete causal pathway to compound selection remains a hypothesis.

I contend that a considerable amount of selection regarding survival in nature operates in this manner. It is hard in the sense that deaths result not directly from conspecific competition but from predation or pathogenesis. Yet it is soft in terms of indirectly influenced by conspecific competition, and is thus, density- and frequency- dependent. Therefore, compound selection may serve as a useful tool in modelling this phenomenon.

However, this does not fully address the question regarding the preponderance of soft or hard selection in natural populations. When modelling compound selection, the contribution of density dependence and frequency dependence can be adjusted, essentially shifting the model towards more hard or soft selection. Assuming that compound selection represents a realistic mode of selection, how should the strength of density- or frequency-dependence be adjusted to accurately reflect real selection pressures in natural populations?

8.2 Biological traits impacting compound selection

The balance between hard and soft components is likely to vary across space. Range cores may experience stronger conspecific competition, whereas range margins may combine lower density with harsher or novel environments. This predicts relatively softer selection in

some cores and harder selection at some margins, but the direction should be tested rather than assumed.

The variation in social behaviours among animal taxa presents another layer of complexity in accurately calibrating compound selection models. The models used in this thesis assume that the presence of conspecifics primarily exerts a detrimental effect on survival by competing for resources, with the only benefit being the availability of mates. However, in natural populations, conspecifics also provide benefits beyond competition. Many herbivores form large groups to reduce predation risk (Makin, Chamaillé-Jammes & Shrader, 2017). Thus, individuals might be more vulnerable to predation when population density is too low. It is challenging to determine whether the protective benefits of grouping against predators are equally distributed among all individuals or if fitter individuals benefit disproportionately. Predators often target the weakest members of a group, implying that fitter individuals might indeed gain more from the group's protective dynamics. Furthermore, fitness in this context may correlate more with age, with juveniles and older individuals being most vulnerable to predation. Therefore, development time and senescence are additional traits that could influence the nature of selection. Synchronous emergence or hatching can satiate predators and is documented in cicadas, mayflies and other taxa (Peplinski *et al*., 2021). The further prediction that these life stages alter the balance of hard and soft selection is an explicit hypothesis, not an established consequence.

Social behaviour becomes even more intricate among primates, which exhibit a range of behaviours including cooperative feeding and group defence against predators. These behaviours likely enhance group fitness, providing better survival chances in large groups of fitter individuals (Albiach-Serrano, 2015). However, primate groups are often hierarchical, with evidence suggesting that higher-ranking individuals gain fitness benefits (Majolo *et al*., 2012). In such taxa, understanding the positive and negative impacts of density- and frequency-dependence is crucial for accurately modelling compound selection.

Overall, determining the balance of density- and frequency-dependent factors in compound selection requires a nuanced understanding of the specific ecological and biological contexts of each species. This understanding is essential for improving the accuracy and predictive power of models used in conservation management and other applications of functional genetics.

8.3 Genetic markers of mode of selection?

A useful test would compare simulations with matched demographic histories and realistic recombination maps under hard, soft and compound selection. Candidate summaries include the site-frequency spectrum, linkage disequilibrium, haplotype structure, temporal allele-frequency change and the relationship between load removal and population growth. Predictive performance should then be validated in experimental or temporal populations.

Computer modelling could be employed to investigate how hard and soft selection impacts the relative abundance of neutral mutations of varying frequencies through linked selection under both hard and soft selection regimes. This would necessitate modelling a genome composed of chromosomes with realistic recombination maps, which presents a challenge as the genome must be sufficiently large to encompass many coding regions where allelic variants are likely to be non-neutral. Without sufficient non-neutral mutations there would not be enough genetic load to meaningfully test different modes of selection. Another potential method of discovery could involve examining changes in genetic sequences in experimental or natural populations. For instance, sexual selection was shown to purge genetic load in the bulb mite, *Rhizoglyphus robini*, by segregating the two male morphs into separate populations (Łukasiewicz, Niśkiewicz & Radwan, 2020). In the populations of aggressive, dominant male morphs, there was strong competition, which purged genetic load, whereas in populations of weaker males, there was no competition and no purging of load.

Manipulating the number of successful males relative to females could vary the strength of sexual competition. Temporal genomic data, functional annotation, haplotype trajectories and direct fitness measures would be needed to distinguish beneficial sweeps from purging deleterious variants; the two processes may remain partly non-identifiable.

Purging during population growth would be suggestive, but not diagnostic, of a soft component. Sufficiently weak hard selection can coexist with positive growth, so selection strength and demographic growth must be estimated jointly rather than inferred from dN/dS alone.

Furthermore, it is not impossible for a population to grow and purge mutations while under hard selection; it merely suggests that the population would not expand as rapidly as it

would in the absence of selection. I propose that soft selection most frequently occurs as part of compound selection, which also necessitates additional selective deaths. This observation may render the search for a distinct marker of hard or soft selection futile. If hard and soft selection do not typically co-exist as components of compound selection, then it is still likely that most populations experience both hard and soft selection across multiple generations. Consequently, the signal from one mode of selection might be obscured by the effects of the other.

Nonetheless, the presence of soft selection within a population implies the existence of competition. This competition potentially indicates that the number of offspring produced and/or the recruitment rate of adults is likely at a sustainable level. It also suggests that the population will maintain adequate levels of fitness. Thus, detecting signals of soft selection could be highly beneficial for monitoring the health and dynamics of populations.

8.4 Neutrality theory revisited

Bruce Wallace (1975) developed the concept of soft selection to address Haldane's dilemma, which questioned how the higher-than-expected levels of within-species genetic variation observed through allozymes could all be subject to selection, given the limitations imposed by the cost of selection in terms of deaths. This dilemma, alongside the relatively consistent rate of protein evolution, led theorists like Kimura (1968) and King and Jukes (1969) to propose that most observed molecular variants evolve neutrally, suggesting that genetic drift is the primary force driving molecular evolution. This neutral theory was further advanced by Ohta (1973), who introduced the idea that many molecular variants are not strictly neutral but nearly neutral. These nearly neutral mutations are primarily affected by natural selection in large populations, whereas in small populations, genetic drift predominantly governs their evolution.

The Neutral Theory of Molecular Evolution, as devised by Kimura (1968), sparked an ongoing debate between those who broadly subscribe to it (neutralists) and those who reject it (selectionists) (de Jong *et al.*, 2024; Galtier, 2024). The disagreement between these two groups primarily concerns substitutions of non-synonymous single nucleotide variants, although it has at times extended to include codon usage bias and the effect of non-coding substitutions on the regulation of gene expression (Galtier, 2024). While both groups broadly agree on the importance of purifying selection, neutralists argue that most single nucleotide variants are

evolving neutrally and that most substitutions result from drift. In contrast, selectionists maintain that variants evolve under selection and that substitutions are adaptive. The neutralists' position has historically been misrepresented as assuming that no substitutions are adaptive when their stance is that such cases are rare. Similarly, the selectionists' position has been mischaracterized as assuming no substitutions are neutral, whereas they do not rule out neutral substitutions (de Jong, 2024).

Wallace's (1975) conceptual framework allows many variants to be selected simultaneously when success depends on rank for a fixed number of opportunities. This makes the selectionist position more plausible, but the strength and biological form of soft selection remain empirical questions.

Finally, a concern regarding the selectionists, particularly Ohta's (1973) nearly neutral interpretation, is the potential for slightly deleterious mutations to drift to fixation, which they view as "anti-Darwinian" since such a process would reduce the fitness of all taxa over time (de Jong, 2024). However, this concern might be mitigated if soft selection is predominant in nature, either in the strict sense or as part of compound selection. It is possible that a mutation slightly deleterious under soft selection, due to its negative effect on relative fitness, is virtually neutral in absolute terms. In other words, within a population, individuals possessing a certain variant might be slightly disadvantaged compared to conspecifics without it, but the population's overall survival would be virtually unaffected if that variant drifted to fixation.

8.5 Soft and hard stabilising selection

For the simulations performed in this study the mode of selection, whether hard, soft or compound, has also been direct. All mutations are conditionally deleterious as this is the most appropriate method of investigating genetic load. However, an alternative to direct selection is stabilising selection which selects for an intermediate optimum phenotype through the conditional selection of quantitative trait loci (QTLs). A QTL is a locus that contributes to the quantitative variation of a continuous phenotypic trait (Zeng, 1994). The phenotypic trait in question is usually polygenic, meaning that its variation is the product of the interaction of multiple QTLs. Stabilising selection is a type of selection that eliminates the extremes of an array of a continuous phenotype (Sanjak *et al*., 2018). Qualitative traits that are under stabilising

selection are for example, bristle and wing development in *Drosophila melanogaster* (Shrimpton and Robertson, 1988), the body weight and morphometry of mice (Flint and Mackay, 2009), and the rate of growth of *Arabidopsis thaliana* (Kroymann and Mitchell-Olds, 2005) and yeast (Steinmetz *et al.*, 2002).

For example, stabilising selection on clutch size would reduce fitness on either side of an intermediate optimum: too few eggs reduce recruitment, whereas too many can exceed parental resources. The example simply illustrates conditionally deleterious alleles around an optimum and is not presented as a novel result.

The fitness of an individual, and by extension the population, results from how well the alleles that shift a phenotypic trait from the optimum in one direction are compensated for by alleles that shift the phenotypic trait from the optimum in the opposite direction. The trait in question may be contributed to by a relatively small number of QTLs that have as strong effect, as is the case with wing length in reed warblers (Hansson *et al.*, 2018), or many QTLs that each have small effects, such as for the timing of flowering of maize (Steinhoff *et al*., 2012). The flowering time in *Arabidopsis* Is controlled by one gene with a major effect (Li *et al*., 2014), as well as many small effect QTLs.

I propose that, as with direct selection, stabilising selection can also be either hard or soft. In the case of soft selection, it would drive the phenotype closer to the optimum phenotype. This would result in a greater loss of the large-effect quantitative trait loci (QTLs) over time and potentially the positive selection of mutations that exert an epistatic effect by fixing the phenotype at its optimum, assuming that the phenotypic optimum does not shift due to environmental variation (Barton, 2017). By contrast, hard selection would select against the phenotypic extremes but have a much weaker effect on maintaining the optimum phenotype, as the benefits of remaining close to the optimum would be relatively small (Figure 6.1). Therefore, a greater amount of QTL diversity may be preserved within the population.

8.6 Final remarks

This thesis shows that fecundity, population size and investment can produce qualitatively different outcomes under alternative selection modes. The main implication is that predictions of load, diversity and extinction depend on how absolute and relative fitness are translated into survival and reproduction. The models are deliberately simplified, and their strongest next test is empirical calibration using temporal genomic, demographic and fitness data. Neutral diversity alone is insufficient: realised and masked load, reproductive variance and population growth should be evaluated together. Compound selection provides a tractable framework for these tests, while its equations and parameters should be validated against species-specific biology.

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