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Article

From Valleys to Peaks: The Role of Evolvability in Fitness Landscape Navigation

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1. Introduction

Mutational robustness and evolvability are fundamental aspects of evolution. The interplay between these two factors presents a complex and often paradoxical challenge in evolutionary biology [1,2]. Mutational robustness refers to an organism's ability to maintain functionality despite genetic mutations, thereby preserving phenotypic stability [1,3]. Conversely, evolvability denotes the potential to generate phenotypic variation, facilitating adaptation to changing environments [4]. The coexistence of these traits appears contradictory: robustness implies resistance to change, while evolvability necessitates variability [5,6].

A key example of this paradox is observed in the "Survival of the Flattest" (SoF) phenomenon. A population at a high but steep fitness peak becomes out-competed by another population at a lower but flatter peak [7]. The reason for that is mutational robustness. More mutants from the population at the higher peak "fall" from the peak than those, mutants from the lower but flatter peak. This difference is sufficient for the survival of the population at the flatter peak.

Beyond simply occupying a steeper or flatter fitness peak, which determines the mutational neighborhood and thus the effect of mutations, organisms can actively modulate their inherent robustness against mutations to prevent changes to their phenotype [8,9]. This means they are not merely passive participants shaped by the fitness landscape; rather, they can adjust their genetic architecture to buffer against mutations when phenotypic stability is advantageous or, conversely, to amplify the effects of mutations, allowing for greater exploration of the fitness landscape. However, how do organisms achieve this ability to evolve their own, mutational robustness or enhance their evolvability? What mechanisms facilitate organisms to dynamically adjust between stabilizing their phenotype and increasing phenotypic variation, thereby optimizing both robustness and adaptability in response to changing environments?

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We, and others, have identified epistasis (ϵ) and pleiotropy (π) to facilitate robustness and evolvability [6,10–13]. Epistasis refers to interactions between different genetic loci, where the effect of one gene mutation depends on the presence of mutations in other genes. This interaction can enhance robustness by buffering the effects of individual mutations, thereby stabilizing phenotypic traits despite genetic variability [14]. For instance, organisms with strong epistatic interactions can maintain functionality even with multiple mutations, effectively "hiding" the impact of deleterious changes. However, this stabilizing role can also limit evolvability by reducing the impact of mutations that might otherwise lead to beneficial adaptations [15]. Thus, epistasis can simultaneously support robustness while constraining the organism's ability to generate novel phenotypic variations [16].

Pleiotropy, on the other hand, occurs when a single gene influences multiple traits. This interconnectedness can contribute to mutational robustness by ensuring that changes in one gene lead to coordinated adjustments across multiple traits, thereby preserving overall functionality [17]. However, the downside is that mutations beneficial to one trait can have detrimental effects on others, thereby limiting the organism's ability to adapt optimally [10]. Lastly, a mutation in a pleiotropic gene can have multiple effects, as the pleiotropic gene is involved in many traits. As such, a mutation can now change the phenotype more drastically, leading to an increase in evolvability, as it allows the faster exploration of the fitness landscape [17,18].

The opposing effects of mutational robustness and evolvability create a conundrum: populations require evolvability to discover and ascend new fitness peaks, yet excessive evolvability can compromise stability, preventing them from maintaining peak positions [2]. The relationship between robustness and evolvability has been explored extensively. Some studies have shown that robustness can facilitate evolvability. For instance, robustness allows populations to accumulate cryptic genetic mutations that are phenotypically neutral under stable conditions but can become beneficial when the environment changes, thereby enhancing adaptive potential [2]. Similarly, large populations often evolve towards greater mutational robustness to maintain functionality, indicating that robustness and evolvability can co-evolve rather than conflict [19]. On the other hand, some researchers argue that these traits are not always aligned. Robustness, while protecting against harmful mutations, can restrict the exploration of new phenotypes, limiting adaptive potential in rapidly changing environments [20]. This tension highlights the paradox where too much robustness may stifle innovation, suggesting that the balance between robustness and evolvability is highly context-dependent.

However, investigating these interactions in natural systems is challenging due to the intricacies of genetic networks and the limitations of experimental manipulation. To address this, computational models provide a powerful alternative, allowing researchers to systematically explore how varying mutation rates and fitness landscapes influence robustness and evolvability [21,22]. In our recent extension of the NK fitness landscape model, we utilized a matrix-based encoding to map genetic loci to phenotypic traits, enabling a detailed investigation of how ϵ, π, and landscape ruggedness (K) affect adaptive potential [23].

Our previous work [13] explored how reducing ϵ, π can prevent populations from shifting toward flatter peaks under high mutation pressures. By minimizing genetic interactions, we showed that populations could maintain their fitness advantage despite elevated mutation rates. However, this research primarily focused on populations already positioned on fitness peaks. Extending this, we have investigated how populations transition from lower to higher fitness peaks under different mutation rates, emphasizing the role of robustness in stabilizing populations once they reach peaks (citation, in review).

In this study, we aim to investigate the conditions under which robustness or evolvability becomes advantageous within a fitness landscape. Our computational model explores how varying mutation rates (μ, from 0.0001 to 0.05), landscape ruggedness ($K = 1, 3, 5, 7$), and initial population placements—whether in valleys, on slopes, or at peaks—impact evolutionary trajectories. By observing how populations navigate fitness landscapes from

diverse starting points, we aim to clarify when evolvability is favored over robustness. Recognizing the context-dependent nature of these traits, our study seeks to uncover the dynamics driving successful adaptation, highlighting the specific roles of robustness and evolvability across different evolutionary scenarios.

2. Materials and Methods

2.1. Extended NK Model

Our computational model is based on the standard NK model framework [**?**], which contains a circular sequence of N binary loci. In this model, each locus interacts with K adjacent loci to contribute to the organism's overall fitness. The model assigns a fitness value to each gene configuration via a lookup table that contains random values. For example, with $K = 1$, each gene is defined by the sequence of two adjacent loci, resulting in four possible binary configurations (00, 01, 10, 11).

As K increases, each gene is influenced by more neighboring loci, increasing the size of the fitness table. Since sites are overlapping, single mutations are affecting more genes, which leads to more changes in fitness values creating a more rugged fitness landscape with multiple peaks and valleys. Unlike the traditional approach of averaging fitness components, we calculate the organism's fitness (W) using the geometric mean of all N fitness values, which has been shown to provide realistic evolutionary outcomes [**? ? ? ?**]:

$$W = \left(\prod_{i=1}^{N} W_i \right)^{1/N} \tag{1}$$

The main difference between the extended NK model[23] used here and the standard NK model[**?**] is the indirect encoding. While fitness is still calculated based on the traits of the organism defined by its phenotype **indirectly encoded** organisms have a genome that defines the phenotype. In this experiment, we utilized the **extended NK model** to investigate the effects of mutational robustness and evolvability across different initial conditions in the fitness landscape. Our goal was to examine how mutation rates (μ), landscape ruggedness (K), and the distinct starting positions of populations—valleys, slopes, or peaks influence evolutionary outcomes. A population of 100 organisms was used in each experimental run, with each organism represented by a genotype vector and a corresponding phenotype vector. Organisms were initialized in distinct fitness landscape positions: valleys (low-fitness regions), peaks (high-fitness regions), and slopes (regions between peaks and valleys). These positions were determined by comparing each genotype's fitness to that of its neighboring genotypes. In each generation, organisms were subject to mutations in both genotype values and mapping matrix values based on the specified mutation rate. These mutations introduced variations, allowing populations to explore the fitness landscape. Selection was performed through a roulette-wheel mechanism, where agents with higher fitness values were more likely to reproduce and pass on their traits.

2.2. Prominence Analysis in the extended NK model

Prominence is calculated by counting the number of neighboring genotypes with fitness values lower than the focal genotype's fitness. A higher prominence value indicates that the genotype resides on a more pronounced peak, surrounded by genotypes of significantly lower fitness. The figure illustrates (see figure 1 how the frequency of prominence values varies across different ruggedness levels. As K increases, the number of fitness peaks decreases, leading to a reduction in regions with high prominence. This shows that as the landscape becomes more rugged, organisms have fewer accessible pathways to climb, making evolution more constrained.

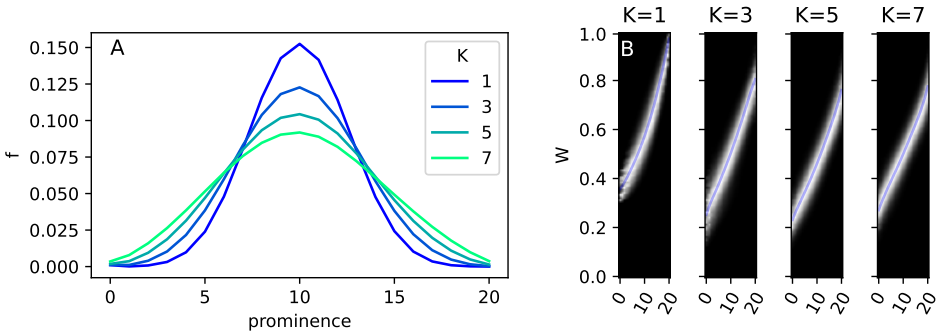

Figure 1. The figure illustrates the impact of landscape ruggedness ($K = 1, 3, 5, 7$) on fitness prominence distributions. (A) The left plot shows the mean prominence values of fitness peaks for different ruggedness levels. Prominence, normalized by the total size of the landscape, decreases as ruggedness (K) increases. The colors represent different K values, with higher K leading to smoother, lower peaks. (B) The right panel displays grayscale heatmaps of prominence distributions for each ruggedness level. Rows represent normalized fitness values, and columns represent prominence. These heatmaps highlight how increasing K disperses fitness prominence, leading to flatter and more widespread peak distributions.

2.3. Indirect Encoding

To isolate the effects of epistasis (ϵ) and pleiotropy (π) from landscape ruggedness (K), we extended the standard NK model by separating the genotype from the phenotype. In our model, each locus in the genotype vector takes a continuous value within the range $[-1, 1]$ rather than binary values. This extension allows us to apply a mapping matrix that transforms these genotype values into a phenotype vector, which is subsequently converted to binary for fitness calculations.

We introduce a mapping matrix, M, of dimension $N \times N$, with values in the range of $[-1, 1]$. This matrix defines the interactions between genes, influencing each phenotypic trait according to the genotype values. The phenotype vector, P, is calculated as the dot product of the genotype vector G and the mapping matrix M:

$$P = G \cdot M \tag{2}$$

Each element of the phenotype vector, representing a trait, is subsequently converted to binary for the purpose of fitness calculation: values greater than 0 are assigned a binary value of 1.0, while values below or equal to 0 are assigned 0.0. This phenotype vector (P), is then treated as the trait vector in the standard NK model, allowing fitness to be calculated based on the value of K (see Figure2). However, unlike in the standard NK model, the degree to which genes can interact is now freely evolvable by changing the expression values G and the weights of the interaction matrix M, independent of K.

2.4. Quantifying Epistasis and Pleiotropy

In our extended model[23], we have separated genetic interactions to allow traits to evolve independently. This separation requires distinct measurements for epistasis (ϵ) and pleiotropy (π).

To achieve this, we calculate an interaction matrix (IM), which identifies how each gene influences specific traits. For an organism with N genes and N traits, the IM is an $N \times N$ matrix[23]. We determine whether each gene i affects a trait j by altering the expression value G_i between -1.0 and 1.0. Using these modified values, we construct the phenotype $P = G \times M$ and check if changing gene i impacts trait j. If it does, $IM_{i,j}$ is marked with a value of 1; if not, it is assigned 0. The row sums in this matrix indicate the number of traits influenced by each gene (π), while the column sums reflect the number of genes affecting each trait (ϵ). This gene-to-trait mapping is governed by the mapping matrix M, which evolves over time. Due to random mutations, directly comparing ϵ and π

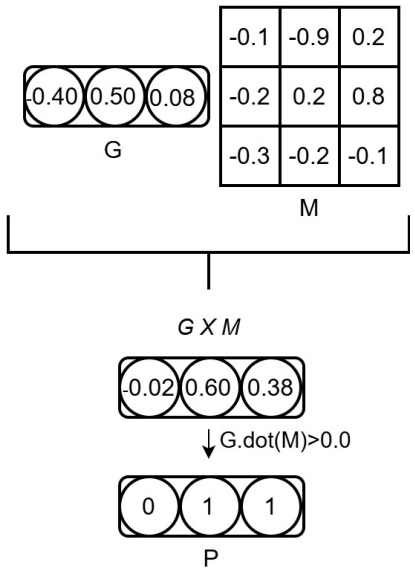

Figure 2. In the indirect encoding method, each organism has a genome composed of N genes. Each gene, represented as a circle, is characterized by a specific value and an associated vector of length N. These gene vectors collectively create a matrix (M), while the gene values form a vector G. The phenotype vector is then produced by taking the dot product of G with M, after which it is discretized so that each component corresponds to one of the N binary traits of the phenotype. Mutations can alter any values within the genome, allowing the interaction between G and M to indirectly shape the phenotype. For direct encoding, M would instead be a fixed identity matrix.

vectors across organisms can be challenging. We resolve this by sorting these vectors in ascending order[23], allowing us to analyze their overall distribution at an organismal level rather than focusing on specific values for individual genes.

We then compare the observed ϵ and π distributions to a baseline random expectation, represented by the black line (see Figure 3). This expectation is calculated from 1000 mapping matrices with randomly generated values between -1 and 1. Values below this baseline indicate lower-than-expected ϵ and π, while values above suggest higher-than-expected levels [23].

To quantify these differences from the expected ϵ and π values, we calculate the area above and below the baseline curves, denoted a and b respectively. The discrepancy Δ, defined as the difference between these two areas, represents how much the observed values deviate from the random expectation.

2.5. Hill-Climbing Optimization for Generating Heterogeneous Populations with Varied Epistasis and Pleiotropy

The indirect encoding method allows for an opportunity to study—how varying genotypes can produce the same phenotype vector, facilitating the study of how changes in ϵ and π affect mutational robustness and evolvability. By varying the G and M while maintaining a constant P, we can investigate the impact of changes in ϵ and π on an organism's adaptability.

To explore these effects, a **hill-climber optimization** is implemented to compare an organism with its mutant counterpart, maintaining an identical phenotype but with varied levels of ϵ or π. We achieve this by selecting organisms with either increased or decreased levels of ϵ or π. Depending on our goal, we can maximize or minimize ϵ and π, or choose one randomly to emulate genetic drift.

Each iteration introduces a random mutation until a mutant with the same phenotype as the original is generated. If this mutant shows a difference in ϵ or π consistent with the desired direction (higher or lower), it is retained. This process is repeated for up to

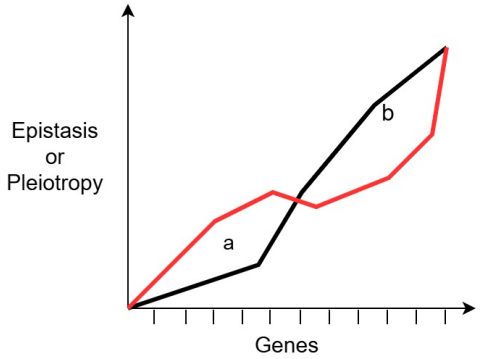

Figure 3. To measure the difference between the expected distribution of epistasis (ϵ) or pleiotropy (π) and a specific measurement, the degree of ϵ and π for all genes is calculated and sorted in ascending order to produce a specific organism's distribution (red line). This is compared to the random expectation (black line). The areas between the red and black lines are calculated: regions above the expectation are marked in orange (a), and regions below are marked in blue (b). The total difference is determined as ($a - b$).

1000 iterations, ensuring the phenotype remains unchanged, thereby fixing the organism's position within the fitness landscape.

Once this process is completed, a heterogeneous population of 100 organisms is created by selecting half of the organisms from the pool of organisms with increased values of ϵ and π, and the other half from the pool with decreased values. This heterogeneous composition enables a comprehensive analysis of how different genetic interaction levels impact mutational robustness and evolutionary dynamics across a diverse population.

2.6. Initialization of distinct Starting Conditions

For each organism in the initial population, the simulation evaluates its fitness along with the fitness of its 20 neighboring genotypes. These neighbors are generated by flipping one bit of the genotype at a time, thereby changing one gene and recalculating the resulting phenotype and fitness. The simulation then counts how many of these neighboring genotypes have fitness levels that are lower, higher, or equal to the fitness of the original organism. Based on this analysis, each organism is assigned a classification value: organisms surrounded entirely by neighbors with lower fitness are assigned the highest value of 20, indicating a strong fitness peak, while those surrounded by neighbors with higher fitness receive a value of 0, indicating a deep fitness dip. Organisms with a mix of neighbors with varying fitness levels are assigned intermediate values from 1 to 19, reflecting their position on slopes or plateaus in the fitness landscape. By starting the population with 20 distinct conditions, we can study how an organism's initial position—whether on a peak, in a dip, or on a slope—affects its ability to adapt, maintain robustness, and explore new phenotypic variations.

2.7. Evolution of Populations Using a Genetic Algorithm

After initializing the population with 100 organisms, all sharing the same phenotype but with varied levels of ϵ and π, the simulation employs a genetic algorithm to evolve the population over multiple generations. In each generation, the fitness of each organism is assessed based on its phenotype's alignment with a predefined fitness landscape. A roulette-wheel selection method is then used to favor organisms with higher fitness for reproduction, ensuring that these organisms are more likely to pass on their genes. However, genetic diversity is maintained through random mutations applied to the genome's expression levels and interaction weights. The process concludes when the population either reaches a genetic sweep or a predefined generation limit(200 generations).

2.8. Quantifying Mutational Robustness ($\overline{HD}$)

In this study, we assess mutational robustness by examining the average impact that mutations have on an organism's phenotype. Specifically, mutational robustness is considered lower when mutations cause a larger effect on the phenotype, facilitating the exploration of new regions in the fitness landscape.

To quantify this impact, we calculate the average Hamming distance ($\overline{HD}$) between the original organism and its mutants. Each organism generates 10000 offspring, with a point mutation rate of 0.01 applied to both the expression levels and interaction weights (M) in the genotype. For each of these 10000 mutants, the phenotype is determined, and the Hamming distances are measured and averaged to obtain $\overline{HD}$.

When correlating the mean epistatic and pleiotropic effects to $\overline{HD}$, we observed that randomly generated organisms exhibit only minor variations in ϵ and π. To address this, we employed a hill-climbing optimization strategy that attempts up to 1000 iterations to generate mutants that maintain the same phenotype but have either increased or decreased levels of ϵ and π[13].

2.9. Simulation of Genetic Drift population

The process begins by creating a root organism with a specific phenotype, which serves as the ancestor for the population. From this organism, a population of 100 organisms is created, ensuring that all organisms initially share the same phenotype but have varied levels of ϵ) and π. The simulation then evolves the population purely through genetic drift for up to 200 generations or until a genetic sweep occurs. During this phase, organisms reproduce randomly to form new generations without fitness-based selection, allowing genetic drift to drive changes in the population. The process ends when either the population converges to a single common ancestor (indicating a genetic sweep) or the generation limit is reached.

2.10. Details of our computational model

The code for the computational model can be found here . Populations were initialized with 100 identical organisms derived from a randomly generated genome and mapping matrix. At every generation, the entire population was replaced with a new one based on fitness values using roulette wheel selection. This stochastic selection method ensured that individuals with higher fitness were more likely to reproduce, while less fit individuals also had a small chance to pass their traits to the next generation. The evolutionary process was strictly asexual, with no recombination or horizontal gene transfer. Mutations occurred probabilistically based on the mutation rate (μ) and the population size of 100 was maintained constant throughout all simulations. Fitness landscapes varied in ruggedness ($K = 1, 3, 5, 7$), allowing us to explore how populations adapted across different levels of complexity.

3. Results

In this section, we present the outcomes of our computational experiments, designed to investigate the interplay between evolvability and robustness within complex fitness landscapes. Previous research has provided two key insights that shaped our hypothesis. First, we demonstrated that populations already positioned on fitness peaks benefit from mutational robustness, particularly under high mutation rates[13]. Second, we found that while high evolvability facilitates crossing fitness valleys and reaching higher fitness peaks, long-term stability at the peak requires mutational robustness, as highly evolvable pioneers are often outcompeted by more robust competitors once the peak is reached (citation). This interplay between evolvability for adaptation and robustness for stability led us to hypothesize that populations beginning in valleys or on slopes benefit from evolvability to explore the fitness landscape, while populations on fitness peaks prioritize robustness to maintain stability under mutational pressure.

To test this hypothesis, we conducted simulations with populations starting at diverse starting positions (see material and methods) across the fitness landscape, ranging from valleys (low fitness) to peaks (high fitness). These populations were subjected to varying mutation rates (μ ranging from 0.0001 to 0.05) and landscape ruggedness levels ($K = 1, 3, 5, 7$) to understand how these factors influence the dynamics of evolvability and robustness. We tracked changes in epistasis (ϵ) and pleiotropy (π) over generations, as these metrics reflect the degree of genetic interaction and the potential for adaptability or stability. Control simulations without selective pressure were also run to ensure the observed dynamics were driven by selection rather than random drift.

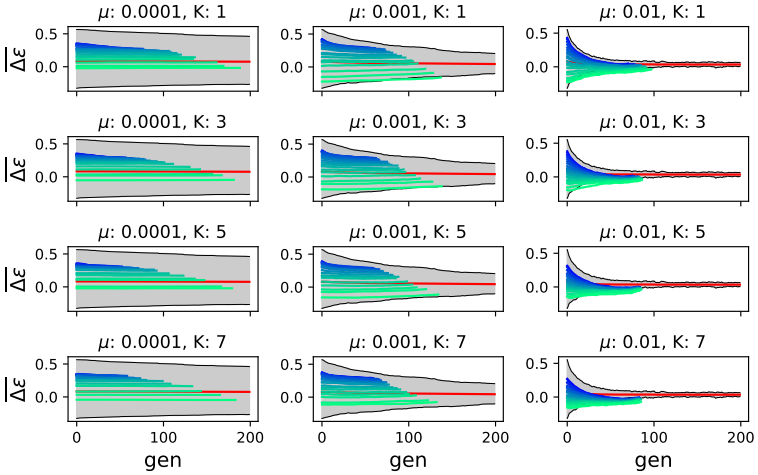

Figure 4. The figure illustrates the changes in ϵ over generations for populations starting in different regions of the fitness landscape. These dynamics are analyzed under varying levels of landscape ruggedness ($K = 1, 3, 5, 7$) and $\mu = 0.0001, 0.001, 0.01$. The x-axis shows the generations, and the y-axis displays the $\Delta\epsilon$ values. The shaded areas indicate the minimum and maximum drift vector, while the red line represents the mean drift across multiple replicates.

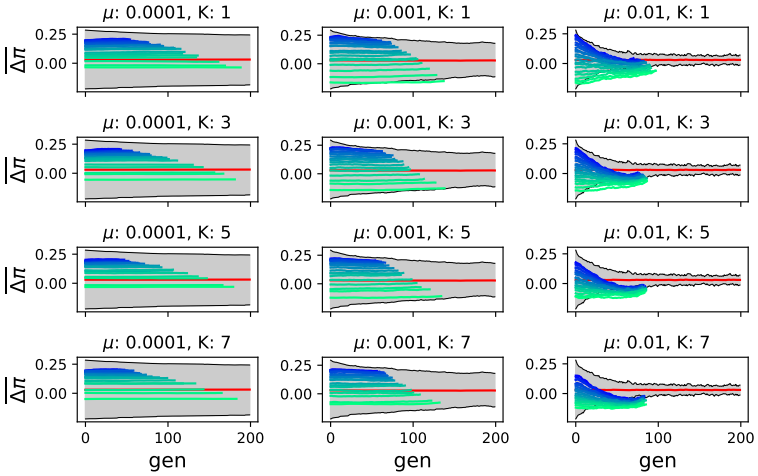

Figure 5. The figure illustrates the changes in π over generations for populations starting in different regions of the fitness landscape. These dynamics are analyzed under varying levels of landscape ruggedness ($K = 1, 3, 5, 7$) and $\mu = 0.0001, 0.001, 0.01$. The x-axis shows the generations, and the y-axis displays the $\Delta\pi$ values. The shaded areas indicate the minimum and maximum drift vector, while the red line represents the mean drift across multiple replicates.

Populations starting in valleys and slopes exhibit higher levels of epistasis (ϵ) and pleiotropy (π) (see figures 4, 5), reflecting a reliance on evolvability. This trend is especially

pronounced at lower mutation rates. As evolution progresses, a noticeable shift emerges. Under high mutation rates, populations starting from higher positions (peaks) increasingly reduce their ϵ and π, often dropping below baseline levels. This reduction indicates a transition toward robustness, allowing these populations to stabilize their fitness and buffer against the destabilizing effects of mutations.

Thus, for populations beginning in valleys or on slopes, evolvability dominates, enabling adaptation and exploration of the fitness landscape. In contrast, populations starting from higher positions prioritize robustness as evolution progresses, particularly under high mutation pressures, to maintain their fitness and resist mutation-driven declines.

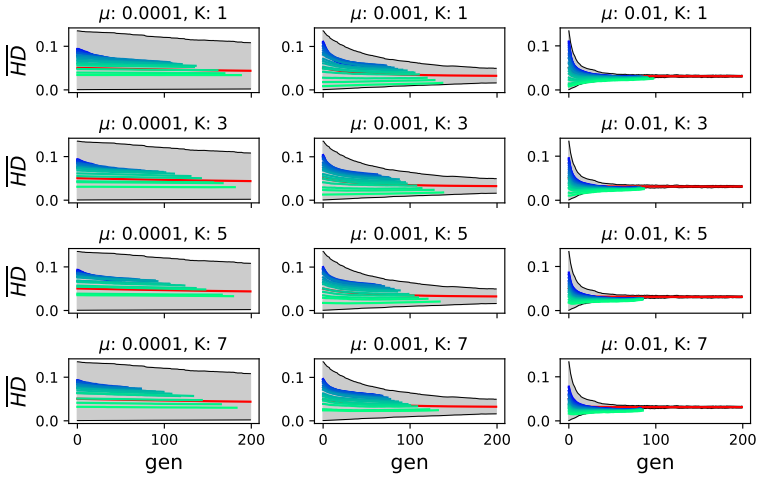

Figure 6. The figure illustrates the changes in HD over generations for populations starting in different regions of the fitness landscape. These dynamics are analyzed under varying levels of landscape ruggedness ($K = 1, 3, 5, 7$) and $\mu = 0.0001, 0.001, 0.01$. The x-axis shows the generations, and the y-axis displays the $\overline{HD}$ values. The shaded areas indicate the minimum and maximum drift vector, while the red line represents the mean drift across multiple replicates.

We also measured mutational robustness using the average Hamming distance ($\overline{HD}$), which quantifies the phenotypic impact of mutations. Higher $\overline{HD}$ indicates lower robustness, while lower $\overline{HD}$ reflects higher robustness and phenotypic stability.

Populations starting in valleys or on slopes exhibited higher $\overline{HD}$, especially at lower mutation rates, demonstrating their reliance on evolvability to explore the fitness landscape. In contrast, populations starting at higher positions consistently maintained lower $\overline{HD}$, particularly under high mutation rates, reflecting a prioritization of robustness to maintain stability.

These findings align with the observed trends in ϵ and π, confirming that populations in lower starting conditions rely on evolvability, while populations in higher starting conditions favor robustness to sustain their advantageous positions.

Next, we wanted to investigate whether the observed evolutionary trajectories of mutational robustness ($\overline{HD}$) significantly deviate from what would be expected under neutral drift, to determine if these changes are driven by adaptation. To address this, we performed a Kolmogorov-Smirnov (KS) test[], comparing the distributions of the start and end points of the observed evolutionary trajectories with the drift baseline. This analysis allowed us to assess whether the observed deviations in $\overline{HD}$ were statistically significant and reflective of adaptive responses, rather than resulting from random drift.

The figure illustrates (see figure 7) the comparison of the start and end points of the observed evolutionary trajectories with the drift baseline using the Kolmogorov-Smirnov (KS) test. The black solid line represents the distribution of the startpoints, the red solid line represents the distribution of the endpoints, and the dotted red line indicates the drift baseline. The asterisks (*) are marked at selected starting conditions to indicate statistically significant differences ($pvalue < 0.05$). For the complete statistical details

across all starting conditions, refer to the table (see table in supplementary material). These significant deviations confirm that the changes in mutational robustness ($\overline{HD}$) observed during evolution are driven by adaptation to selective pressures, rather than being the result of neutral drift.

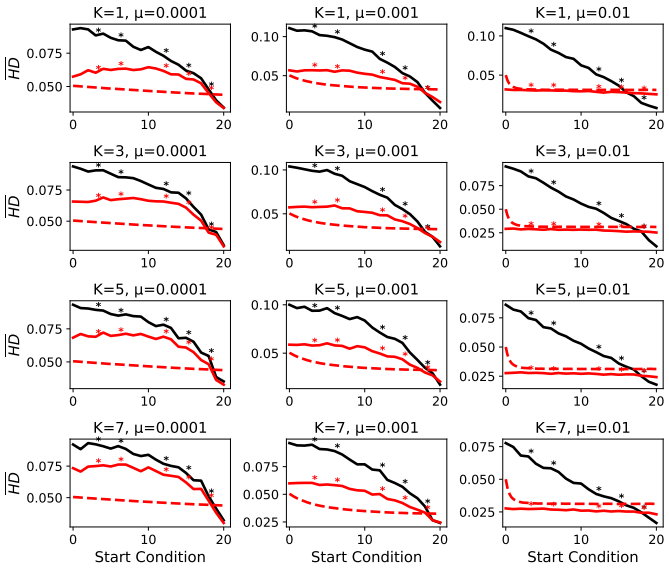

Figure 7

To further validate this interplay between evolvability and robustness, we examined how mutational robustness, measured by the Hamming distance (HD), correlates with prominence. We observe that (see figure 8), populations residing on fitness peaks, characterized by high prominence, exhibited lower HD indicative of high mutational robustness. These populations are better equipped to maintain stability, as their phenotypes remain largely unaffected by mutations. This robustness allows them to safeguard their advantageous position on the fitness peak, minimizing the risk of deleterious phenotypic changes.

On the other hand, populations in valleys or on slopes, where prominence is low, demonstrated higher HD. This reflects greater phenotypic variability, indicating increased evolvability. These populations leverage this adaptability to explore the fitness landscape, seeking pathways to climb toward higher fitness peaks. This correlation underscores the dual pressures shaping evolutionary dynamics: the need for robustness in stabilizing fitness at peaks and the demand for evolvability to navigate rugged landscapes from valleys and slopes.

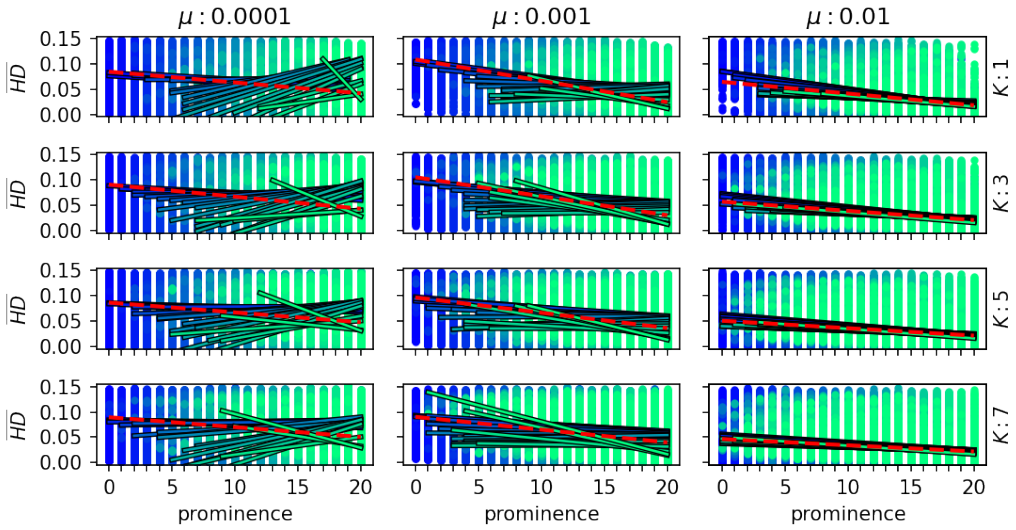

Figure 8

4. Discussion

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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Conflicts of Interest: Declare conflicts of interest or state "The authors declare no conflicts of interest." Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. Any role of the funders in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. If there is no role, please state "The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results".

Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

Appendix A

Appendix A.1

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data are shown in the main text can be added here if brief, or as Supplementary Data. Mathematical proofs of results not central to the paper can be added as an appendix.

Table A1. This is a table caption.

Title 1	Title 2	Title 3
Entry 1	Data	Data
Entry 2	Data	Data

Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled, starting with "A"—e.g., Figure A1, Figure A2, etc.

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