Evolvability and organismal architecture

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Evolvability and Organismal Architecture

the Blind Watchmaker and the Reminiscing Architect

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We (the indivisible divinity that works in us) have dreamed the world. We have dreamed it resistant, mysterious, visible, ubiquitous in space and firm in time, but we have allowed slight, and eternal, bits of the irrational to form part of its architecture so as to know that it is false.

Jorge Luis Borges, Avatars of the Tortoise

Blindness has not been for me a total misfortune; it should not be seen in a pathetic way. It should be seen as a way of life: one of the styles of living.

Jorge Luis Borges, Blindness

CONTENTS

SUMMARY

In evolutionary biology, adaptive evolution is often envisaged as a "Blind Watchmaker". Like a watchmaker, evolution can create complex and intricate systems, but it is blind because it is an undirected process that does not follow a laid-out plan. Evolution thus proceeds by trial and error and can find solutions only to present circumstances. This perspective, however, overlooks a crucial aspect of the evolutionary process. Organisms have developed features throughout their evolutionary history that enhance their "evolvability," allowing them to adapt to new conditions more effectively. Hence, evolution is not as blind as standard evolutionary theory depicts it. There are numerous examples of traits that do not confer an immediate advantage but instead promote long-term adaptability. For instance, bacteria can increase their mutation rate under stressful conditions to hasten the production of new adaptive mutants, and developmental systems are structured in such a way that random genetic changes are biased toward functional phenotypes. Life has existed on Earth for 3.5 billion years and throughout this time, conditions have continually changed, and life has continually adapted to these changes by evolving. Accordingly, the ability to adapt to new circumstances through evolution, i.e. evolvability, must be a crucial factor in the success of biological lineages. However, standard evolutionary biology does little to accommodate this idea within its framework. It does not enquire as to why there could be organisms that are more evolvable than others, nor as to how these differences in evolvability could be achieved. I argue that when we consider organisms as interdependent and interconnected systems, instead of the haphazard combination of components, it is possible to see how they have accumulated evolutionary "memories" of past selection within their architecture. These memories can be invoked when adapting to present-day challenges. Under this perspective, evolvability is incorporated into the organisms' architecture and evolution becomes more akin to a "Reminiscent Architect" than to a "Blind Watchmaker".

The purpose of this thesis is to explore the role of organismal architecture in evolvability and to incorporate these concepts into a more cohesive theoretical framework.

Chapter 1 introduces the concept of evolvability, and gives a general overview of the themes of this thesis. It provides reasons for the importance of evolvability and goes through the various definitions given to this term. It then moves on to analyse the limitations of standard theory that make it difficult to study evolvability within its framework. To facilitate the study of evolvability a new approach is proposed which focuses on organismal architecture. Organismal architecture can accumulate information about successful adaptations across long periods, making these past adaptations more readily deployable when similar situations occur in the future. To achieve this new framework I advocate for a multidisciplinary approach. Insights from evolutionary disciplines in the computational fields are listed which already highlight the importance of architecture in allowing evolvability. Chapter 1 concludes with a discussion of the methodological approach taken in this thesis.

Chapter 2 aims to provide a clearer framework for understanding the concept of evolvability. Evolvability can refer to many different biological phenomena; this multifaceted nature can sometimes cause misunderstandings about what one exactly means by this term. To disentangle matters, the genetic, developmental and ecological factors that influence evolvability are classified into three categories: factors that provide genetic variation, factors that bias the effects of variation on fitness, and factors that shape the selective process. Timescale is also important, as some evolvability factors exert effects over many generations whereas others on a narrower timescale. We also distinguish between mechanisms that broadly influence an organism's genome and traits, versus those with more restricted impact on specific adaptations. Additionally, we identify two major perspectives on evolvability in the literature: as a general, enduring capacity of lineages over evolutionary timescales, or focused on adapting to new environments or niches. By providing this structured overview, the chapter clarifies some of the complexity around evolvability and facilitates more accessible discussion among researchers, hoping to minimise misunderstandings.

Chapter 3 addresses the second category mentioned above: factors that bias the effects of variation on fitness. It is one of the central dogmas of biology that genetic mutations are random concerning the "requirements" of natural selection. Yet, there are many examples of adaptation-enhancing biases in the occurrence of phenotypic variation. Employing simple models for the evolution of gene-regulatory networks, we demonstrate how such adaptation-enhancing biases can readily evolve. Despite the inherent randomness of the genetic mutation process, even small gene networks swiftly evolved an architecture that influences the phenotypic effects of mutations. This bias leads to a significant acceleration of adaptive evolution when the environment changes. We call the specific configuration of the network that translates random genetic mutations into evolution-enhancing phenotypic effects a "mutational transformer". Moreover, we identify two different types of mutational transformers: "mutational amplifiers", which allow large changes in the phenotype through few mutations (amplifying their effect), and "mutational canalisers", which instead allow for the switching between two different phenotypes via few mutations (effectively canalising mutations in one of two outcomes).

Chapter 3 is followed by two "intermezzos". Both are theoretical contributions to published articles, which are included in this thesis because they helped to shape my views on organismal architecture and formed the starting point of other thesis chapters.

In **Chapters 2** and **3**, I plead for a mechanistic approach to modelling evolutionary processes. In Intermezzo 1, I sketch such an approach for the study of the evolution and the evolutionary consequences of non-genetic inheritance. I first provide an overview of the various theoretical approaches to incorporate non-genetic inheritance in an evolutionary framework, highlighting their strengths and weaknesses. I then describe a more mechanistic model consistent with my methodology that can incorporate recent molecular and evolutionary insights on non-genetic inheritance.

Intermezzo 2 is the modelling component of an empirical study that demonstrates experimentally that parasitic wasps can induce their fat metabolism. However, according to conventional wisdom, they should have lost the corresponding metabolic pathway millions of years ago. We argue that the wasps never really lost the trait, but simply plastically switched it off for long periods of time. But then the question arises: how can

functional plasticity be maintained if it is only sporadically employed? To answer this, we developed a simple model for a plastic switch which can turn a metabolic pathway on or off. We demonstrate that such a switch can readily evolve and subsequently stably persist despite mutational erosion, even if the switch is rarely used.

Chapter 4 expands on the study from **Intermezzo 2** and investigates how complex adaptations can persist even when selection occurs only sporadically. Many organisms possess adaptations that are beneficial only occasionally, once every few generations. The study aims to understand how such adaptations evolve and remain intact despite being eroded by genetic drift and the accumulation of mutations. Previous research on this topic has mainly focused on simple traits, but most adaptations in nature are complex. To address this, I conducted simulations to study the evolution of gene-regulatory networks encoding for traits varying in complexity. It turned out that complex adaptations readily emerge and remain stable at high quality for thousands of generations if the sporadicity of selection is moderate (selection occurs once every 5 or 10 generations). When selection is more infrequent (once every 100 generations), the adaptation still emerges and persists, but the performance of the evolved networks is of lower quality. More complex network architectures are able to maintain higher fitness, particularly when the selective task is intricate. Hence, complex adaptations can arise and persist over extended periods, even when they are fine-tuned only sporadically by selection.

In **Chapter 5**, I use biological insights to improve the design of Genetic Programming algorithms used in machine learning. Genetic programming is a branch of computer science which uses evolutionary algorithms to find new solutions to computational problems. Specifically, I propose a new mutation method called Facilitated Mutation, which enhances the efficiency of "Grammar-Guided Genetic Programming". This is a Genetic Programming technique that uses sets of grammatical rules of varying relevance to translate a solution from a genotype to a phenotype. Facilitated Mutation introduces different mutation rates for different grammatical rules. More important rules mutate less frequently to preserve the core functionality, while less important ones are allowed to mutate more often and explore different variations. This idea is inspired by observations in biology, where organisms maintain the functionality of critical genes while allowing less important genes to undergo more rapid mutations. The effectiveness of Facilitated Mutation is compared against a traditional mutation approach on the task of evolving neural network optimisers for image classification. The results show that Facilitated Mutation performs better in key metrics, improving overall optimisation performance, generating higher-quality solutions, and discovering a greater variety of high-quality solutions.

Finally, **Chapter 6** contains an overall discussion of my thesis. This chapter includes two parts. First, I outline some broad philosophical considerations developed during the production of this thesis. I advocate for a more pluralistic view of evolutionary theory, where different types of theory and approaches should not be seen as conflicting, but as different angles from which one can tackle a scientific question. Second, Chapter 6 reviews and summarises the research findings of this thesis and identifies interesting future directions for this field of study. The work in this thesis advocates for the importance of organismal architecture in retaining evolutionary memories, enabling organisms that are shaped by a blind process to improve their capacity to adapt to new environments.

SAMENVATTING

In de evolutiebiologie wordt adaptieve evolutie vaak gezien als een "blinde horlogemaker". Net als een horlogemaker kan evolutie complexe en ingewikkelde systemen creëren, maar het proces is blind omdat het ongestuurd is en geen vastomlijnd plan volgt. Evolutie werkt dus met vallen en opstaan en kan alleen oplossingen vinden voor de huidige omstandigheden. Dit perspectief ziet echter een cruciaal aspect van het evolutieproces over het hoofd. Organismen hebben in de loop van hun evolutionaire geschiedenis eigenschappen ontwikkeld die hun "evolueerbaarheid" vergroten, waardoor ze zich effectiever kunnen aanpassen aan nieuwe omstandigheden. Evolutie is dus niet zo blind als de standaard evolutietheorie zou doen vermoeden. Er zijn talloze voorbeelden van eigenschappen die geen direct voordeel opleveren, maar in plaats daarvan het aanpassingsvermogen op de lange termijn bevorderen. Bacteriën kunnen bijvoorbeeld hun mutatiesnelheid onder stressvolle omstandigheden verhogen om de productie van nieuwe adaptieve mutanten te versnellen en ontwikkelingssystemen zijn zo gestructureerd dat willekeurige genetische veranderingen in de richting van functionele fenotypes worden gestuurd. Er bestaat al 3,5 miljard jaar leven op aarde en gedurende deze tijd zijn de omstandigheden voortdurend veranderd en heeft het leven zich voortdurend aan deze veranderingen aangepast door te evolueren. Het vermogen om zich aan te passen aan nieuwe omstandigheden door middel van evolutie, oftewel evolueerbaarheid, moet dus een cruciale factor zijn in het succes van biologische lijnen. De standaard evolutiebiologie heeft echter weinig mogelijkheden om dit idee binnen haar raamwerk te plaatsen. Er wordt niet onderzocht waarom er organismen zouden kunnen zijn die beter evolueerbaar zijn dan andere, noch hoe deze verschillen in evolueerbaarheid bereikt zouden kunnen zijn. Ik beweer dat als we organismen beschouwen als onderling afhankelijke en verbonden systemen, in plaats van lukrake combinaties van componenten, het mogelijk is om te zien hoe ze evolutionaire "herinneringen" van vroegere selectie in hun architectuur hebben ingebouwd. Deze herinneringen kunnen worden opgeroepen wanneer de organismen zich aanpassen aan hedendaagse uitdagingen. Vanuit dit perspectief is evolueerbaarheid een onderdeel van de architectuur van organismen en lijkt evolutie meer op een "Herinnerend Architect" dan op een "Blinde Horlogemaker".

Het doel van dit proefschrift is om de invloedvan de architectuur van organismen op evolueerbaarheid te onderzoeken en de rol van architectuur in het evolutieproces in een theoretisch raamwerk te integreren.

Hoofdstuk 1 introduceert het concept van evolueerbaarheid en geeft een algemeen overzicht van de thema's van dit proefschrift. Er worden redenen gegeven voor het belang van evolueerbaarheid en er wordt ingegaan op de verschillende definities van deze term. Vervolgens worden de beperkingen van de standaardtheorie geanalyseerd die het moeilijk maken om evolueerbaarheid binnen het raamwerk van de standaardtheorie te bestuderen. Om de studie van evolueerbaarheid te vergemakkelijken wordt een nieuwe benadering voorgesteld die zich richt op organismale architectuur. De architectuur van

organismen kan informatie verzamelen over succesvolle aanpassingen over lange perioden, waardoor deze aanpassingen uit het verleden gemakkelijker kunnen worden ingezet wanneer soortgelijke situaties zich in de toekomst voordoen. Om dit nieuwe raamwerk te realiseren, pleit ik voor een multidisciplinaire aanpak, en ik geef een opsomming van nzichten uit evolutionaire disciplines in andere computationele velden die het belang van architectuur in het mogelijk maken van evolueerbaarheid al benadrukken. **Hoofdstuk 1** sluit af met een bespreking van de methodologische aanpak in dit proefschrift.

Hoofdstuk 2 heeft als doel een duidelijker kader te bieden voor het begrijpen van het concept van evolueerbaarheid. Evolueerbaarheid t kan verwijzen naar veel verschillende biologische fenomenen; deze veelzijdigheid kan soms leiden tot misverstanden over wat men precies bedoelt met deze term. Om de zaken uit elkaar te halen, worden de genetische, ontwikkelings- en ecologische factoren die invloed hebben op evolueerbaarheid ingedeeld in drie categorieën: factoren die genetische variatie verschaffen, factoren die de effecten van variatie op fitness beïnvloeden en factoren die het selectieve proces vormgeven. De tijdschaal is ook belangrijk, omdat sommige evolutionaire processen een effect hebben over vele generaties terwijl andere een beperkter tijdsverloop hebben. We maken ook onderscheid tussen mechanismen die de evolutie van tal van eigenschappen beïnvloeden, versus mechanismen die een beperktere invloed hebben op specifieke aanpassingen. Daarnaast identificeren we twee belangrijke perspectieven op evolueerbaarheid in de literatuur: als een algemene, blijvende capaciteit van lijnen over evolutionaire tijdschalen, of gericht op aanpassing aan nieuwe omgevingen of niches. Door deze gestructureerde benadering hopen wij de discussies rond evolueerbaarheid te verduidelijken en het gesprek tussen vakgebieden te vergemakkelijken.

Hoofdstuk 3 gaat in op de tweede categorie die hierboven is genoemd: factoren die de effecten van variatie op fitness beïnvloeden. Het is een van de centrale dogma's van de biologie dat genetische mutaties willekeurig zijn wat betreft de "vereisten" van natuurlijke selectie. Toch laten veel voorbeelden zien dat de effecten van genetische mutaties op fenotypische niveau vaak adaptatiebevorderend zijn. Met behulp van eenvoudige modellen voor de evolutie van genregulatie netwerken laten we zien hoe willekeurige genetische mutaties omgevormd kunnen worden in adaptatiebevorderende fenotypische variatie en hoe zo'n omvorming relatief makkelijk kan evolueren. Ondanks de inherente willekeurigheid van het genetische mutatieproces, evolueerden zelfs kleine gennetwerken snel een architectuur die de fenotypische effecten van mutaties beïnvloedt. Deze bias leidt tot een aanzienlijke versnelling van adaptieve evolutie wanneer de omgeving verandert. We noemen de specifieke configuratie van het netwerk dat willekeurige genetische mutaties vertaalt in evolutiebevorderende fenotypische effecten een "mutatie omvormer". Bovendien identificeren we twee verschillende typen mutatie omvormers: "mutatie versterkers", die door weinig mutaties grote veranderingen in het fenotype mogelijk maken (hun effect versterken), en "mutatie kanaliseerders", die de overgang tussen twee specifieke fenotypes vergemakkelijken (d.w.z., die mutaties effectief kanaliseren in een van de twee uitkomsten).

Hoofdstuk 3 wordt gevolgd door twee "intermezzo's". Beide zijn theoretische bijdragen aan gepubliceerde artikelen, die in dit proefschrift zijn opgenomen omdat ze mijn opvattingen over de architectuur van organismen hebben helpen vormen en het uitgangspunt vormden voor andere hoofdstukken van het proefschrift.

In **hoofdstuk 2** en 3 pleit ik voor een mechanistische benadering van het modelleren van evolutionaire processen. In **Intermezzo 1** schets ik zo'n benadering voor de evolutionaire modellering van niet-genetische overerving. In een eerste stap geef ik een overzicht van reeds bestaande pogingen om niet-genetische overerving op te nemen in het evolutionair raamwerk en belicht ik hun sterke en zwakke punten. Vervolgens beschrijf ik een meer mechanistisch model dat recente moleculaire en evolutionaire inzichten over niet-genetische overerving incorporeert en nauw aansluit bij mijn missie om de architectuur van organismen meer aandacht te geven.

Intermezzo 2 is de modelcomponent van een empirische studie die experimenteel aantoont dat sluipwespen nog steeds in staat zijn, hun vetmetabolisme te induceren. Dit is verrassend, want de vakwereld ging er vanauit dat deze wespen hun vetmetabolisme al miljoenen jaren geleden zouden hebben verloren. Wij beargumenteren dat de wespen de eigenschap nooit echt hebben verloren, maar gedurende lange perioden alleen heel af en toe hebben ingeschakeld. Maar dan rijst de vraag: hoe kan zo'n plasticiteit behouden blijven als deze slechts sporadisch wordt gebruikt? Om dit te beantwoorden hebben we een eenvoudig model ontwikkeld voor een schakelaar die een metabolische route aan of uit kan zetten. We laten zien dat zo'n schakelaar gemakkelijk kan evolueren en vervolgens stabiel kan blijven bestaan ondanks mutatie-erosie, zelfs als de schakelaar zelden wordt gebruikt.

Hoofdstuk 4 borduurt voort op het onderzoek uit **Intermezzo 2** en onderzoekt hoe complexe aanpassingen kunnen blijven bestaan, zelfs als selectie slechts sporadisch plaatsvindt. Veel organismen bezitten aanpassingen die slechts af en toe, eens in de paar generaties, gunstig zijn. Wij begrijpen nog steeds niet goed hoe dergelijke aanpassingen kunnen evolueren en intact blijven ondanks dat ze worden aangetast door genetische drift en de opeenstapeling van mutaties. Eerder onderzoek naar dit onderwerp heeft zich gericht op eenvoudige eigenschappen, maar de meeste aanpassingen in de natuur zijn complex. Om dit te onderzoeken heb ik met behulp van simulaties de evolutie van genregulatie netwerken bestudeerd die coderen voor eigenschappen die variëren in complexiteit. Het bleek dat complexe aanpassingen gemakkelijk ontstaan en gedurende duizenden generaties stabiel blijven mits selectie eens in de 5 of 10 generaties voorkomt. Wanneer selectie minder frequent optreedt (eens in de 100 generaties), ontstaat de aanpassing nog steeds en blijft ook bestaan, maar de kwaliteit van de aanpassing is duidelijk lager. De kwaliteit blijft op een hoger niveau als de onderliggende netwerkarchitectuur complexer is, ondanks het feit dat complexe netwerken makkelijker door mutaties ontregeld kunnen worden.

In **hoofdstuk 5** gebruik ik biologische inzichten om het ontwerp van algoritmen voor genetisch programmeren, die worden gebruikt bij machinaal leren, te verbeteren. Genetisch programmeren is een tak van de computerwetenschap die evolutionaire algoritmen gebruikt om nieuwe oplossingen te vinden voor KI toepassingen. In dit hoofdstuk bespreek ik de "Grammar-Guided Genetic Programming" methode, een veelbelovende KI techniek die grammaticale regels introduceert om een concrete probleemstelling te vertalen naar een computerprogramma. Ik stel een nieuwe methode voor, genaamd "Facilitated Mutation", die het mutatieproces van de grammaticale relgels laat afhangen van de specificiteit van deze regels. Algemene regels muteren maar heel af en toe, waardoor de kernfunctionaliteit van het programma niet vaak wordt aangetast. Daarentegen mogen

specifieke regels vaker mogen muteren, waardoor en verschillende varianten van het programma kunnen worden verkden. Deze benadering is geïnspireerd op observaties in de biologie, waar organismen de functionaliteit van kritieke genen behouden terwijl ze minder belangrijke genen toestaan om sneller mutaties te ondergaan. In **hoofdstuk 5** wordt de effectiviteit van Facilitated Mutation voor een uitdagend KI probleem vergeleken met de traditionele mutatie benadering. We laten zien dat Facilitated Mutation ten opzichte van alle onderzochte benchmarks beter presteert: er worden niet alleen betere oplossingen gevonden, maar ook een grotere variëteit aan oplossingen van hoge kwaliteit.

Hoofdstuk 6 tenslotte bevat een algemene discussie van mijn proefschrift. Dit hoofdstuk bestaat uit twee delen. Eerst schets ik enkele brede filosofische overwegingen die tijdens het schrijven van dit proefschrift zijn ontwikkeld. Ik pleit voor een meer pluralistische kijk op de evolutietheorie, waarbij verschillende soorten theorieën en benaderingen niet gezien moeten worden als conflicterend, maar als verschillende invalshoeken van waaruit men een wetenschappelijke vraag kan benaderen. Ten tweede geeft **hoofdstuk 6** een overzicht en samenvatting van de onderzoeksresultaten van dit proefschrift en identificeert het interessante toekomstige richtingen voor dit studiegebied. Het werk in dit proefschrift pleit voor het belang van de architectuur van organismen bij het vasthouden van evolutionaire herinneringen, waardoor organismen die gevormd zijn door een blind proces hun vermogen om zich aan te passen aan nieuwe omgevingen kunnen verbeteren.

1

INTRODUCTION

Stefano TISO

...if one could understand a flower as it has its Being in God
this would be a higher thing than the whole world!

attributed to *Meister Eckhart*

↓

Little flower—but if I could understand
What you are, root and all, and all in all,
I should know what God and man is

Alfred Tennyson, Flower in the Crannied Wall

↓

To see a World in a Grain of Sand.
And a Heaven in a Wild Flower.
Hold Infinity in the palm of your hand.
And Eternity in an hour.

William Blake, Auguries of Innocence

↓

...if we could understand a single flower we would know who we are and what the world is

Jorge Luis Borges, A Personal Anthology

1.1. TENETS

More than three and half billion years ago, while Earth's molten crust was still cooling down, life took root on this planet, and to this day, it is still flourishing. In this dynamic world where astronomical, geological, and ecological phenomena lead to ever-shifting conditions, living beings face perpetually changing trials. Their only way to survive and thrive is to adapt through the process of evolution by natural selection. Life and Evolution are thus the two sides of the same coin since their inception. For this reason, I argue that living beings throughout their long history must have, to an extent, evolved the ability to evolve.

Like other scientists, I, therefore, consider "evolvability", this capability to undergo adaptive evolution, a fundamental driver of the success or downfall of biological lineages. Yet, standard evolutionary theory has for a long time overlooked this topic, focusing on a more short-term view of evolution. The long-term influence of evolvability has been dismissed as negligible since, as Richard Dawkins eloquently expressed, evolution is considered akin to "a blind watchmaker".

In this thesis, I question some of the assumptions of standard evolutionary biology. I try to show that, when these assumptions are relaxed, evolution does not seem so blind, and that evolvability plays an important role, revealing a "reminiscing architect" next to the "blind watchmaker". Building upon past successes, evolvability empowers organisms to meet ever-new challenges. The solutions to past adversities remain ingrained in the architecture of those who survive, making them more prepared for future struggles. With only a few additions to the mainstream perspective, we can arrive at new conclusions; integrating evolvability and its consequences in a comprehensive evolutionary framework. These additions are:

- To consider evolution as taking place in a dynamic environment, where selective targets are never static but constantly changing. Organisms, therefore, do not adapt to a single and unchanging selective target but evolve adaptive response strategies to a series of challenges.

- To see organisms not as a sum of independent traits determined by independent genes, but as the emergent result of the coordinated action of many interconnected components. These components do not individually contribute to the organism's fitness, but structured together they build adaptive response mechanisms.

- Finally, and most importantly, to recognise that a fundamental determinant of evolvability is the structural relationship between the mechanisms that give rise to an organism i.e.: organismal architecture. It is in the architecture of organisms that we can read the records of the steps they took to increase their evolvability.

In the following introduction, I will expand on and articulate this brief declaration of intent. Firstly, I will elucidate the significance of the concept of evolvability and provide an overview of its many definitions and key features. Secondly, I will critically evaluate the limitations associated with the standard theoretical approach to studying evolvability. In doing so, I will highlight the need for a more nuanced and comprehensive understanding of this concept. Thirdly, I will present my rationale for exploring the role of architecture in

facilitating a deeper understanding of evolvability. Specifically, I will argue that studying architecture can offer unique insights into higher-level evolutionary dynamics and can help shed light on the fundamental principles that govern the evolution of complex biological systems. Fourthly, I will explain the methodology that I intend to use to show the relevance of architecture and evolvability, providing a detailed justification for its suitability. Finally, I will give a brief outline of the research chapters contained in this thesis and their significance to my overall goal.

1.2. EVOLVABILITY

One of the most striking aspects of life on Earth is the remarkable diversity and success of different organisms, ranging from microscopic bacteria to complex humans. Despite their vast differences, many types of organisms have managed to thrive and avoid extinction. What sets successful organisms apart from those that go extinct? For instance, how can we explain the almost complete absence of multicellular organisms that exclusively use asexual reproduction (only one animal clade, Bdelloid rotifers, seems to exclusively display this reproductive mode) [1–3] ? This question puzzles biologists, as asexual reproduction has clear short-term advantages in replication efficiency. This question is one of the greatest dilemmas in evolution. One explanation, for instance, is that the persistence of multicellular sexual reproduction might be a necessary requirement for multicellular organisms to escape the constant extinction threat of unicellular parasites [4]. Although many multicellular organisms almost exclusively reproduce asexually they can switch to sexual reproduction under stressful conditions. This way they can respond by recombining their genetic material and find new solutions that allow them to survive. The scarcity of obligate asexual reproduction in multicellular organisms can thus, at least in part, be explained by the fact that many lineages that were obligatory asexual might have been wiped out from the evolutionary record due to a fatal parasitic invasion.

Sexual reproduction could not only help escape the parasitic onslaught but could also grant other selective advantages. For instance, it is also proposed it could help avoid other deleterious factors such as mutational meltdown, which happens in asexual populations and would not allow sustained survival of organisms of a certain complexity [5]. Thus, while less efficient at replication than asexuality in the short-term, given the right initial conditions (heterogeneous environment or selective pressures, small population and/or frequent bottlenecks) sexual reproduction can persist long enough to then triumph over asexuality thanks to its long-term benefits [1, 6].

On the other hand, one might ask: How can bacteria be able to survive their own parasites (i.e. bacteriophages) if they are asexual? And indeed it might very well be that they just developed a different design, which allows them to employ a strategy that differs from that of multicellular organisms. Their simpler and streamlined design makes them more robust to abrupt genetic changes and thus they are able to use other, somehow more radical, mechanisms for genetic recombination such as: integrating random DNA fragments picked up from the environment (transformation [7]), transferring plasmids to one another, even across very different bacterial families (horizontal gene transfer or conjugation [8]), and even by using some of their virus parasites themselves as vectors for exchanging genetic materials (transduction [9]). This is a clear example of how cer-

tain designs can prove more advantageous than others in favouring adaptive evolution under certain conditions (e.g. sexual reproduction for complex multicellular organisms; transduction, conjugation, and transformation for the more streamlined bacteria), and that organismal designs are driven by the necessity of being able to adapt to constantly new selective challenges.

An organism's ability to adaptively respond to new conditions is crucial for its survival and propagation. However, it is not trivial to explain how the capability to produce these adaptive responses evolved in the first place. Selection is blind, acting only on present, short-term conditions and variation, and cannot target features that facilitate long-term evolution to conditions that have not yet been met. Nevertheless, I will argue in this thesis that long-term features can and did evolve along evolutionary history. Lineages that accumulate short-term adaptations that eventually lead to long-term advantages will be able to produce better-matched phenotypes to new conditions. Thus, these serendipitously long-term oriented lineages will be more likely to persist and dominate the evolutionary record compared to those lineages whose short-term adaptations did not confer them the ability to respond adaptively to unforeseen conditions. For instance, when talking about sexual reproduction it has been shown that it is advantageous to use it only in a restricted set of circumstances [10, 11], but those few multicellular organisms who luckily acquired it because of the initial short-term need for it, and then managed to maintain it, were also those that to this day have been able to survive catastrophic events such as new pathogens outbreaks and long term genetic melt-down. The ability to maintain and gather long-term adaptation acquired through short-term selection is, in my opinion, greatly influenced by how organisms structure the relationship among their different traits, i.e. their architecture.

For this reason, I consider evolvability and evolvability-enhancing architecture fundamental to understanding long-term evolutionary dynamics, the factors that govern the success or failure of biological designs, and the evolution of adaptive responses. By including evolvability-enhancing architecture as a relevant factor, the framework of evolutionary theory is expanded. While standard evolutionary theory mainly focuses on single adaptation events through gradual changes it often treats long-term evolutionary phenomena as simple statistical events dictated by speciation and extinction rates. Standard theory to this day struggles to provide a proximate explanation of how microevolution leads to macroevolution [12, 13]. Evolvability allows for a description of the different strategies and constraints that different organisms have evolved while faring through ever-changing conditions and therefore helps explain the emergence and disappearance of groups of organisms (and their design) over time.

Evolvability can be defined broadly as "a system's capability to undergo adaptive evolution" (where capability here indicates the degree to which an organism is capable of undergoing adaptive evolution, not the presence/absence of this ability). However, as Pigliucci [14] argues in his opinion paper "Is evolvability evolvable?", evolvability encompasses a family of related but distinct phenomena. When discussing evolvability, we are referring not to a single concept but rather to overlapping ideas that illuminate different facets of evolution in action. Therefore a general definition has limited utility for focused

research questions, as this concept has a very broad range of potential applications and spans multiple fields of research. As a result, scientists have proposed more specific definitions tailored to their areas of study.

Pigliucci identifies three different types of definitions based on the different evolutionary levels/scales on which they focus: evolvability as the genetic variation within a population which can act as the raw material for selection (genetic scale) [15], evolvability as the ability of an organism to produce variation, i.e. variability (genotype-to-phenotype scale) [16], and finally, evolvability as the capacity to undergo major evolutionary transitions (macroevolutionary scale) [17]. Other definitions have been proposed in evolutionary biology (see [18, 19], and Box 1 of Chapter 1 of this thesis), but they more or less adhere to the same pool of ideas. On the other hand, different fields of study have come up with definitions on their own. These fields "cross-fertilize" with biology, while, on the other hand, pursuing their own research interest, providing different definitions of evolvability. For instance, Charles Ofria, Christoph Adami, and other students of Artificial Life systems such as Avida, often define evolvability in terms of the ability to perform different or more complex (algorithmic) operations [20–22]. The goal of this research is to see if organisms can be seen in terms of algorithmic logic, and therefore if this type of logic could help us through its principle to better describe and reproduce biological life and evolution. Researchers coming from the field of artificial intelligence such as Kenneth Stanley often see Evolvability as the ability of a system to produce innovative behaviours not observed before [23, 24]. The goal of their research is to exploit the unsupervised process of evolution as an automated optimisation/creation process, that would allow them to find new interesting solutions to difficult problems, without the need for a human designer and unshackled from human cognitive biases. Finally, Richard Watson applies an information theory and theory of learning approach to the concept of evolvability [25]. He defines it as an organism's capacity to encode (e.g. in its genome) adaptive solutions to past selection pressures in a generalisable way, allowing effective responses to novel situations. More evolvable lineages will better extract useful information from past selection events and flexibly apply it to new conditions. Watson, using this perspective, is interested in understanding if an unguided and unconscious process such as evolution can display "cognitive-like" properties similar to learning.

While this plurality of definitions reflects the rich array of ideas that fall under the banner of evolvability, it can also introduce conceptual confusion as sometimes different definitions will point towards aspects of evolvability that are almost incommensurable. For instance, for a researcher who studies evolvability as the amount of genetic variation in a population [26], it can be very difficult to communicate with someone who researches evolvability as the capability to generalise previous selective events and re-use them for future challenges [25]. For this reason, if we want plurality to lead to richness instead of confusion, we need to acknowledge the diversity, and potential incommunicability, between different lines of research both when giving or scrutinising a definition.

The concept of evolvability not only helps expand fundamental theory and research but has relevant practical implications as well. Evolvability can influence applied domains like medicine, agriculture, and engineering, demonstrating the concept's relevance beyond theory. Evolvability could help to predict future events that would threaten hu-

man stability such as the rise of new pandemics [27], or the accumulation of antibiotic resistance [28]. On top of being used to prevent risks, research on evolvability could also inform future policies aimed at improving our ecological impact on the planet, such as biodiversity and conservation efforts, containment of invasive species, assessment of the risk and impact of climate change [18]. Moreover, the concept of evolvability could drive research into new advancements to increase our quality of life, for instance, breeding crops for evolvability is a promising approach for tackling complex real-world problems [29, 30]. Evolvability is not only a subject of interest in topics that are related to biological evolution. Evolution has already been employed in the fields of engineering, robotics, and informatics with increasingly impressive results [31–33]. Many researchers in these fields would like to understand more about evolvability in this sense and extrapolate its general properties. Their interest lies in the ability to apply unbounded evolutionary potential for novelty to systems where solutions to real-life problems such as engineering or software development are evolved instead of hand-crafted by humans. This ability of evolution to produce unbounded complexity and novelty is sometimes referred to as open-ended evolution, one of the main research goals especially of the artificial life community [34].

1.3. THE VIEW OF STANDARD EVOLUTIONARY THEORY

Despite a growing consensus, there remains significant debate regarding the importance and prevalence of evolvability in shaping the course of evolution. Standard evolutionary theory has historically paid relatively little attention to this topic and has yet to fully integrate it within its framework [14].

The standard theory of evolution emerged in the early 20th century as a rigorous formalisation of evolution in the language of mathematics and statistics. It led to significant advances in our understanding of evolutionary processes, including the reconciliation of discrete inheritance with continuous traits by Ronald Fischer [35, 36], the concepts of genetic linkage and genetic mapping by J.B.S. Haldane [37, 38], and the metaphor of fitness landscapes by Sewall Wright [39–41]. This work has not only revolutionised the way evolution was understood after its inception. To this day, its principles underpin the fields of population and quantitative genetics, arguably the two most widespread and relevant approaches to the study of evolution.

However, standard theory is not without limitations. At the time of its formulation, scientists lacked the empirical and mechanistic understanding of an organism's cellular, physiological, and developmental processes, and appropriate techniques for studying them. The limited computational power of the time also inhibited the amount of complexity that could be modelled in evolutionary dynamics.

As a result, mathematical and statistical models were developed. These models, by their nature, allow researchers to overlook the intricate mechanics of biological life focusing on observable outcomes. Despite not being fully solvable analytically, equilibrium and stability analyses, as well as approximation techniques can be applied to these models without requiring extensive computational efforts. Studying the dynamics of natural selection, however, is intrinsically complicated and to be able to use these models, limiting assumptions have to be made. For instance, genotype-to-phenotype (G-P) mappings are mainly described as simple 1:1 relations between genotype and phenotype, the effects of

genetic mutation are often considered to be unbiased/random and infinitesimal, as the number of loci underlying a trait, and evolution is studied at stable equilibrium conditions with a single fixed selective optimum [36, 42].

This is not to say that standard theory r ejects the possibility of relevant complex interactions between genotype and phenotype, the existence of non-random and non-uniform mutation effects, or the importance of dynamic selective regimes. However, accounting for non-linear interactions, except for particular sub-cases, is extremely hard with just analytical tools, as it is for non-uniform mutation effects or studying evolution outside of equilibrium conditions. Therefore, given these limitations, researchers at the time wisely decided to focus their efforts in directions where the techniques available could be most effectively used to guide research.

1.4. SEEING PAST THE BLIND WATCHMAKER

The focuses and limitations of standard theory limit its scope of inquiry and make it inadequate for the study of evolvability, as they encourage a phenomenological approach to evolutionary study and consequently a gene-centric, short-term view of evolution.

A phenomenological approach aims to describe only the observable outcomes of an event, disregarding the internal mechanisms that lead to it. Classical theory, in this spirit, mainly considers genes (the carriers of information) and phenotypes (the decoded information which is the target of selection) minimising the importance of the internal processes that lead from one to the other.

A gene-centric view of evolution logically ensues from this perspective. Genes independently and directly translate into selectable phenotypes, with no significant intermediate step, and are therefore considered the fundamental unit of selection and the only relevant variable for understanding evolution. Genes are thus seen as selfish entities, in constant competition with one another, coming together with the sole purpose of maximising their own reproduction. It is the selective advantages of individual genes that drive the fate of evolutionary dynamics.

This gene-centric view naturally leads to a “short-term” conception of evolution, eloquently described by Richard Dawkins in his famous analogy of “evolution as a blind watchmaker”. In this metaphor, genes can be seen as the individual pieces of a complex clockwork mechanism (the organism), shaped in myriads of infinitesimally different variations by the effect of small random kinks (mutations), and drawn and assembled by a blind watchmaker (evolution). The watchmaker is blind and has no intention nor foresight, shuffling components randomly with no knowledge of their current role nor future usefulness. Evolution thus can only select for organisms in the present moment, discarding those who fail and continuing to build upon those who survive, unable to select an organism designed to face future challenges; as the watchmaker is blind and therefore oblivious to them.

Evolvability, however, has not been completely dismissed by the standard theory and those aspects that are treatable by a short-term population and quantitative genetics approach have been successfully integrated [15, 26]. This line of research mainly focuses on the role of present variation as raw material for selection and has a fruitful theoretical history : from considering evolvability as a quantity similar to heritability (conceptualised

by Houle in his seminal paper [26]), to, more recently, defining it as the alignment of genetic variation with the gradient of selection (the G-Matrix, as done in the on-going work of Hansen [43]).

What these studies do not consider is another crucial aspect of evolvability: the capability to create new variation and to bias it towards adaptive outcomes. Framing evolution this way (phenomenological, gene-centric, short-term, blind) naturally tends to preclude other crucial aspects of evolvability from being a relevant factor to evolutionary dynamics: the capability to create new variation and to bias it towards adaptive outcomes. When organisms cannot be selected for their capability to adapt to new selective challenges and genes constantly fight for their own survival disrupting interactions beneficial in the long-term, it is hard to envision how the ability to produce new variation or to bias it could ever arise.

A broader view of evolvability encompasses both adaptations in response to selection and innovation through generating novelty.

To do so, it is necessary to move from a phenomenological approach toward a mechanistic one [44], where the aim is to explain the nature of the outcomes of evolution in terms of biological processes. This approach is more in line with recent progress and discoveries in the biological sciences. With the omic revolution and the rise of systems biology, it is becoming more and more evident that gene interactions, biased effects of mutations, and environmental conditions play a fundamental role in determining an organism's phenotype [45–47], and theoretical models show that by taking these elements into considerations changes the outcomes of evolution [48–50]. Only when one looks at the inner response mechanism, the innovative potential of the evolutionary process can be understood and explained. Without a knowledge of the inner mechanism, no interesting nor significant explanation can be given as to how organisms generate more or less variation, nor how they can potentially shape this variation in their favour.

In particular, I argue that the design principles and motifs in organismal architecture that facilitate evolvability reveal the otherwise hidden potentials of evolution (e.g. the ability of population to adapt to new challenges not only based on their standing genetic variation, but for instance as well thanks to the ability of the individual's to produce new and possibly adaptively biased variation). By examining the interplay between architecture and evolvability, we gain a richer sense of what evolution can achieve and how it might operate.

Evolutionary developmental biology offers a wealth of examples highlighting the role of organismal architecture in evolvability. The astounding success of arthropods is attributed by many to the modular and flexible architecture of their developmental program, especially of their appendages [51]. The developmental genes of arthropods are organised in such a way that they can be easily combined in different places at different times [52, 53] to produce a wide variety of appendages. This ingenious architecture allowed arthropods to easily evolve appendages to accomplish almost any task: from evolving mouthparts for feeding, to antennae for perception, wings for aerial locomotion, and even gills for breathing [54]. The best instance to display the success of this architecture in enhancing the evolvability of arthropods is the so-called Cambrian explosion. Most of the genes and developmental programs responsible for the burst of diversity in body plans during that period are still being retained to this day [55]. While this variety has

been shaved down and fine-tuned to a restricted series of main solutions it still retains the capability for incredible innovative potential, re-exhuming ancestral configurations to face new or returning challenges, as it has happened for instance in the re-convergent evolution of mantis-shrimp raptorial appendages [56].

This example highlights the point that to understand the aspect of evolvability tied to innovative potential, we must turn our attention to organismal architecture and its history.

1.5. A REMINISCENT ARCHITECT

Understanding evolvability through the lens of organismal architecture can provide insights into the higher-level dynamics that play out over long evolutionary timescales such as those of lineages' successes and downfalls. As a first thing, it is important to define what organismal architecture means for the purposes of this thesis. A general definition of organismal architecture can be given as: "the structural relationship among the elements that compose an organism". What these components are and how they influence evolution changes depending on the scale being analysed, from the genetic to the morphological level. At the genetic level, for instance, architecture refers to the organisation and regulation of gene expression. For example, prokaryotic genetic architecture is often streamlined, with genes linked spatially and functionally, allowing to easily switch on and off entire metabolic functions, while on the other hand, eukaryotic architecture scatters functionally related genes throughout the genome allowing for more interconnected and fine-grained regulation [57]. At the developmental level, architecture describes how different parts of the developmental program interconnect and build upon each other to form morphological structures. For example, it is the architecture of the interactions with other developmental factors of the Hox gene family that allows it to produce many different structures e.g.: limbs, central nervous system, and vertebrae, making it fundamental during development across the tree of life [54]. A similar example can be seen in the expression of Distal-less (Dll) in the formation of arthropod appendages mentioned in the previous section [58]. At the morphological level instead, architecture involves the arrangement and interaction of physiological processes, body parts, and systems that determine an organism's morphology and functioning. The architecture of the chambered heart and lungs in vertebrates varies to accommodate the energetic needs of different clades: the reptilian three-chambered heart allows for partial recharge of anoxic blood, apt for sustaining the low-level metabolism of cold-blooded organisms, while the mammalian four-chambered heart grants maximal efficiency in oxygenation, sustaining high-demand metabolism [59]. These are just a few examples of what the concept of architecture could mean, but as with the concept of evolvability, this term encompasses a wide range of phenomena and its definition cannot be univocal.

The pivotal question this thesis asks is: How can an organismal architecture - how an organism is structured and organised - influence the ability to evolve adaptively?

If natural selection acts in a short-term/blind manner, solely on the basis of an organism's current phenotype and fitness, how can evolvability and adaptive capacity nonetheless emerge over evolutionary time? Together with other scientists [18, 60–62], I argue that this is because the solutions that enable organisms to overcome selective chal-

lenges become ingrained in their architecture and that of their lineages. As environments change in the future, this accrued evolutionary baggage can facilitate new adaptations.

This can be exemplified by the increase in the rate of acquisition of antibiotic resistance in bacteria. Bacteria are becoming faster at responding to antibiotic treatments, and more and more bacteria are acquiring resistance to the vast majority of antibiotic treatments [63, 64]. One reason for this lies in their capacity to store past solutions in their genetic architecture. Consider a population of bacteria exposed to a new antibiotic. Mutations that happen to confer resistance often involve the re-purposing of influx/efflux pumps to rid the cell of toxins as well as the modification of the composition of the cell membrane [65]. Those individuals with mutations that apply the right membrane modifications or recruit more of the correct pumps under stress will thus survive and reproduce. If the same bacterial lineage then encounters a similar antibiotic in the future, under similar stress conditions, this genomic memory may aid in evolving resistance more quickly or easily (multi-drug resistance) as often antibiotics belonging to the same or to a closely related family act in similar ways [66].

This example illustrates how the “ghost of selection past” [67, 68], stored in organisms’ architecture, could shape evolvability, making it possible for organisms to create and bias variation towards adaptive outcomes. While natural selection alone cannot explain the evolution of features that facilitate evolution itself, the architecture shaped by natural selection gains a form of reminiscent foresight. Over vast timescales, the accumulation of such evolutionary solutions and "memories” in developmental, genetic, cellular, and morphological architectures enable increasing adaptability and resilience. Examples of this are for instance, the plastic tuning of limb length depending on terrain type in anole lizards [69], or the constraint of beak development only in the three relevant dimensions useful to adjust to food type in Darwin’s finches [70, 71]. The ensemble of historical adaptations equips the organism with latent abilities to meet new challenges. This view suggests that the dichotomy between "blind" natural selection and the concept of evolvability is false. Dawkins’s watchmaker is blind and unguided, mixing genes into combinations, which sometimes by chance, happen to work. But besides it is a reminiscent architect who takes care to integrate and preserve the watchmaker solutions into the organisms’ architecture, and uses them as the base upon which to build future adaptations. Thus, the reminiscing architect gradually constructs robust and functional organisms with the help of the blind watchmaker. These organisms contain the record of their past hurdles and can, thanks to this, weather through new conditions. Natural selection forges the structures that enable evolution, broadly conceived, to become an open-ended creative process. Evolvability-enhancing architecture arises inevitably from accumulated experience. The effectiveness of natural selection at solving short-term ever-changing problems thus yields evolvability for the long run.

However, as much as this conjecture might be interesting and the examples brought in its favour compelling, it is extremely difficult to empirically capture the long-term evolutionary dynamics of organismal architecture and its impact on evolvability. Whilst we have sufficient empirical examples that show that organisms possess mechanisms and strategies to create and bias variation toward favourable outcomes [44, 62, 72–76], we understand little of how this mechanism can come to exist in the natural world. Theory can come to our aid and has produced many different models that point towards

promising directions for the role of the reminiscing architect in the game of the blind watchmaker.

1.6. INSIGHTS FROM COMPUTATIONAL THEORY

The role of theoretical modelling in the study of evolvability has been paramount since its inception. Being related to long-term dynamics, and involving a plethora of phenomena, evolvability is often difficult to pinpoint in empirical experiments. In particular, this is true for what concerns those studies that analyse the aspects that interest this thesis: the production of adaptive variation in relation to organismal architecture.

To my knowledge, mathematical/computational models addressing the role of architecture, variation production, and its canalisation towards adaptive outcomes, are relatively recent, starting to appear in the literature less than two decades ago. Before that time, the topic of the role of variation and architecture in evolvability was already being discussed (e.g. see the seminal paper from Gunter Wagner and Lee Altenberg [16], the work of Kirschner and Gerhart [19, 62, 76], and way before that the work of Waddington [77]), but the discussion remained on the conceptual level and was mainly backed by verbal arguments. Evolvability was already being modelled, however, this was done from a population genetic approach seeing evolvability as the existent variation in a population [26] and with no regard to organismal architecture (as I already mentioned previously).

In most cases for the models analysing the role of architecture and variation with respect to evolvability, the attention is focused on the regulatory architecture at the genetic level (not to be confused with the structural architecture of genes in the nucleus). These models usually employ various representations of a Gene Regulatory Network (GRN) that leads to the expression of a phenotype. GRN models consider gene-to-gene interactions as the key mechanism from which the phenotype emerges, contrary to the more standard view (population genetics, quantitative genetics, adaptive dynamics, etc.) in which the allelic values are sufficient to derive the phenotype. GRN models often include nonlinear interactions (typically disregarded by standard theory) and model a much higher number of loci than models in population genetics and assume much stronger gene effects than models in quantitative genetics. Overall GRN models encapsulate a more mechanistic approach, which makes them more apt to study the evolution of evolvability and organismal architecture. This type of modelling is not only used to study theoretical evolutionary questions, other disciplines like evolutionary developmental biology (evo-devo) [78] and evolutionary systems biology [79] make as well ample use of these tools and are arguably the fields that first conceived this approach. To my knowledge, a comprehensive review of the different GRN representations in more theoretical/conceptual research is not yet available. Although in no way complete, I will try to provide a brief overview of some of the relevant models and the insights they provided in the coming paragraphs. In particular, I will focus on those models and insights that have guided me to formulate my conception of the relation between evolvability and organismal architecture. These insights are:

- The capability of architecture to evolve through natural selection to bias mutations towards adaptive outcomes.
- The fact that complex architectures can easily evolve to robustness: i.e. reduce the

negative effects of deleterious mutations and enlarge the search space of evolutionary solutions.

- The proof that architectures in changing environments can evolve to become modular i.e.: having an independent functional component that can be flexibly changed and recombined
- And finally but most importantly that architectures indeed have a form of "developmental memory" or as I call it reminiscent foresight: i.e. they can retain solutions to the past selective challenge and redeploy them when in need.

Some of the first models to explicitly address the relationship between evolvability and architecture, are those from Pauline Hogeweg [59, 61, 72]. Hogeweg and collaborators show how natural selection can spontaneously restructure architecture to lead to the production of adaptively biased variation. They model an organism's GRN with a complex and evolvable architecture representing its metabolic pathways. GRNs evolve in a regime of two changing environments (i.e. selective challenges) that require switching between two different types of metabolism. Individuals in these simulations evolve evolvability through simple natural selection, becoming increasingly faster at adapting to changes in the environment. This is made possible as the architecture of the GRNs evolves to minimise the number of mutations needed to switch from one optimal phenotype to the other and reduce the number of deleterious mutations that could afflict the network. Some architectures adapt so well that they are able to evolve a "switch" structure, that with a single mutation makes the organism adapt from one environment to the other.

The models of Andreas Wagner address the influence of architecture on the effects of mutation and variation as well. A good part of their work focuses on a specific architectural property: robustness [80–83]. Robustness is the capability of a system (i.e. an organism) to undergo multiple perturbations (i.e. mutations) without disrupting its overall function (i.e. changing the phenotype) [84]. A. Wagner and his collaborators show in a series of models of both GRNs [80] and RNA sequences [83] that robustness is a property that can easily evolve in these systems and that, as already claimed by other researchers in systems biology [84–87], robustness favours evolvability. This at first glance might seem counterintuitive, since weaker effects of mutations should lead to less phenotypic variation and innovation, thus reducing evolvability. However, A. Wagner and collaborators demonstrate that this is a false dichotomy. Robust systems allow the evolutionary process to travel along "pathways of neutral mutations" (consecutive mutations in the genotype that do not perturb the phenotype); joining adaptive phenotypes that would otherwise seem impossible to reach through "blind" natural selection, for instance, because the most direct path between the two is made of maladaptive steps. Being able to circumvent maladaptive pitfalls to reach more adaptive phenotypes provides robust architectures with a higher "heritable phenotypic variation", making them more evolvable [83]. A. Wagner imputes robustness to how the architecture influences the mapping from genotype to phenotype (G-P map). In particular, he advocates that it is the disparity in the degrees of freedom/dimensionality between genotype and phenotype spaces that makes this possible. When modelling genotype-to-phenotype translation as a network of interactions (i.e. mechanistically), the number of possible connections and combinations between its elements is exponentially higher than the number of possible outcomes (in a

similar way to how the number of possible neural connections in the brain is much bigger than the atoms in the universe, while the possible number of behaviours a brain can express arguably is not). This naturally leads multiple genotypes to map onto the same phenotype, thus making it easier to find neutral phenotypic paths that can lead to adaptation. The idea of the role of high-dimensionality and redundancy in G-P landscapes playing a role in evolution is reprised by other researchers, see for instance Gavrilets 2010 [88] and [50].

Another important line of research that focuses on the effects of architecture on the G-P landscape (like Andreas Wagner) and variation bias in changing environments (like Hogeweg) is that of Günter P. Wagner and Jeremy Draghi [89, 90]. Together with Lee Altenberg, G.P. Wagner is the proposer of the first "variability-based" definition of evolvability: "Evolvability as the ability of random variations to sometimes produce improvement." [16]. In the same article, he also advocates the importance of another "architectural" concept for studying evolvability: modularity [70]. Modularity is the property of a system where functionally distinct parts work independently from each other [70]. In an organism with a modular architecture, different components will perform different functions (i.e. during development different sets of genes will take care of developing different sets of organs), making it so that mutation on one component won't hinder the functioning of another. Like robustness, modularity is a design principle that has been long recognised as important in organismal architecture and evolvability. Modularity allows evolution to freely re-shape one component to a new task without compromising the functionality of the whole organism, thus increasing the range of possible solutions to a selective challenge [16, 86, 87, 91]. G.P. Wagner and Draghi show in a modelling study both that architectures evolving in changing environments can imprint onto themselves the different selective challenges they face, organising different adaptations into separate functional modules [89], and that architectures can bias variation toward adaptive outcomes, strategically positioning themselves in the G-P space so that few mutations can switch from one to another adaptive phenotype if the environment changes [90].

G.P. Wagner has contributed to a wealth of theoretical studies on evolvability and architecture, often collaborating with researchers from other disciplines that are more computationally oriented. One collaborator was Richard Watson [60], a researcher with a background in computational and cognitive sciences. Watson and collaborators demonstrate with a GRN model how evolution can display "learning-like" behaviour. They evolve GRNs to display target expression patterns. Once the GRNs are perfectly adapted, researchers scramble the network's configuration to become completely random and show that nonetheless, if the architecture is preserved, said GRNs are able to "remember" and eventually re-express the expression pattern they were selected for. Even more surprisingly, if the GRNs are evolved to display not only one but various target patterns, once disturbed they not only can reproduce one of the previous patterns but are able to produce patterns that are the synthesis of the ones they adapted to. This indicates that evolution is not only able to remember but to generalise over past adaptations. Watson, together with Eörs Szathmáry, articulates the vision and possible implications of comparing evolutionary processes and their relation to evolvability to learning processes in a subsequent paper [25]. Here they state:" evolvability is to evolution as generalisation is to learning" and I would add, as they implicitly do in the paper that "Organismal architecture

is to evolvability as memory is to generalisation". Indeed what this, as well as subsequent research from Watson shows, the place in which "developmental memories" are stored and engrained, is none other than the structural relations between the components of their GRNs [92].

The conceptual framework that Watson applies to his research and the way he frames his insights are not native to evolutionary theory. Most of the concepts he adopts originate from the field of computational science. In particular, the terminology Watson uses when discussing evolution as a learning process is rooted in the concept of learning or training in Artificial Intelligence (see [25, 92]). This is not exclusive to Watson's research, in the line of research on evolvability and architecture there is no lack of crossovers from other disciplines. For instance, many papers on the evolution of modularity often model the evolution of circuitry or robot controllers, clearly showing their roots in engineering disciplines that stepped over into evolutionary theory [91, 93, 94]. This is not a coincidence as the evolutionary, engineering, and computational fields share many points of interest, the most evident one being: the adaptation of complex systems to complex tasks [59, 95]. The use of evolution is indeed no stranger to many lines of research that have nothing to do with biology, and are instead interested in solving more practical problems such as optimal circuit configuration, algorithm design, and artificial intelligence [96, 97]. Most of these practical problems, in one form or the other, are often related to some form of computation, and indeed the field of Evolutionary computation has been existing now for almost half a century [97]. As the example from Watson clearly shows, integrating decades-long research on evolution and evolvability from other disciplines could prove to be very fruitful for evolutionary biology, and the opposite is also true. For this reason, I would also like to give a quick overview of studies outside biology that also focus on architecture and evolvability.

1.7. CROSSOVER: EVOLVABILITY AND ARCHITECTURE IN COMPUTATIONAL SCIENCES AND OTHER DISCIPLINES

A first example is that of research conducted by Jeff Clune (who also collaborated with Richard Watson in) together with Jean Baptiste Mouret and Hod Lipson [98]. All these scientists come from purely computational or robotic engineering backgrounds, nonetheless, their research had important resonance also in the biological community. In their paper, Clune and collaborators show that modularity can evolve in networks in very simple conditions, namely: by simply imposing a cost on connections. They evolve networks similar to Artificial Neural Networks (widely employed in Artificial Intelligence) and select for their proficiency in pattern recognition and Boolean logic tasks that change over time. They demonstrate that networks that pay a fitness cost for their connections, evolve to minimise this cost and become more modular, while those networks that are not subjected to connection costs, never evolve a modular structure (they remain integrated [91]). Moreover, and more importantly, they go on to show that modular networks are more evolvable, being able to adapt to new tasks faster and better than those that remain integrated. Despite this paper originating from researchers whose backgrounds are only tangentially related to biology, the similitude is obvious between this system and how we

think modularity could favour evolvability in organisms [70, 99–103]. A similar study was conducted by Kashan a few years earlier on the evolution of logic-gate circuits [93] (the building blocks of computational hardware).

Another interesting research line is that of Kriegman [94] in the field of evolutionary robotics [104, 105]. In this discipline, researchers use evolution to find new and innovative robot designs or robot controllers. This often involves a form of development, where the body of the robot and/or its controller are not directly implemented, but instead emerge dynamically from the instruction of a genetically encoded developmental program. For example, Kriegman and colleagues evolved soft-bodied robots for a locomotory task [94]. The development of the body design as well as the locomotory controller of the robot are divided into two separate modules. This decoupling increases evolvability, through a process called differential canalisation. During evolution, the two developmental systems take two different evolutionary pathways: the development of the body canalises [77], becoming robust to mutations, and reliably presenting a working body design independently of the controller (modular behaviour). This, in turn, allows evolution to experiment with a range of controller solutions without the risk of disrupting the overall ability for locomotion. This way the overall evolvability of this system is increased, as it can explore more solutions through the controller, and at the same time it buffers the effects of maladaptive variations with a robust body design. Kriegman and collaborators speculate that this pattern of differential canalisation could be driven by the evolutionary advantage of having a high bias towards neutral or adaptive mutations (for the controller) at the cost of losing variability (canalisation) in the function of another component (the body design). Finally, they relate this concept to the idea of genetic assimilation by Marie Jane West-Eberhard [106] (the constitutive expression of the previously plastic expression of a trait) and propose it as a possible explanation.

Both examples display how insights and approaches from different disciplines can enrich our understanding of biological evolution, helping us to illuminate the role of architectural principles in guiding evolvability. Nonetheless, the two pieces of research clearly nod in the direction of evolutionary biology, and their goals remain largely theoretical and aligned with those of a good portion of evolutionary biologists. There are however branches of research in computational sciences, that specifically focus on using evolutionary principles to resolve very practical problems, and have very little interest in the biological coherence of the underlying concepts. These fields are for instance Neuroevolution (evolution of the architecture of Artificial Neural Networks) [107], AI evolutionary algorithms (the design of different modes of evolution, normally for evolving/training such networks) [31], and genetic programming (the evolution of programs) [108]. What is a fascinating aspect of these disciplines is that the scientists involved often approach evolution from a completely different perspective than a biologist would. Their final goal is to evolve either an artificial neural network, an algorithm, or a program that is the best at executing a certain task. Little interest is given to the soundness of the rationale behind design choices. Instead, the value of a result is usually established based on clear and practical benchmarking tests, which often involve outperforming or matching the quality of results of other state-of-the-art methods. Therefore, researchers in this field do not try to reproduce evolution as it is in the biological world. They, instead, retro-engineer it, taking inspiration from biology only when useful, and try to create the optimal design

for evolution to solve a problem of interest (i.e. playing Atari games, robot locomotion, controlling virtual football players [109]). As scientifically flawed as it might sound to a biologist, this more unrestricted way of studying evolution (not biological evolution, just evolution as an algorithm) has led to many interesting insights and creative evolutionary outcomes [110]. I argue that achievements from these foreign disciplines can serve as an inspiration to biology [111] and advance its line of research (of course the opposite is also true, see Ch 4 of this thesis).

Of particular interest to me is the fact that despite their detachment from biological principles and evolution, we find that architecture and evolvability are still central ideas in this research.

Research from Kenneth Stanley and Risto Miikkulainen proves that an algorithm that allows neural networks to evolve by gradually augmenting their topologies (NEAT) can outperform fixed-topology trained networks in many tasks [112]. The intention behind NEAT is to allow evolution to "both optimise and complexify solutions simultaneously, offering the possibility of evolving increasingly complex solutions over generations" [112]. NEAT does so by maintaining and incrementally improving via evolution parts of the network architecture. The idea of evolvability (defined by Stanley as the ability to produce innovation) is also very involved in the conception of NEAT, and indeed in many cases, NEAT has been proven to enhance the evolvability of the system in which it is used [113, 114]. NEAT has given rise to a very prolific line of research and is one of the best-known representations of ANN evolution [94, 115–119]. Its framework has now been expanded for almost two decades in a family of architecturally oriented encodings that allow a more efficient evolution of artificial neural networks for a plethora of tasks.

Another interesting architectural insight provided by Kenneth Stanley and Sebastian Risi is their proof that evolution can form very complex and high-performing ensembles by being able to find a way to efficiently connect high-dimension components (in their case architectural blocks of artificial neural networks with hundreds of thousands of connections/parameters each) through low-dimension interfaces (i.e. through only a few connections) [120]. This process mirrors the fashion in which different developmental programs interact only via a few of the many genes that compose them [70]. Even more interestingly, they show that mutations on these "compressed" low-dimensional interfaces produce coordinated and functional changes across the "expanded" high-dimensional components. This behaviour is well-known in artificial intelligence and is specifically exploited by the "Auto-encoder" architectures [121, 122] (sometimes referred to as hourglass networks). Autoencoders are famous for being able to produce generative art and the (sometimes hilarious, sometimes scary) so-called deep-fakes [123, 124]. They can do so via compressing and expanding information, thus forming information bottlenecks. Scientists have shown that these bottlenecks contain the crucial features of the incoming information and are thus much easier to fine-tune for a specific task [121, 122]. This leads to speculation that biological organisms might be able to bias variation adaptively in a similar way. Bowtie architectures are well-known across biology and very similar to auto-encoders. They have the same low-dimensional information bottlenecks that connect different high-dimensional components [125, 126]. Mutating preferentially these variational hotspots at the interface of different functional systems thus, like autoencoders, could favour the production of coordinated and functional variation. This idea is

not completely new in evolutionary biology and it has, in different terms, already been presented by Kirschner and Gerhart in their theory of facilitated variation [62]. I suspect, however, that Kirschner and Gerhart were oblivious to this "convergence" with Artificial Intelligence, as auto-encoders became popular only in the recent decade [124]. No one to my knowledge has ever explicitly made the connection between these two ideas until now. However, it has to be said that autoencoders are being used in evolutionary computation already and that Richard Watson as well has been recently working with this representation [127].

Lastly, I would like to focus on Grammatically Guided Genetic Programming (GGGP) [128], a branch of genetic programming (GP) [108]. The goal of GP in general, as already mentioned previously, can be outlined as the attempt to design programs to solve specific computational tasks via evolution. This is thought to be a particularly fruitful approach for those computational tasks to which no known optimal solution exists (i.e. calculus optimisation, path-making algorithms, robotic arm kinetic control, ANN training optimisation), and where therefore evolution's creative potential can shine. GP represents programs as binary trees, which can be easily parsed recursively and executed, and the structure of these trees is genetically encoded in a variety of ways. Practitioners of GGGP explicitly design all the different operators of the program and encode them into a grammar system. In this grammar different types of operators cover different grammatical functions (e.g. a sum operator conjoins two other operators, a parenthesis operator forms a new branching point in the grammatical sentence, a numerical constant closes the sentence, etc.). Although this is referred to as 'grammar', the base idea of this line of research is to use expert knowledge about a problem to design a system of relationships between operators so that evolution can more easily explore the solution space i.e.: to design the architecture of a system to increase its capacity to adapt - evolvability. This approach has been successfully applied to a variety of tasks, from designing neural architectures [129] to image recognition [130], and pathfinding [131]. Its explicit attention to architecture, as well as its general applicability [132], make GGGP a fertile ground for applying and gleaning insights on architecture and evolvability (see Chapter 4 of this thesis).

1.8. METHODOLOGY

Now that I have stated my ideas and provided sufficient conceptual background, I can illustrate how I intend to research the role of organismal architecture in evolvability and the rationale behind my choices. A rationale is indeed necessary for this line of research, since, as I hope to have sufficiently demonstrated in the previous sections, one defining aspect of evolvability research is the astounding plurality of all its aspects, from the conceptual (discussed above) to the methodological (discussed below). The examples given in the previous two sections provide a good survey of the diversity of techniques and approaches one could apply to the study of architecture. From different choices of representation of architecture e.g.:

1. a GRN described by interconnectable metabolic reaction rates,

2. a GRN that is a fully connected binary network,

3. a GRN that resembles a simple ANN,
4. a GRN that mimics a logic gate circuit,
5. a complex state-of-the-art Artificial Intelligence Neural Network Architecture,
6. a robot morphological and motor developmental program,
7. a grammatical system (see GGGP)

to different choices in the set of selective challenges in which individuals evolve e.g.:

1. Optimal metabolic end-product expression via equation system optimisation,
2. Stable matching of one or multiple expression patterns,
3. Execution of alternating Boolean logic operations,
4. Pattern recognition of modularly changing bit sequences,
5. Simultaneous and sequential binary output matching,
6. Emergent speed of locomotion,
7. Image recognition, path-finding, or robotic arm coordination

Modelling choices are almost limitless, and this introduction does not even get close to covering all of them (for other systems in use see RNA landscapes used by Hogeweg and Wagner [59, 83], or Artificial life systems such as AVIDA [20], Tierra [133] and the various iterations of the cellular automata models [134]). On top of this, I would like to mention that I did also not cover in detail the role that different selective challenges play as well in influencing evolvability (as this exceeds the already hefty scope of this introduction).

In the face of so many choices, one can easily feel overwhelmed. Moreover, one needs to recognise that, despite some of these models do try, and in some rare cases even succeed, to accurately capture some aspects of biological reality, there is a huge gap, both qualitative and quantitative (see Discussion), between the outcomes predicted by theoretical models and the natural world. Therefore, it is important when making a model to face the immense sea of possible implementations with clear goals and intentions, as well as to acknowledge the difference between models and reality. To do so, I choose an approach of intermediate complexity (in line with the philosophy of my research group). The idea of intermediate complexity is enucleated in the eloquent (yet so devilishly vague!) quote from Albert Einstein that says: "Make everything as simple as possible, but not simpler." [135] which I would rephrase, to help the reader understand, as: "Keep everything as simple as possible, then add one grain of complexity, and then, again, keep this grain as simple as possible.". The idea of such an approach is to start from simple yet reasonable assumptions, understand where the limits of those assumptions are, and in a methodical and almost naively simple manner, relax one assumption. One should strive to make the most simple and yet most significant change possible, and then let the new properties and outcomes of the so-changed process spontaneously emerge. Once this is done, and ideally all ramifications have been assessed, the process

can be iterated again. Intermediate complexity is a "what if" approach, that seeks not to predict real events, but to give us a glimpse of what other potential dynamics we might be missing out on by restricting ourselves to a certain set of assumptions. This is the idea behind my research methods: through models, not to describe and predict what the interplay between organismal architecture and evolvability actually does, but to prime our intuition (mine and that of every other scientist interested in the topic) to what it could be doing. To ground this idea more concretely, I will now illustrate my modelling choices in researching evolvability and organismal architecture. Let me first recapitulate the principles and limitations of standard evolutionary theory. This theory finds its strengths in its simplicity and overall cohesiveness. Evolutionary processes are described in a mathematical and statistical framework, where individuals are often subsumed into populations and specific traits to average quantities. This allows it to describe a wide range of complex dynamics in simple terms, and, accepting the simplifications that come with it, to rigorously predict their outcomes. However, as I already explained, this approach also leads to a phenomenological and short-term view of evolution. Under this view, only individual traits and not their interactions are selected, and long-term evolutionary advantages cannot be accounted for by natural selection. For the theory to move on from this impasse, and be able to account for phenomena like evolvability, it is necessary to take a mechanistic approach, acknowledging in particular that organismal architecture can provide new and important insights. Therefore I aim, in line with my rationale, to implement the simplest expansion possible to be able to model systems so that variation between individuals is explicitly accounted for, and interactions between genes and their architectures are relevant to evolution. This way I hope to observe in minimal conditions if and how evolvability emerges from their newly expanded dynamics. For this reason, my simulations have two key expansions from classical theory: each individual is explicitly modelled (instead of being averaged at the population level) and its phenotype is determined by the outcome of the activity of a GRN with a minimally complex architecture (instead of separate genes with little interaction among each other). A good approximation of the modelling method I use can be seen in the research from G. Wagner and Draghi [90], as well as van Gestel and Weissing (specifically the GRN) [50]. Individuals' GRNs are usually composed of a few, but tightly interconnected, genes. The GRNs have (innately) an architecture of connections, but this usually has an extremely simple topology that cannot evolve. Overall, their functioning and behaviour are very similar to some of the most elementary ANN architectures i.e.: multilayer perceptrons [136, 137]. This similarity is not a coincidence, as it has been mathematically proven that these basic ANN architectural templates have all the minimal requirements (if scaled to a sufficient size i.e.: number of genes/neurons) to be able to accomplish any given task (i.e. approximate any given function/ be Turing complete) [138]. This makes them ideal for my goals as they are the simplest possible mechanistic representation of a gene network that still allows for new (and potentially any) behaviour to evolve. These choices, however, exclude the observation of many interesting dynamics, such as the evolution of modularity, particular design motifs, or highly complex behaviours. At the same time, this allows me to focus my analysis on other aspects more clearly (in a similar way to how the standard theory foregoes most organismal complexity in exchange for exceptional clarity in its formalism). By maintaining such a low degree of complexity, it

is possible to capture dynamics in systems so simple that they are easy and intuitive to understand for a wide variety of scholars, making this concept more accessible to the entire community. Moreover, simulations, in some cases, are simple enough that they can be translated from a computational model into a mathematical formula. This re-formalization could help to bridge the gap between the phenomenological/mathematical approach of the standard theory with the mechanistic/computational approach that evolvability studies normally adopt. In conclusion, I hope to highlight the advantages this approach offers, and why I find it particularly appealing in trying to "pick up" a general pattern across many phenomena and combine knowledge from different fields. Overall my modelling does not want to produce digital experiments, but instead aims to provide computational metaphors; continuing Waddington's conviction that models should be "Tools for Thought" [139].

1.9. THIS THESIS

This final section will give a brief overview of the contents of this thesis. The work in this thesis will first deal with conceptual work on evolvability (Chapter 2), it will move on modelling (Chapter 3 and 4 and the two intermezzos), to then give an example of multidisciplinary research between evolutionary fields (Chapter 5), and finally will conclude with a few philosophical consideration on the field of evolutionary studies and a distillation of this thesis' results (Chapter 6 / Discussion).

Chapter 2 discusses the many facets of 'evolvability' and argues that a mechanistic perspective can clarify and resolve long-standing debates in the field and facilitate communication between different disciplines. Thinking in terms of mechanisms can help to more clearly identify what facet of evolvability one is studying, allowing one to more easily pinpoint the scope and limitations of one's research. We also review the current definitions used for evolvability and highlight a previously unremarked difference in the way different groups of researchers conceptualise it.

Chapter 3, provides a 'proof of principle' that adaptive developmental biases at the phenotypic level can readily evolve, even if the mutation process at the genetic level is unbiased and random. We term the particular type of mutational bias we are interested in: "mutational transformation". A mutational transformer is a mechanism through which the phenotype switches from one adaptive state to another via a single or few genetic mutations. Using individual-based simulations, we show how mutational switches can evolve in gene regulatory networks with simple and complicated architecture. We observe two distinct modes of mutational switching depending on the type of allowed gen interactions: mutational amplifiers for linear interactions and mutational canalisers for nonlinear.

My thesis also includes two 'intermezzos': research that I conducted in the initial stages of my PhD. Both intermezzos were published as part of a larger study. I here only reproduce the theoretical aspects of the study, as they are relevant to Chapter 4 of this thesis.

In the **first intermezzo**, which is part of the published paper. I discuss another potential application of the mechanistic modelling approach to study the evolution and evolutionary consequences of non-genetic inheritance. I first give an overview of the

many different models with which evolutionary biology has described and treated non-genetic inheritance, highlighting their strengths and pitfalls. I then proceed to describe a potential model in line with my methodology, which would allow the incorporation of the molecular and evolutionary insights collected in the publication.

The **second Intermezzo** includes a modelling study part of the published paper [140] that provides an explanation for the puzzling fact that parasitic wasps can be induced to switch on their fat metabolism, although they seemed to have lost the corresponding metabolic pathway millions of years ago. This finding seems to contradict Dollo's law of irreversibility, which states that complex traits cannot be re-evolved once lost. The model demonstrates that a switching device (switching on or off a metabolic pathway) can be stably maintained in the face of mutational erosion, even if the switch is only rarely used. This supports that the wasps' metabolic pathway persists plastically dormant rather than re-evolving after irreversible loss.

Chapter 4 extends the study in Intermezzo 2 by considering the evolutionary emergence and robustness of gene-regulatory networks with a complex task (not just a switching device) in situations where the adaptation is only sporadically under selection. The model reveals complex reaction norm phenotypes evolve under regimes of sporadic selection. Both genotypes pre-adapted under constant selection, and those not pre-adapted perform equally well. Counterintuitively, larger genotypes withstand mutations better in certain conditions. Overall, the results demonstrate complex regulation can emerge even with minimal selective pressure.

Research in **Chapter 5** leverages recent insights from evolutionary theory to improve the design of Genetic Programming (GP) algorithms aimed at Machine Learning. Specifically, mutational regimes are designed that improve the search efficiency and performance of a certain class of Neural Network optimisers. The new mutational regimes prove to be computationally efficient while at the same time increasing the quality and diversity of an image recognition task, demonstrating cross-fertilisation between fields.

Finally, **Chapter 6** contains an overarching discussion of my thesis. This chapter contains two parts. First, I lay out some considerations and conclusions of a broad scientific/theoretical and philosophical nature. Second, I review and summarize the findings of my research and outline interesting future directions for this field of study.

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2

CAPTURING THE FACETS OF EVOLVABILITY IN A MECHANISTIC FRAMEWORK

Stefano Tiso*, Jana M. Riederer*, Timo J.B. van Eldijk*, Franz J. Weissing

If Simorgh unveils its face to you, you will find that all the birds, be they thirty or forty or more, are but the shadows cast by that unveiling. What shadow is ever separated from its maker? Do you see? The shadow and its maker are one and the same, so get over surfaces and delve into mysteries.

Farid ud-Din Attar Or Attar of Nishapur, The Conference of the Birds

* These authors contributed equally to the paper

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ABSTRACT

"Evolvability" – the capability to undergo adaptive evolution – is a key concept for understanding and predicting the response of biological systems to environmental change. Evolvability has various facets and is applied in many ways, easily leading to misunderstandings among researchers. To clarify matters, we first categorize the mechanisms and organismal features underlying evolvability into determinants providing variation, determinants shaping the effect of variation on fitness, and determinants shaping the selection process. Second, we stress the importance of timescale when studying evolvability. Third, we distinguish between evolvability determinants with a broad and a narrow scope. Finally, we highlight two contrasting perspectives on evolvability: general evolvability and specific evolvability. We hope that this framework facilitates communication and guides future research.

2.1. EVOLVABILITY IS AN IMPORTANT YET ELUSIVE CONCEPT

Understanding adaptation to changing environments is more important than ever. Climate change, antibiotic resistance, and viral vaccine evasion represent major societal challenges: is an endangered species able to adapt to environmental change? Will a bacterial pathogen evolve antibiotic resistance? Can a virus evade vaccine-based immunization? At the core of these issues lies a common element: the capability of organisms to adapt – evolvability [2]. Evolvability research sheds new light on genomic architecture [3], the structure of regulatory networks [4, 5], and many other features of biological systems (see Glossary). It has yielded surprising new insights, such as: adaptive evolution can proceed at a similar pace as ecological change, resulting in intricate and unexpected eco-evolutionary dynamics [6, 7], evolvability and robustness do not conflict but mutually reinforce each other [4, 8, 9]; and organisms with high evolvability can "generalize" over environments [10, 11]. Furthermore, evolvability research may add new perspectives to formulating a predictive theory of evolution (see Box 1 and Outstanding Questions). Evolvability is studied by diverse approaches. For instance, Johansson et al. [12] inspect the genetic variance-covariance matrix; Woods et al. [13] compare the speed of adaptation of bacterial strains; and Martín-Serra et. al [14] focus on morphological integration and modularity. These approaches, though all valid, are widely disparate: all aim to understand evolvability, yet each focuses on a different facet. This plurality is also reflected in the fact that evolvability has been defined in many different ways (see Box 1). We aim to highlight the different facets of evolvability and how they relate to each other, to facilitate a more nuanced and cohesive discourse on the topic. Throughout, we define evolvability as the capability of a biological system to undergo adaptive evolution (see Box 1 for a justification).

BOX 1. DEFINITIONS OF EVOLVABILITY

Here we will briefly discuss some definitions of evolvability, as they provide a good overview of the diversity of approaches in the field of evolvability research [15–17]. One important early definition revolves around the additive genetic coefficient of variation. It defines evolvability as the ability to respond to selection as governed by the presence or absence of standing genetic variation (often assessed in the G matrix) [18–20]. Another important perspective was given by Wagner and Altenberg [21], who made a distinction between variation and variability, i.e. the propensity of characters to vary. Evolvability is then considered not as the currently present variation but instead as the ability to generate new variation. A third, different perspective on evolvability considers the ability to generate major innovations [22, 23]. For a comprehensive review of these developments, the reader is referred to [2]. Note that these definitions of evolvability are reflected in our first category of determinants, as they all view evolvability as determined by the presence or provisioning of variation. One additional aspect of the definitions of evolvability (also called "evolutionary potential" or "adaptive capacity") is the relationship between variation and adaptation. While earlier treatments (as discussed in [2]) define evolvability as the ability of a biological system to evolve, irrespective of whether evolution is adaptive or not, recent definitions tend to restrict the concept to adaptive processes. For example, Payne and Wagner [24] combine the aspects of variation and adaptation when they define evolvability as "... the ability of a biological system to produce phenotypic variation that is both heritable and adaptive". Other definitions in this vein have been provided by [8, 18, 25–30]. Following the general trend in the field, we here focus on adaptive evolution as well. When defining evolvability as the "capability of a biological system to undergo adaptive evolution" we do not mean the presence or absence of this capability, but rather we consider its degree in a continuous fashion. Adopting an adaptive perspective does by no means imply that non-adaptive processes (e.g. genetic drift) are irrelevant; in fact, many of the determinants of evolvability reflect such processes (e.g. mutation). However, there are at least two reasons for focusing on adaptive evolution. First, many applications of evolvability (e.g. evolution of antibiotic resistance, adaptation to anthropogenic change) consider the adaptation to environmental challenges. Second, relating the rate and outcome of evolution to underlying selection pressures provides a yardstick, making it possible to differentiate between organismal and environmental features [18] and allowing comparisons across organisms. Both features are important first steps toward a predictive theory of evolution [31].

2.2. TOWARD A MECHANISTIC APPROACH TO EVOLVABILITY

Evolvability is often viewed in terms of outcomes (e.g. speed of adaptation). As the same outcome can be achieved in many ways, it is useful to study evolvability by a mechanistic

approach [24]: viewing evolvability not as a phenomenon per se but as a product of the mechanisms and organismal features that underlie it. A mechanistic perspective also clarifies discussions on the evolution of evolvability [2, 24]: while questions regarding the evolution of "the capability to undergo adaptive evolution" easily turn abstract, they become more obvious and transparent when translated into questions regarding the evolution of concrete mechanisms (e.g. the mutation rate).

2.3. CATEGORIZING THE DETERMINANTS OF EVOLVABILITY

We refer to the mechanisms and organismal features that govern evolvability as determinants of evolvability. These affect different aspects of adaptive evolution, and consequently shape evolvability in different ways. We here identify three ways in which determinants can shape evolvability, based on what aspect of adaptive evolution they affect, and categorize them accordingly (figure 1). Firstly, determinants may affect evolvability by providing variation. The mutation rate is the most obvious example of such a determinant [3, 33–35]. Secondly, determinants may affect evolvability by influencing the effect of variation on fitness. For example, developmental biases may predispose mutations towards being beneficial [36–39]. Thirdly, determinants may affect evolvability by shaping the selection process – shorter generation times, for example, may speed up adaptation [40, 41].

2.3.1. CATEGORY 1: PROVIDING VARIATION

Heritable variation serves as the raw material for evolution. Hence, our first category refers to those mechanisms that generate and maintain variation. For example, mutations generate variation in many ways, ranging from point mutations to genome rearrangements. Interestingly, mutation rates vary widely between organisms as well as within genomes [42–44], and they can be regulated based on the environment (e.g. stress-induced mutagenesis, [45]) – indicating that evolvability can evolve through the evolution of the mutation rate. Examples of determinants maintaining variation include evolutionary capacitors such as heat shock proteins. HSP-90 in Saccharomyces cerevisiae, for instance, acts as a chaperone protein aiding correct protein folding. Chaperoning can shield sequence mutations from selection, thus maintaining variation. This can later be released under stressful conditions [46, 47]. Developmental canalization can affect evolvability in a similar manner, in that it allows the accumulation of cryptic genetic variation [48]. Furthermore, capacitors may also be behavioural in nature, as parental care and thermoregulatory behaviour also allow cryptic genetic variation to accumulate [49, 50]. Horizontal gene transfer may also be viewed as a category 1 determinant, as it allows variants to be maintained that would otherwise be lost from the population, for instance by establishing an "accessory genome" [51, 52] or through the so-called rescuable gene hypothesis [53]. Not all heritable variation is genetic: epigenetic inheritance, inheritance of environmental features, and cultural inheritance can also affect adaptive evolution [54, 55]. Hence, category 1 also includes mechanisms providing non-genetic heritable variation.

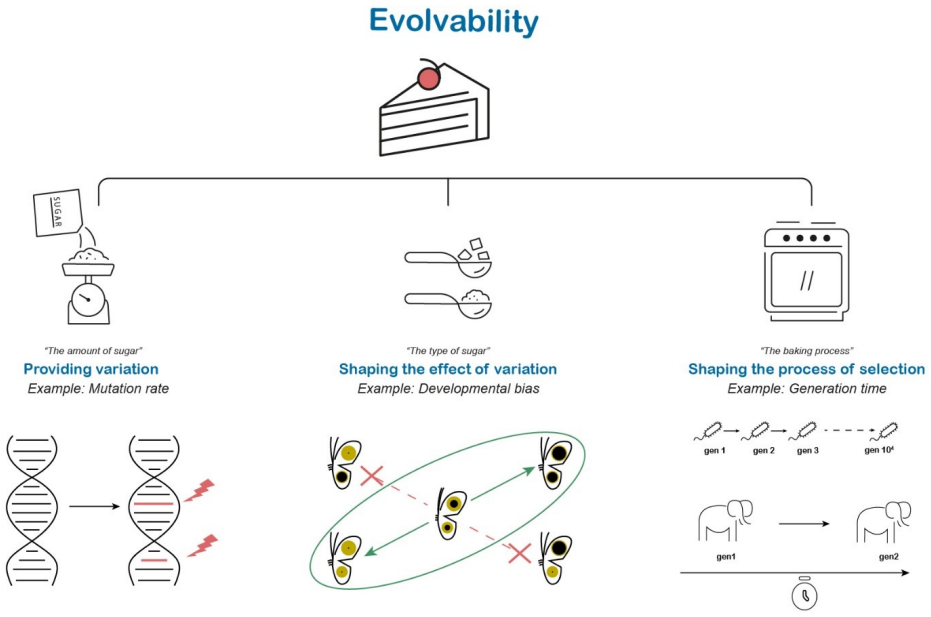

Figure 1 | The way that mechanisms and organismal features affect evolvability can be classified into three categories. Each one contributes to evolvability in a different way. This can be compared to the process of baking a cake: the end result depends on several fundamentally different aspects - the amount of ingredients, the quality of ingredients and the baking process. We suggest that evolvability is analogously affected by three different classes of determinants: those providing variation ("the amount of sugar"), those shaping the effect of variation on fitness ("the type of sugar"), and those shaping the selection process ("the baking process"). An example of a determinant providing variation is the mutation rate, where mutation encapsulates a wide variety of phenomena ranging from point mutations to genome rearrangements. Developmental biases are examples for determinants that shape the effect of variation on fitness. Consider for instance the developmental system underlying the eyespot pattern on the wings of the butterfly *Bicyclus anynana*. This system is organized in such a manner that mutations can easily change the colour composition of the two wing eyespots in the same direction, whilst mutations changing the colour composition in opposite directions are extremely rare (depiction based on [32]). Depending on whether the selective pressure favours eyespots with the same colour composition or not, this bias may facilitate or impede evolvability. Finally, an example of a determinant that shapes the selection process is generation time: a shorter generation time allows faster adaptation – in absolute time, bacteria evolve faster than elephants.

2.3.2. CATEGORY 2: SHAPING THE EFFECT OF VARIATION ON FITNESS

The mapping from mutation to fitness is affected by a variety of mechanisms: mutations may be random with respect to the genotype, but their effects on the phenotype and consequently fitness are often not [9, 56]. Through features such as genomic, developmental, and regulatory architecture, the genotype-to-phenotype-to-fitness map can bias the fitness effects of mutations [18, 37]. Category 2 thus contains determinants that influence the effect of variation on fitness. Examples can be found in the evo-devo literature [32, 37, 39, 57], which describes various biases introduced through the developmental process (developmental bias, for an example see the butterfly *Bicyclus anynana* in Fig. 1). The effect of variation on fitness can also be biased by genomic and regulatory architecture [5, 58]. In yeast, for example, genes for which upregulation is selectively favoured in a higher-temperature environment are grouped on the same chromosome. Therefore, a duplication of this chromosome suffices to achieve upregulation of all relevant genes; without such genome organisation, many independent mutations would be required to obtain an equivalent high-temperature adaptation [59].

2.3.3. CATEGORY 3: SHAPING THE SELECTION PROCESS

Starting from the same variation, evolution can still proceed at a very different pace and/or can lead to very different outcomes. Thus, category 3 contains determinants that impact evolvability not by shaping variation, but rather by shaping how the selective process acts on this variation. Examples are organismal features influencing population structure (e.g. dispersal tendency or mating patterns), as population structure may strongly affect adaptive evolution [60]. For instance, limited dispersal is hypothesized to have aided the rapid evolution of eusociality in diverse clades of insects [61]. Two other examples of category 3 determinants are generation time and the mode of reproduction. In coevolutionary host-pathogen arms races, the shorter generation time of pathogens provides them with an evolvability advantage, as they can evolve faster per time unit than their host [23]. Considering the Red Queen hypothesis, it becomes evident that hosts need other adaptations (e.g. sexual reproduction or a variation-generating immune system) to cope with pathogens on a longer-term perspective [62]. In the coevolution of hosts and their symbionts, the rapid evolution and/or diversification of the symbiont is often not in the interest of their host. Accordingly, the hosts of various symbiotic systems reduce the symbiont's evolvability by actively interfering with the symbiont's sexual reproduction [63].

2.4. HOW A MECHANISTIC CATEGORISATION AIDS OUR UNDERSTANDING OF EVOLVABILITY

Some determinants of evolvability can be classified into more than one category. This is a deliberate feature of the proposed categorisation, as it highlights that a determinant can affect evolvability in different ways. The categorisation prompts the researcher to critically consider how mechanisms and processes shape adaptive evolution. An illustrative example can be found in the literature on evolvability and plasticity. Some have argued that

plasticity impedes evolvability: plastic responses shield organisms from selection, preventing genetic adaptation (category 3) [64]. Others have argued that plasticity allows the accumulation of cryptic genetic variation (category 1) [11, 65], thus potentially enhancing evolvability, because plastic traits are only expressed under particular environmental conditions. Finally, arguments derived from Gene Regulatory Network models conclude that the evolution of plasticity can restructure the genotype-phenotype map in such a way that random mutations are more likely to produce adaptive phenotypes (category 2) [66, 67]. Our categorisation of determinants thus showcases these often subtle but nevertheless crucial distinctions. The effect of modularity on evolvability provides another example. Inspection of the underlying mechanisms reveals that modularity has not one but two impacts on evolvability. Firstly, it facilitates innovation by allowing pre-existing modules to be combined in different configurations, thus providing variation (category 1) [68]. Secondly, it also allows individual modules to vary independently, without affecting the functionality of the entire system (reducing antagonistic pleiotropy): this makes deleterious mutations less impactful, thus creating an adaptive bias that shapes the fitness effects of variation (category 2) [69]. This reduced impact of deleterious mutations (category 2) may also allow organisms to tolerate higher mutation rates (category 1) - showing that determinants in different categories can interact in a reciprocal manner: the processes that provide variation (category 1), bias the fitness effects of variation (category 2), and shape the selection process (category 3) are not independent of each other. Our view on the determinants of evolvability is suited to different approaches to evolution and evolvability. The first category (providing variation) contains not only mechanisms that provide new mutations, but also mechanisms that facilitate major innovations (however, the relationship between evolvability and major innovations is not yet well understood). Similarly, the second category considers not only instances of genotypic and developmental biases, but also includes broader ideas such as phenotypic accommodation and the theory of facilitated variation [38, 70–72]. Finally, the third category considers not only genetic mechanisms (such as horizontal gene transfer, which also allows beneficial variants to spread more quickly [73]) but also - amongst others - niche construction, where organisms shape their own selective environment [74].

2.5. EXPLICITLY CONSIDERING TIMESCALE RESOLVES APPARENT INCONGRUENCIES

Determinants differ in the timescale on which they act – thus, when comparing evolvability across biological systems, the outcome is crucially dependent on timescale (see figure 2). Considering timescale can help to resolve several apparent discrepancies.

This is exemplified by comparing determinants that provide variation (category 1): consider the impact of standing genetic variation [19] and the impact of mechanisms generating variation [21] on evolvability ([2], see Box 1). In the short term, adaptation is more strongly influenced by standing genetic variation, whereas mechanisms generating and maintaining variation are of greater significance when considering longer-term evolutionary trajectories [26]. Some approach evolvability in terms of speed of evolution [75], whilst others approach it in terms of attained level of adaptation [18, 76]. Both aspects are

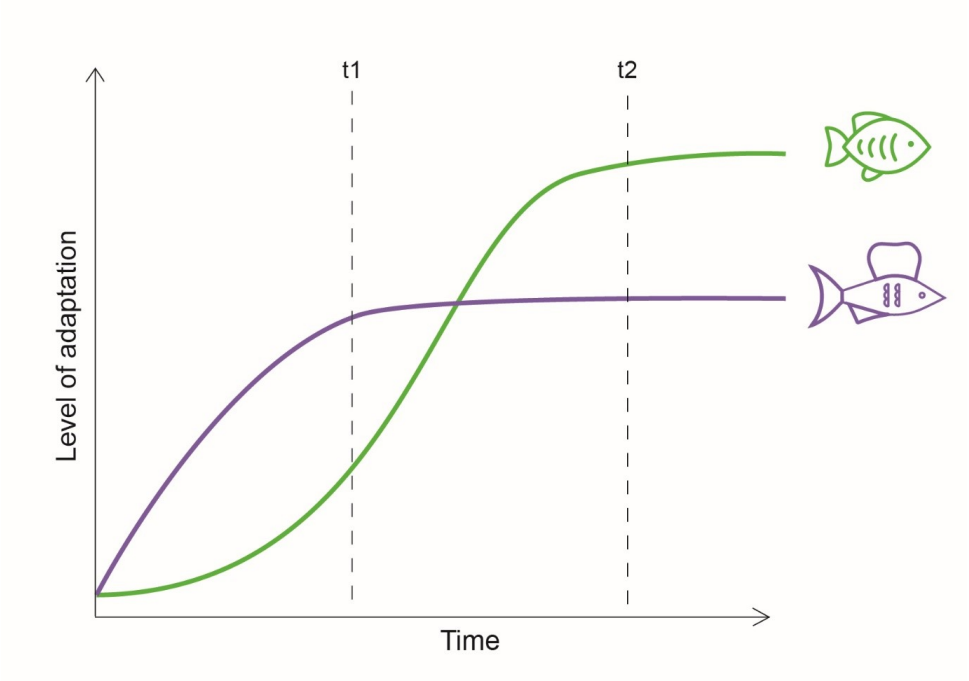

Figure 2 | When studying evolvability it is important to explicitly consider timescale; observing at different times can lead to different conclusions. Suppose that we observe the ability of two fish species to adapt to a new food source. Our conclusions on which of the two is more evolvable (is better able to adapt to the new selective challenge) will depend on the time at which we observe their level of adaptation. At time t1, the purple fish species is more adapted (and hence seems more evolvable), but at time t2, the green fish species is more adapted (and hence seems more evolvable). This occurs because rates of adaptation are not constant across time - thus, being explicit about the timescale of observation can resolve apparent discrepancies when comparing the evolvability of different organisms.

relevant [15], but they are often two sides of the same coin, which becomes apparent when explicitly considering timescale. Consider, for example, different modes of inheritance. Epigenetically inherited traits can provide fast adaptation - yet this adaptation is often relatively inaccurate, given that the relative instability of epigenetic marks impedes the reliable maintenance of a certain optimal phenotype. In contrast, genetic adaptation proceeds more slowly, but in view of the high fidelity of genetic inheritance it may, in the long term, result in a higher level of adaptation. Therefore, epigenetic inheritance confers higher evolvability in the short term, and genetic inheritance confers higher evolvability in the long term [54]. Another discrepancy that can be resolved by considering timescale is the debate over the evolvability benefits of sexual reproduction, with both sexual and asexual reproduction being linked to increased evolvability [2, 77]. All other things being equal, the response to selection (and hence the rate of adaptive evolution) is higher under asexual reproduction, as in case of sexual reproduction selection can only act upon the additive component of genetic variation [78] (category 3). Therefore, asexual reproduction facilitates evolvability in the short term. In the longer term, sexual reproduction confers a higher evolvability, as the slower speed of evolution is outweighed by the ability to better explore the fitness landscape and reach global rather than local peaks. The claims that sexual reproduction increases evolvability, and the claims that asexual reproduction increases evolvability can thus both be true, just at different timescales (see figure 2). Overall, the above examples show that the effects and relative importance of determinants vary over time. Therefore, explicitly considering timescale is crucial when studying evolvability.

2.6. ACCOUNTING FOR ENVIRONMENTAL CONTEXT SHOWS THAT DETERMINANTS DIFFER IN SCOPE

Evolvability is the capability to undergo adaptive evolution; it is therefore necessary to consider in relation to which environmental challenge such adaptation arises. This reveals an additional property of determinants: their scope. Some determinants affect evolvability across many different environmental challenges; we consider these to have a broad scope. For example, mutation rates impact evolvability in virtually all environments. Other determinants have a narrow scope as they only shape evolvability in a restricted set of environments. For example, the grouping of temperature-relevant genes on one chromosome in yeast [59] only enhances evolvability to a change in temperature; it does not impact adaptation to other environmental challenges. The scope of determinants pushes the researcher to consider the range of environments in which a determinant is relevant. Determinants relevant for adaptation to one environment may not be as relevant when considering adaptation to another. For example, in the radiation of Darwin's finches, developmental biases in beak development have been implicated in their adaptation to different seed sizes [79, 80]. However, evolvability with regards to beak shape will not be relevant with regards to other environmental challenges, such as temperature regulation or predator escape. By contrast, a higher mutation rate will affect evolutionary adaptation with regards to many different environmental challenges.

2.7. TWO PERSPECTIVES ON EVOLVABILITY

A very different distinction does not refer to the determinants of evolvability, but to the scholars studying evolvability. Depending on their scientific discipline, research question, or model system, scholars differ in whether they view evolvability as "general" or "specific" with regards to environmental challenges (figure 2).

Scientists adopting a specific evolvability perspective refer to the capability of a biological system to undergo adaptive evolution to a specific environment or a specific challenge – the main focus therefore is on how well a biological system can meet a specific selective target. This perspective is useful when studying adaptation to a particular challenge, e.g. when exploring the capability of bacteria to evolve resistance to a particular antibiotic, the capability of a virus to evolve resistance to a vaccine, or the ability of an endangered species to evolve adaptations to a specific anthropogenic threat [81, 82]. In contrast, scientists adopting a general evolvability perspective view evolvability as the capability of a biological system to adapt to a wide spectrum of environments or of challenges – thus effectively considering evolvability irrespective of the environmental context. This perspective is useful when considering adaptation to unpredictable environments, and is also frequently used in studies exploring the link between evolvability and diversification [76, 83, 84]. Whilst specific and general evolvability are both useful conceptualizations of evolvability, insights gained from one do not necessarily translate into insights about the other. Depending on the chosen perspective, the same observation can lead to different conclusions (figure 2), it informs what questions are asked, and affects how results are interpreted. Consider a population that is able to adapt rapidly to a specific challenge, such as a bacterial strain quickly evolving resistance to a particular antibiotic. From the perspective of specific evolvability this strain has a high evolvability, whilst viewed from the perspective of general evolvability this single instance of rapid adaptation says nothing about the ability of the strain to adapt to other challenges (heat stress, pH stress, etc.). The distinction between general and specific evolvability should not be confused with the scope of a determinant: the latter is a property of a determinant, whereas the former concerns two different ways of viewing evolvability.

2.8. CONCLUDING REMARKS

Evolvability is an intricate concept with many facets. Different facets are at centre stage in different lines of research. Furthermore, evolvability is conceptualized in two different ways: specific and general evolvability. Being aware of these differences is crucial for fostering an integrated and structured view on evolvability research. Throughout we argue that evolvability should not be studied as a phenomenon per se, but as a product of the mechanisms underlying it. Moreover, it is useful to clearly distinguish between determinants that provide variation, shape the effect of variation on fitness and shape the selection process. A structured mechanistic approach clarifies debates in the literature and provides a sound basis for studying the evolution of evolvability. Evolvability cannot be quantified by a single number. Both speed of evolution and level of adaptation are relevant, but they are not independent. Scholars should explicitly consider this when conducting evolvability research. We hope that the proposed mechanistic approach

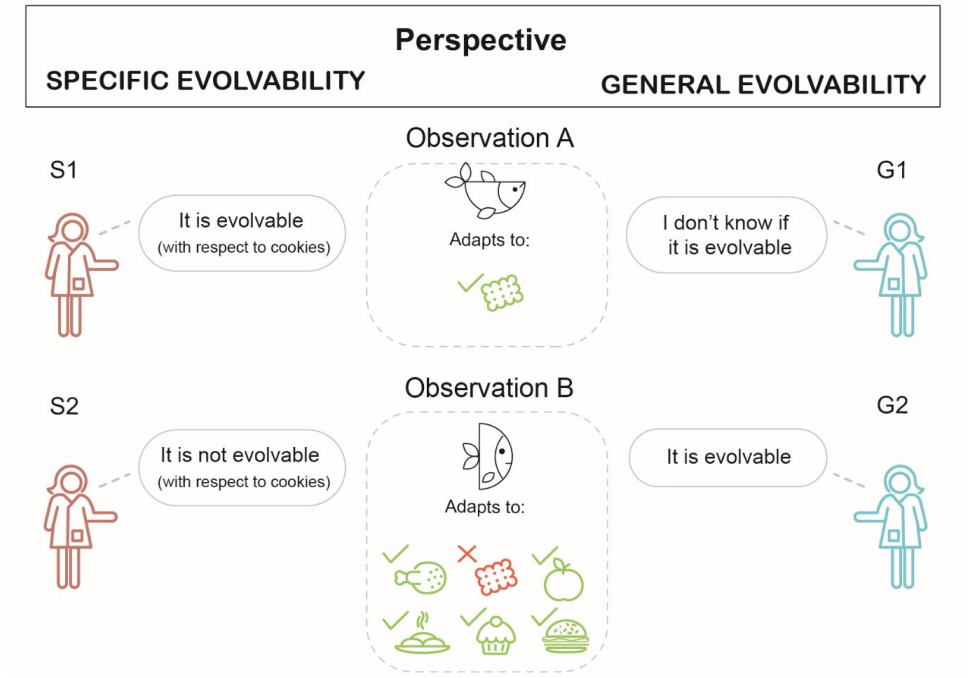

Figure 3 | Specific and general evolvability represent two different perspectives on evolvability. This influences questions and interpretation of results in evolvability research. Different scientists can reach different conclusions from the same observations. This figure illustrates how scientists with different perspectives (on the left: specific evolvability, on the right: general evolvability) interpret the same observations regarding adaptation very differently. Observation A: A fish species can easily adapt to using cookies as a food source. From the specific evolvability perspective (S1), this is interpreted as high evolvability with respect to cookies. From the general evolvability perspective (G1), it is not possible to draw conclusions, since no information is available regarding the ability to adapt to other food sources (environments). Observation B: A fish species cannot adapt to using cookies as food source, but (e.g. due to modular mouth parts) can undergo adaptation to a wide range of other food sources (environments). From a specific evolvability perspective (S2), this is interpreted as a lack of evolvability with regards to cookies. From a general evolvability perspective (G2), the ability to adapt to a wide range of different environments (ability to deal with dietary shifts) indicates a high evolvability. Notice that the two perspectives characterize scholars of evolvability, rather than the determinants of evolvability. The two perspectives should therefore not be confused with determinants acting at short vs long timescales, or with determinants having narrow vs broad scope.

facilitates communication across disciplines, helps to address major questions regarding evolvability (see Outstanding Questions), and provides guidelines for designing future studies on evolvability.

2.9. ACKNOWLEDGMENTS

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GLOSSARY:

Biological system: We here define a biological system to be any biological entity that can be subject to evolution by natural selection. Cryptic genetic variation: Standing genetic variation that has little effect on phenotypic variation under normal conditions, but generates variation under changed conditions. The release of this variation can facilitate (or hamper) adaptation and thus impact evolvability. Developmental bias: The developmental mechanisms underlying a trait can introduce biases in the variation in the phenotype, even if the underlying mutations are unbiased. These biases can be (but need not be) aligned with the direction of selection, in which case they facilitate adaptive evolution. Developmental canalization: Robustness to genetic or environmental perturbations frequently exhibited by developmental systems, leading to a stable phenotypic outcome. Evolutionary capacitor: Mechanism that prevents the expression of genetic variation under some conditions, thus allowing the accumulation of cryptic genetic variation, and 'releases' this variation under other conditions, thus exposing it to selection. Gene regulatory network (GRN) model: Model that explicitly represents the genotype-to-phenotype-map, as a complex network of regulatory interactions between genes. GRN models have been studied extensively in the context of evolutionary developmental biology and evolvability. Modularity: The ability of subsets of a system ("modules") to function independently of other parts of the system (see [68]). Modularity can impact evolvability in various ways: for example, independent modules can be easily combined in different ways, and furthermore do not interfere with each other's functioning. Phenotypic plasticity: The expression of different phenotypes by the same genotype in response to environmental conditions. The impact of phenotypic plasticity on evolvability is subject to much debate (see for example [11, 64–67]): their relationship is complex and not yet well understood. Robustness: The capability of the state of a biological system to persist under (environmental or genetic) perturbation. For example, robustness may refer to the ability to maintain a certain phenotype in the face of environmental fluctuations or genetic mutations. Intuitively one might consider evolvability (the ability to change) and robustness (the ability to withstand change) to be opposed, however, it has been shown that they can be two sides of the same coin [4, 8].

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3

THE EVOLUTION OF MUTATIONAL TRANSFORMERS SPEEDS UP ADAPTATION IN A CHANGING ENVIRONMENT

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* These authors contributed equally to the paper

ABSTRACT

At the genetic level, mutations are generally assumed to be random with respect to the 'demands' of natural selection. Yet, it has been demonstrated that mutations are often biased with respect to their phenotypic effects. Such biases can be important, as they allow the rapid adaptation of a population to changing conditions. Here, we demonstrate that adaptation-enhancing biases can readily evolve in a fluctuating environment. To this end, we consider a population that is exposed to a stochastically changing environment. The environment can be in two states for which different phenotypes are adaptive. The phenotype of an individual is determined by a gene regulatory network that cannot sense the environmental state and can therefore only adapt to environmental change through mutation and selection. In our model, the mutation process at the genetic level is random and not evolvable. Yet, even small networks rapidly evolve a structure that biases the phenotypic effects of mutations in such a way that adaptive evolution after environmental change is speeded up considerably. We term the mechanism responsible for this increase in evolvability a "mutational transformer". A mutational transformer is a network configuration where the phenotypic effects of genetic mutations are distributed in such a way that substantial adaptation to new conditions can be achieved via a single or few genetic mutations. We show that in our model mutational transformers are based on two distinct mechanisms: mutation amplification and mutation canalisation.

3.1. INTRODUCTION

Evolvability concerns "the capability of a biological system to undergo adaptive evolution"[1–3]. The mechanisms that govern evolvability are subject to evolution themselves; hence, evolvability is far from a static property but is shaped by selection and other evolutionary processes. For instance, mutation rates can evolve to increase the production of variation under stressful conditions, thereby enhancing evolvability [4–6]. Evolvability can also change over the generations through the evolution of mechanisms that amplify, buffer, or bias the effect of (genetic) variation on fitness [7–9], phenomena that are sometimes referred to as developmental bias and/or canalisation [1, 10, 11]. These mechanisms alter the genotype-to-phenotype map that describes how changes at the genetic level translate into phenotypic differences. Any alteration of this map can have implications for the dynamics and outcome of evolutionary processes [12–14]. Some genotype-to-phenotype maps enhance evolvability more than others [1]. This may simply be a coincidence or a side-effect of other processes. However, it is worth considering the possibility that the structure of the genotype-to-phenotype map has been selected for evolvability, so as to facilitate rapid adaptation to frequently encountered environmental changes. In several empirical systems, there is evidence for this, as after a commonly encountered environmental change, a highly adaptive phenotype can be achieved via a single or very

few mutations with large phenotypic effect. A first example concerns the rapid emergence of antibiotically resistant subpopulations that quickly arise in bacterial populations grown from a single susceptible clone ('heteroresistance'). This extremely fast acquisition of resistance is associated with the spontaneous tandem amplification of specific resistance-enhancing genes [15–19]. A second example concerns the adaptation of the yeast *Saccharomyces cerevisiae* to heat stress. Rapid adaptation to a higher-temperature environment can be provided by the duplication of a single chromosome. This duplication simultaneously upregulates a multitude of genes that provide adaptation to a higher temperature [20]. In these and other examples, the structure of the genotype-to-phenotype seems to have evolved to facilitate rapid adaptation. The idea that the structure of the genotype-to-phenotype map evolves to facilitate the generation of adaptive variation has also been proposed and extensively discussed in the theory of facilitated variation [21].

But how can the genotype-to-phenotype map evolve in a way that random genetic mutations have phenotypic effects that speed up adaptive evolution? A key theoretical study on this question was performed by Anton Crombach and Paulien Hogeweg [10], who considered the evolution of gene regulatory networks (GRNs) that generated expression patterns, which in turn resulted in a specific phenotypic effect. A population of individuals evolved in an environment that alternated between two states, each selecting for a different target GRN expression pattern. The authors observed that throughout evolution the GRNs re-organized to allow for increasingly rapid switching between the two target expression patterns. Moreover, the increasing rate of adaptation was accompanied by a reduction in the number of mutations required to switch between expression patterns. These findings clearly demonstrate that structural features can evolve to enhance evolvability: whilst the frequency and type of genetic mutations remain random and constant, the GRNs evolve to be structured in such a way that a single or very few mutations are sufficient to switch to a phenotype that is well-adapted to the new environment. In a follow-up study, Hogeweg and colleagues [22] showed that such a mutational configuration can also evolve in GRNs that are able to sense the environment and, hence, can evolve phenotypic plasticity. The results of [10] and [22] showcase clear instances of the evolution of developmental biases toward an adaptive phenotype. We here term the underlying configuration a "mutational transformer": the GRN is structure transforms the phenotypic impact of mutations in such a way that a single or very few mutations allow for a switch to a different adaptive phenotype, facilitating rapid adaptation. The name indicates that a population has evolved to use mutation (and not plasticity) to rapidly switch between alternative adaptive phenotypic states.

The studies of Hogeweg and colleagues provide proof of principle that mutational transformers can evolve. However, the GRNs and their phenotypic effects in [10] and [22] are quite complex, making it difficult to see how the mutational transformers arise and how they function. Moreover, it remains unclear whether such complexity is essential for mutational transformers to evolve. Here, we therefore take a minimal modelling approach that allows us to study in detail whether, when, and how the distribution of phenotypic effects of random genetic mutations evolves under changing environmental conditions. To this end, we consider simple heritable GRNs consisting of a small number of interacting nodes

and a single phenotypic output value. The performance of a network is determined by how well this output value matches the current state of the environment. In line with [10], we evolve these GRNs in an environment that randomly alternates between two different states. The GRNs considered cannot sense their environment, making it impossible to evolve phenotypic plasticity. Accordingly, individuals whose phenotype matches one environment will inevitably be mismatched to the other. Thus, the only way in which an individual's phenotype can track environmental change is through mutation and genetic evolution of their GRN. Over a long period of evolution, in which the population has been repeatedly selected to adapt to a transition between environmental states, we expect the evolution of a mutational transformer, that is, the evolution of networks that rely on a smaller number of mutations to change their phenotype from one optimal phenotype to the other. In a first step, we will demonstrate that mutational transformers do indeed evolve even in very simple networks, consisting of merely two nodes and encoded by just three gene loci. This allows us to investigate the evolution and functioning of a mutational transformer in considerable depth and detail. Subsequently, we study how the complexity of the GRN influences the evolution of a mutational transformer. Besides varying the number of nodes, we also investigate whether non-linear interactions in the gene regulatory network allow for the evolution of more efficient mutational transformers.

3.2. MODEL

3.2.1. MODEL OVERVIEW

The model structure is illustrated in Figure 1. Each simulation follows a population of fixed size (1,000 individuals) over a period of 1 million generations. Generations are discrete and non-overlapping and reproduction is asexual. The individuals are living in an environment that can be in one of two states (E1 or E2). The state is constant throughout a generation (and the same for all individuals), but it switches stochastically to the other state once in a while. Each individual has a phenotype y that is determined by a heritable gene regulatory network. Phenotype $y = +0.5$ is optimal in environment E1, while $y = -0.5$ is optimal in environment E2. The 'fitness' (expected reproductive success) of an individual is negatively related to the distance of its phenotype y from the optimal value y_{opt} of its environment.

Each individual harbours a GRN that is encoded by N gene loci (where N depends on the network architecture). The alleles at these loci are real numbers whose values represent the strength of gene-gene interactions and basal gene-activity levels (Figure 1A). Together, the GRN alleles of an individual determine the individual's phenotype. These alleles are transmitted from parent to offspring, subject to rare mutations. At the start of each generation, 1,000 offspring are produced. In a first step, a parent is assigned to each offspring by drawing an individual from the previous generation (with replacement) by means of a weighted lottery where the weighing factor of each potential parent is proportional to the fitness of that individual. This procedure ensures that the 'fitness' of each individual in the parental population is proportional to the individual's expected reproductive success. Each newly produced offspring inherits all GRN alleles from its parent. Subsequently, mutation takes place: at each GRN locus, a mutation takes place

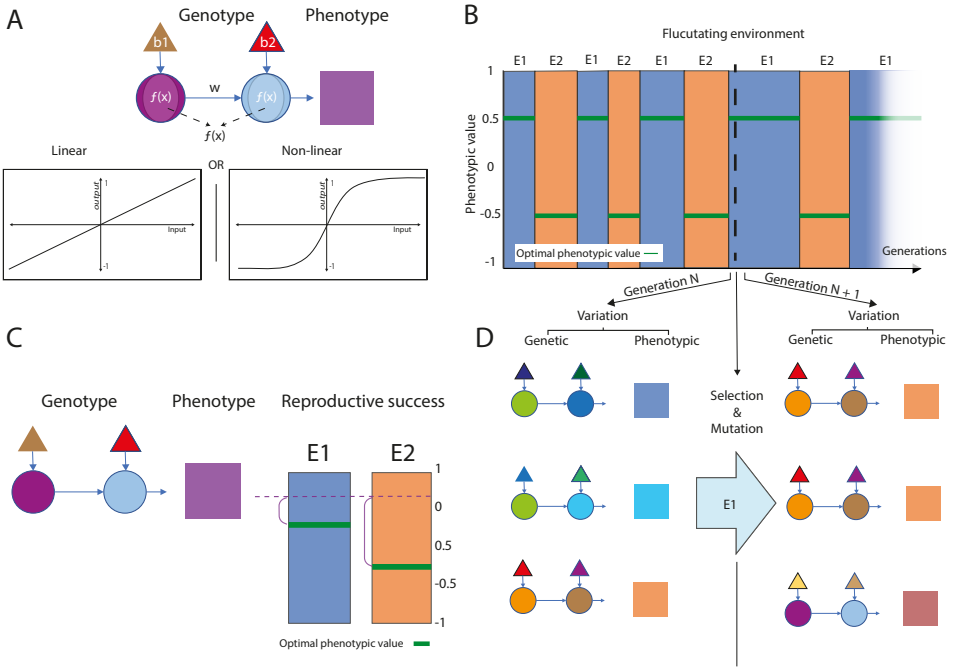

Figure 1 | Graphical illustration of the model. A. Determination of the phenotype of an individual. Each individual harbours a heritable gene regulatory network (GRN) that determines the individual's phenotype. The panel shows the simplest network, which consists of two connected nodes (coloured circles). The working of the network is characterised by two baseline activation levels $b1$ and $b2$, the connection weight w, and the expression function $f(x)$, which is either the identity function (left) or a sigmoidal function (right). The phenotype y of an individual is fully determined by these ingredients. The parameters $b1$, $b2$, and w are encoded by alleles at three gene loci and transmitted from a parent to its offspring, subject to rare mutations. All three loci have the same mutation rate and the mutational step size is normally distributed around zero. **B.** The environment fluctuates stochastically between two states (E1 and E2). The duration of the periods between environmental switch events is geometrically distributed, with a mean value of 100 generations. In each state, there is a different optimal phenotypic value (indicated in green). **C.** Individuals are selected to minimise the mismatch between their phenotype y and the optimal phenotype y_{opt} in the current environment: the expected reproductive success of an individual is given by a Gaussian function of this mismatch and therefore decreases with the squared mismatch between y and y_{opt} **D.** Selection and mutation determine (together with genetic drift) determine how the genotype distribution (and the corresponding phenotype distribution) changes from one generation to the next.

with a certain mutation probability that is fixed and the same for all loci. If a mutation takes place, the parental allele a is changed by adding a small number (the mutational step size) to it. For all loci, the mutational step sizes are drawn from the same normal distribution with mean zero and a small mutational variance. After mutation has taken place, the offspring's phenotype is determined, which, in turn, determines the fitness of the offspring. This procedure results in an evolutionary trajectory that is governed by the interplay of selection, mutation, and genetic drift. At the genetic level, the mutation process is completely random, that is, not affected by the state (or the state dynamics) of the environment: the mutation rate and the distribution of mutational step sizes remain constant throughout each simulation. However, the phenotypic effect of a mutation depends on the allelic values at the GRN loci (see below). Accordingly, the distribution of phenotypic mutational effects can evolve. We are interested in the evolution of this distribution and the corresponding evolution of the 'evolvability' of the population, which we quantify by the speed with which the new phenotypic optimum is approached after each change of the environment.

3.2.2. ENVIRONMENTAL CHANGE

In each generation, the environment is in one of two states (E1 or E2). At the start of each generation, the environment switches from the previous state to the alternative state with probability $x = 0.01$. Hence the duration of environmental stasis is geometrically distributed with mean value $x^{-1} = 100$ generations.

3.2.3. FITNESS

The optimal phenotype in environmental state E1 is $y_{opt} = +0.5$, while it is $y_{opt} = -0.5$ in state E2. The fitness of an individual with phenotype $y = y_{opt}$ decreases with the mismatch with the environmental optimum in a Gaussian manner:

$$F(y) = exp(-S * (y - y_{opt})^2)$$

. The parameter S quantifies the strength of selection. In all our simulations $S = 2$.

3.2.4. GENE REGULATORY NETWORKS

The phenotype of an individual is encoded by a gene regulatory network. There are various ways to model GRNs. We here follow [23], where the flow of information through the network (via gene activation and inhibition) is modelled in close analogy to that of artificial neural networks. The GRNs considered are multilayered and feedforward: they consist of one initial node, multiple layers of internal nodes, and one output node. The type of network will be indicated by the notation $n_1 - n_2 - n_3 - 1$, where n_k is the number of nodes in layer k, and the 1 to the right denotes the output node. For Instance, 1-2-2-2-1 refers to a network with one initial layer of one node and three internal layers with two nodes per layer. The simplest network considered does not have internal layers and is of type 1-1. This network is shown in Figure 1A. Each node j of the GRN has an activation state x_j and produces an output $z_i = f(x_i)$, where f is the so-called transfer function of

the node. The activation state of a node j is given by the node's baseline activation and a weighted sum of the outputs of the nodes in the previous layer:

$$x_i = b_j + \sum_i w_{ij} * z_i = b_j + \sum_i w_{ij} * f(x_i)$$

, where i ranges over all nodes of the previous layer. The baseline activation values b_j and the connection weights w_{ij} are real numbers that are transmitted from parents to their offspring (subject to rare mutations). Together with the transfer function, they fully determine the functioning of the GRN and the resulting phenotype: The initial nodes have no inputs from other nodes. Accordingly, their activation state is $x_i = b_i$ and its output is $z_i = f(b_i)$. Together with the weighing factors w_{0j}, where j ranges over the nodes of the second layer, this determines the activation states x_i and the output $z_i = f(x_i)$ of these nodes. These again determine the activation states and the output values of the nodes in the third layer, and so on. In the end, the output node ω is reached. As before, the activation level x_ω of this node is determined by this node's baseline activation level b_ω and a weighted sum of the outputs of the nodes of the previous layer. In our model, the output of the terminal node $z_\omega = f(x_\omega)$ corresponds to the phenotype of the individual ($y = z_\omega = f(x_\omega)$). The simplest network of type 1-1 (illustrated in Figure 1A) has only two nodes: the input node 0 and the output node ω. Hence, there are only three heritable parameters: the baseline activation levels b_0 and b_ω and the weighing factor $w_{0\omega}$, which, for simplicity, we call w. In view of the above rules, the phenotype of an individual with a 1-1 network is therefore given by:

$$y = f(x_\omega) = f(b_\omega + w * z_0) = f(b_\omega + w * f(b_0))$$

. We will consider two transfer functions. The simplest is the identity function $f(x) = x$. In this case, the phenotype of an individual is given by the relationship $y = b_\omega + w * b_0$. The second is the sigmoidal function (illustrated in Figure 1A) $f(x) = \frac{x}{1+|x|}$.

3.2.5. INHERITANCE AND MUTATION

Individuals are haploid and have an allele at all GRN loci, that is, at the loci encoding the baseline activation values b_j and the connection weights w_{ij}. The alleles are real numbers that are transmitted from parents to their offspring, subject to mutation. The per-locus mutation probability is $\mu = 0.01$. If a mutation occurs, a mutational step size is drawn from a normal distribution $N(0, \sigma)$ with mean sigma and mutational standard deviation $\sigma = 0.1$. This step size is added to the parental value, yielding the new mutated value.

3.2.6. QUANTIFYING EVOLVABILITY

To assess whether long-term evolution in a fluctuating environment enhances evolvability, we need to quantify the rate of adaptation to a new environmental state after a change in the environment. To this end, we operationally define the "time to adaptation" as the number of generations it takes until the mean fitness of the population reaches a threshold value of 0.9. In two situations, no "time to adaptation" is recorded: (a) if the

threshold value is not reached before another change in environmental state occurs, and (b) if immediately after a change in environment the mean fitness of the population already exceeds the threshold value. However, we record how often situations (a) and (b) do occur.

3.3. RESULTS

3.3.1. NETWORK STRUCTURE AND THE EVOLUTION OF MUTATIONAL TRANSFORMERS

The example simulation in Figure 2 illustrates that, in the course of long-term evolution, the time to adaptation substantially decreases even in the case of a simple 1-1 GRN with identity transfer function. The substantial decrease in the time to adaptation from the start of the simulation (Figure 2A) to the end of the simulation (Figure 2B) suggests that a "mutational transformer" has evolved, that is, a change in the way how random mutations at the genetic level translate into changes of the phenotype. To check for the generality of this result, we run multiple replicate simulations for a wide range of GRN architectures. GRNs varied in the number of layers, the number of nodes per layer, and the type of transfer function. Figure 3 shows the results for four network architectures (1, 1-1, 10-2-1-1-1 and 5-5-5-1) and both transfer functions. In each case, the time to adaptation was reported in relation to the duration of evolution. If a mutational transformer evolves, the time to adaptation should decrease in the course of evolution. This does not happen for the trivial "network" 1 (consisting of just one output node and therefore arguably not really a network). As shown in Figure 3A, the time to adaptation did not noticeably decrease in the course of evolution, neither for the identity nor for the sigmoidal transfer function. In contrast, there is a pronounced decrease in the time to adaptation in GRNs of type 1-1 (Figure 3B), both in the case of an identity transfer function (for which this phenomenon is illustrated by Figure 2) and even more so in case of a sigmoidal transfer function

The boxplots in Figure 3CD show, for the GRNs considered, the time to adaptation in the second half of the evolutionary trajectory. In all cases, the initial time to adaptation was typically 50 generations or more. With the exception of the trivial "network" 1, the time to adaptation decreased in all cases to a median value between 5 and 10 in the second half of the simulations (the final 500,000 generations). In other words, all non-trivial GRNs allowed for the evolution of a faster rate of adaptation (i.e., higher evolvability). There is no clear relationship between network complexity and the evolved time to adaptation. However, for any network architecture considered, the evolved time to adaptation was markedly shorter for the sigmoidal transfer function than for the identity function. We will now investigate in more detail how the reduction in adaptation time was achieved. For this, it will be useful to consider the two transfer functions separately.

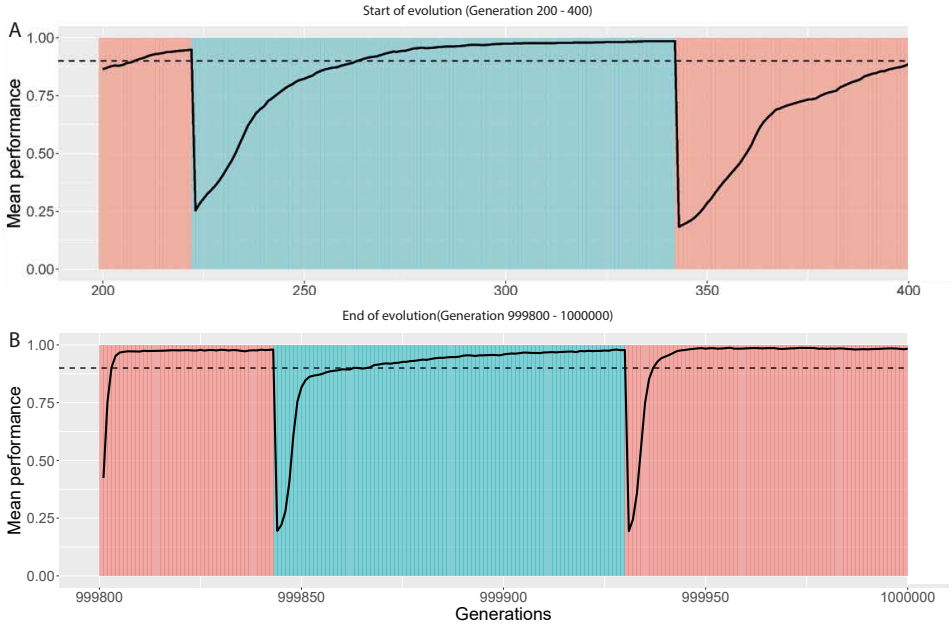

Figure 2 | Time to adaptation at (A) the start and (B) the end of long-term evolution. For an example simulation based on a 1-1 network with identity transfer function, the graphs show the drop and increase in mean fitness after two instances of environmental change. The state of the environment is indicated by the background colour (blue: $y_opt = 0.5$, red: $y_opt = -0.5$). The dashed line indicates the threshold value of 0.9 used to quantify the time to adaptation. In the early part of the simulation (Panel A: generations 200 to 400), the mean fitness of the population recovers much more slowly after environmental change than in the end of the simulation (Panel B: the final 200 generations).

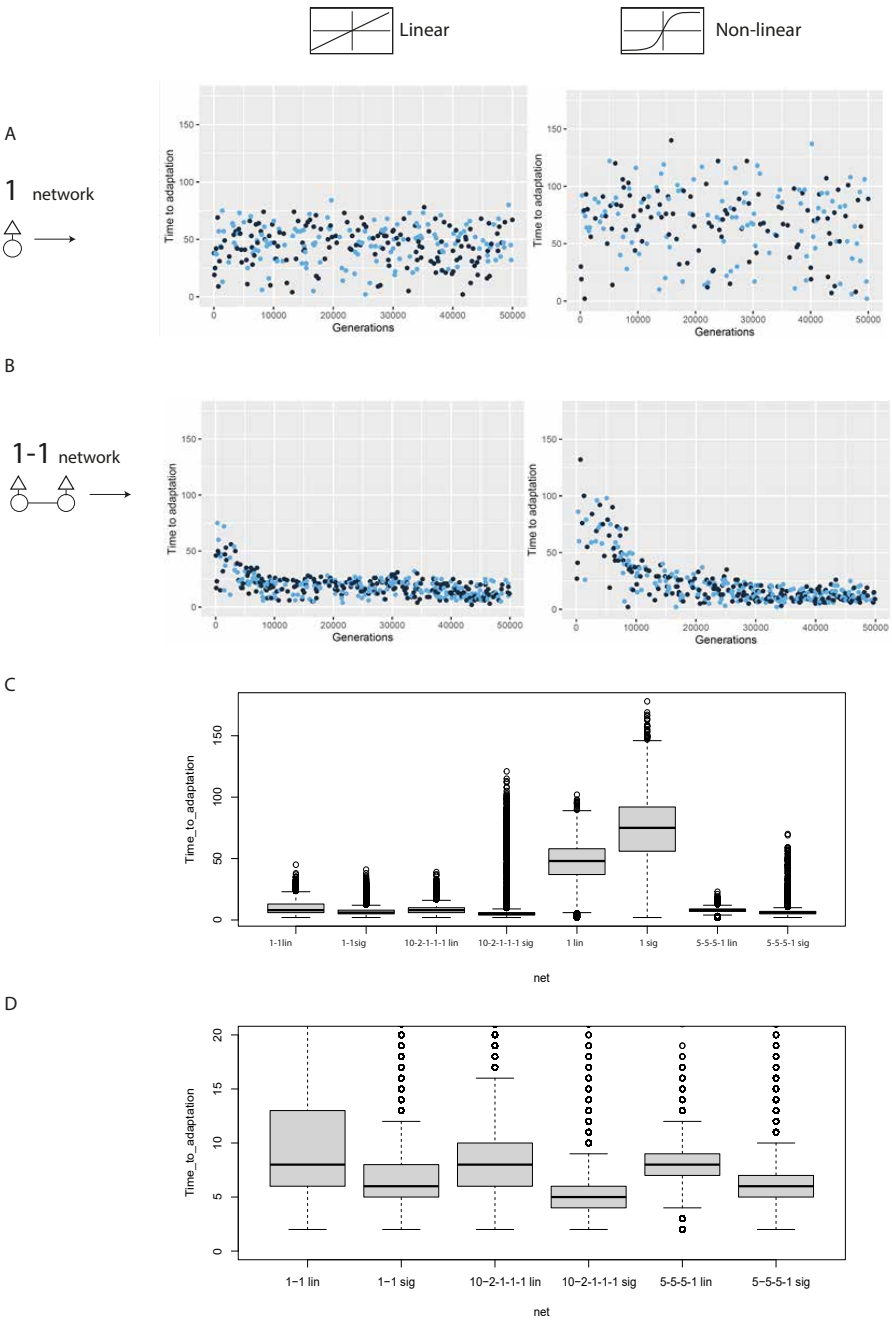

Figure 3 | Evolution of the time to adaptation for GRNs differing in complexity and transfer function. A. For the trivial "network" with just one output node, no evolutionary trend in the time to adaptation was observed, neither in the case of the identity transfer function (left panel) nor in the case of a sigmoidal transfer function (right panel). Point colours indicate the two different environments. **B.** For the 1-1 network, the time to adaptation markedly decreased in the initial phase of evolution (first 10,000 generations), for both transfer functions. **C.** Evolved time to adaptation during the last 500,000 generations of the simulation for GRNs differing in architecture (1, 1-1, 10-2-1-1-1 and 5-5-5-1) and transfer function (identity or sigmoidal). In the boxplots, the data of 7-10 replicate simulations are combined. With the exception of the trivial "network" 1, a fast adaptation rate evolved in all GRNs. **D.** A zoomed-in version of panel C, showing only those types of GRN that evolved a fast rate of adaptation. This panel shows that the GRNs with a sigmoidal transfer function evolved a faster rate of adaptation than GRNs with the same architecture but an identity transfer function.

3.3.2. MUTATIONAL AMPLIFIERS CAUSE MUTATIONAL TRANSFORMATION IN LINEAR GRNS

To understand the inner workings of a network that evolved a fast mutation rate, we first study the simplest type of GRN: a network with two nodes and a linear transfer function (1-1). The behaviour of this GRN is controlled by only three loci: $b1$, w, and $b2$. When analysing the results of many replicate simulations, we observe that in this linear GRN, either the w locus or the $b1$ locus evolves to become responsible for the fast adaptation; this locus is the sensitive locus that acts as a mutational transformer. This is illustrated by Figure 4, which shows for an evolved example network the phenotypic effects of genetic mutations at each of the three loci. While the phenotypic effects of mutations at the $b1$ and $b2$ locus are very small (Figure 4AC), the sensitive w-locus acts as a "mutation amplifier": mutations at this locus have a large effect on the phenotype, allowing the rapid evolution of the phenotype to a new optimum, should the environment require this. In fact, the distribution of phenotypic effects induced by small-scale and normally distributed mutations at the w-locus encompass both phenotypic optima. Hence, if a GRN is adapted to one phenotypic optimum, the other phenotypic optimum can be produced by just one or two mutations. To summarise these findings: The amplification of small mutations on the sensitive locus to large phenotypic changes, allows the alternate optimal phenotype to be reached through a single mutation, thereby facilitating rapid adaptation when the environment changes, creating a mutational transformer. These results can be explained by the fact that for the network considered the phenotype of an individual is given by (see Methods). This shows that $b1$ and w have a similar effect on the phenotype and explains why both corresponding loci can be sensitive. The phenotypic effect of mutations at the w locus are amplified by a factor $b1$, the current value of $b1$. Hence, the allele at the $b1$ locus regulates the phenotypic effects of w-mutations, and vice versa.

3.3.3. NON-LINEAR GRNS EVOLVE MORE REFINED MUTATIONAL TRANSFORMERS

For all network architecture considered, the evolved time to adaptation was markedly shorter for the sigmoidal transfer function than for the identity function (Figure 3D). We hypothesise that non-linear gene interactions allow for the evolution of a more refined mutational transformer. To understand this, we now examine the simplest nonlinear GRN that managed to evolve a mutational transformer (i.e. 1-1, see Figure 5). Figure 5A illustrates that in the initial phase of evolution (in generations 200 to 400, left panel) it takes more than 50 generations before, after a change of the environment, the mean fitness of the population has regained the value 0.9, while the time to adaptation is much faster (between 5 and 10 generations) in the final stage of evolution (right panel). This is achieved by a marked differentiation of the alleles $b1$, w, and $b2$ at the three GRN loci (Figure 5B). This raises the question: how does the evolution of these loci translate to the evolution of a mutational transformer? To investigate this, we record the phenotypes

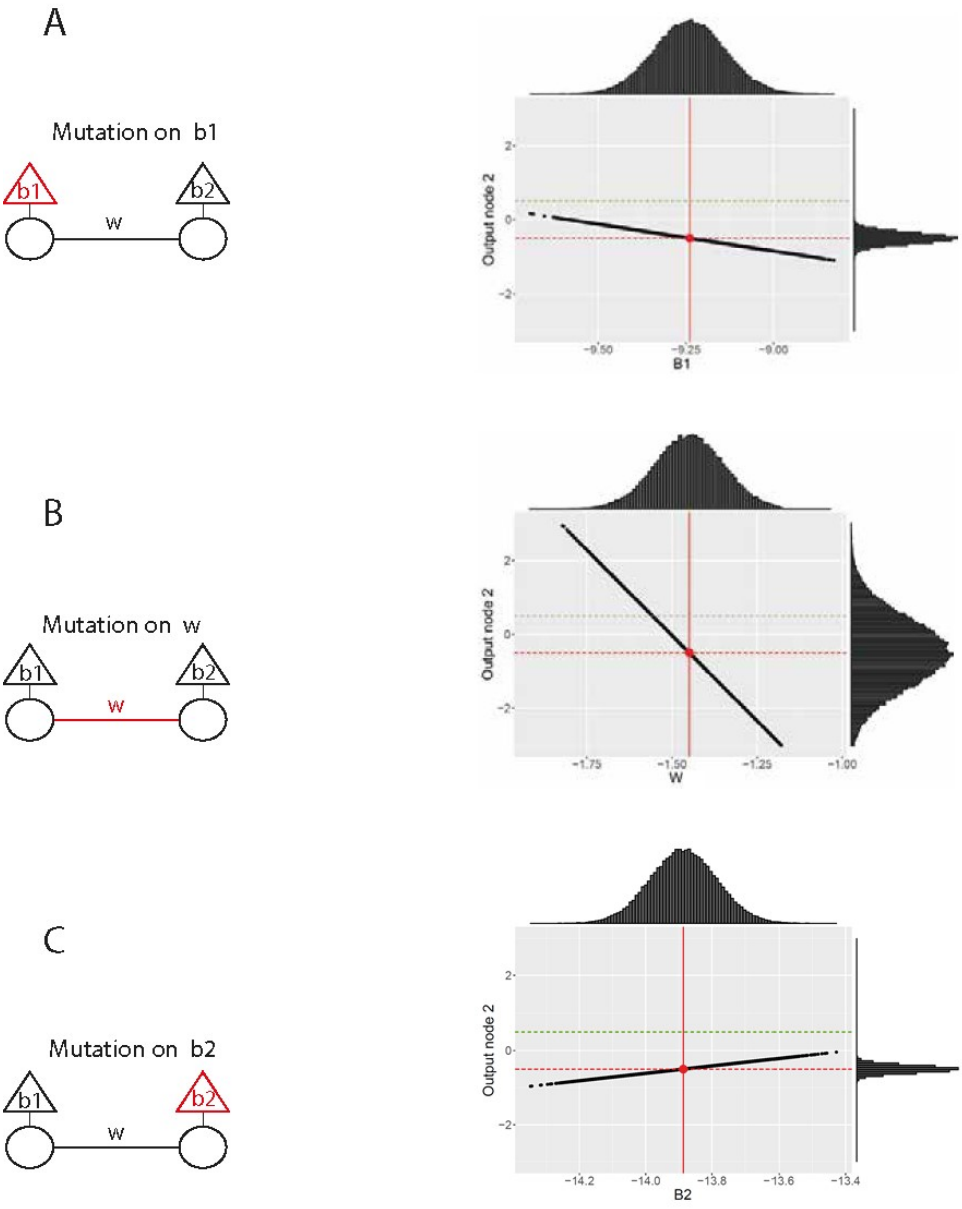

Figure 4 | Phenotypic effects of mutations at the three loci of a 1-1 GRN that evolved a fast rate of adaptation. Data in (A-C) reflect a mutational analysis of a 1-1 GRN with linear transfer function that had evolved a fast rate of adaptation. The panels show the phenotypic effects of random mutations at **A.** the $b1$ locus, **B.** the w locus, and **C.** the $b2$ locus. The allelic values before mutation are indicated by the solid red line. The phenotype produced by the unmutated GRN (indicated by the dotted red line) matches the current environmental optimum (which happens to be equal to -0.5). The dotted green line indicates the alternative optimum (0.5), the "desired" value after a change in environment. While the mutational effects on the phenotype are relatively small in case of the $b1$ and the $b2$ locus, the w locus acts as a mutation amplifier: the other phenotypic optimum can be reached with just one or two mutations, which is the defining property of a mutational transformer.

produced by 1000 different mutations at each locus of the best-performing GRN at a certain time point (Figure 5C). Initially, all loci are similarly sensitive to mutations and the distribution of the phenotypes produced is unimodal. Most mutations will only slightly alter the value of the currently expressed phenotype. This explains why, at the start of the simulation, the recovery of the population after an environmental change is slow and gradual: after an environmental change, many mutations are required to shift to the new optimal phenotype. Towards the end of the simulation, when the population is able to recover rapidly from environmental change (i.e. the mutational transformer has evolved), we observe that the phenotype is highly sensitive to mutations at a particular, sensitive, locus. We observed this same pattern in the linear GRNs, where a single gene evolved to be sensitive to mutations. However, instead of being unimodal as for the 1-1 GRN with an identity transfer function (Figure 4), the distribution of phenotypes produced by mutations at the sensitive locus of non-linear 1-1 GRN is bimodal, that is, with two peaks (Figure 5C, panels 3 and 4). The two peaks coincide with the two optimal phenotypes showing that the GRN has evolved to bias the effect of random mutations toward these phenotypes. The distribution of phenotypic effects of mutations on the sensitive locus is shaped such that the phenotype to which the population is currently adapted is most frequently produced. The second peak in the distribution of the phenotypic effects of mutations corresponds with the optimal phenotype of the alternative environment. This mechanism seems to be symmetrical: after adaptation to an environmental change, the new optimal phenotype is now produced more often. If mutation leads to an intermediate phenotype that is between the two different optimal phenotypes, mutations are equally likely to produce either of the two optimal phenotypes. In all non-linear GRNs considered (1-1, 10-2-1-1-1 and 5-5-5-1), a sensitive locus with similar properties evolved and it always occurred in the initial part of the GRN (either at the b0 locus or at one of the loci encoding the weighing factor w_{0j} of the connection between the initial node and a node j in the first processing layer).

3.3.4. THE INNER WORKINGS OF A REFINED MUTATIONAL TRANSFORMER: A MUTATION CANALISER

The mutational transformers that evolve in non-linear GRNs represent a more refined solution when compared to the mutational amplifiers observed in linear GRNs. This is highlighted by the faster recovery of performance after an environmental change (Figure 3C-D). The non-linear GRNs evolve their architecture, not only to put the two optimal phenotypes in the range of a single mutation step, but also to canalise the phenotypic outcomes of mutations towards particular phenotypic values. We term this more refined mechanism underlying the evolution of a mutational transformer in nonlinear GRNs a mutation canaliser. The behaviour of the non-linear 1-1 GRN is governed by only three parameters which allow for numerical analysis (Figure 6). This allowed us to study how mutations on the sensitive locus affect not only the output of the second node (phenotype) but also how they affect the output of the first (intermediate) node. Applying normally distributed mutations to the evolved value of locus $b1$ leads to a slightly skewed distribution of node 1 output values. The values of the $b2$ and w loci have evolved so that when the skewed distribution of outputs of node 1 is fed into node 2 it is transformed to a bimodal

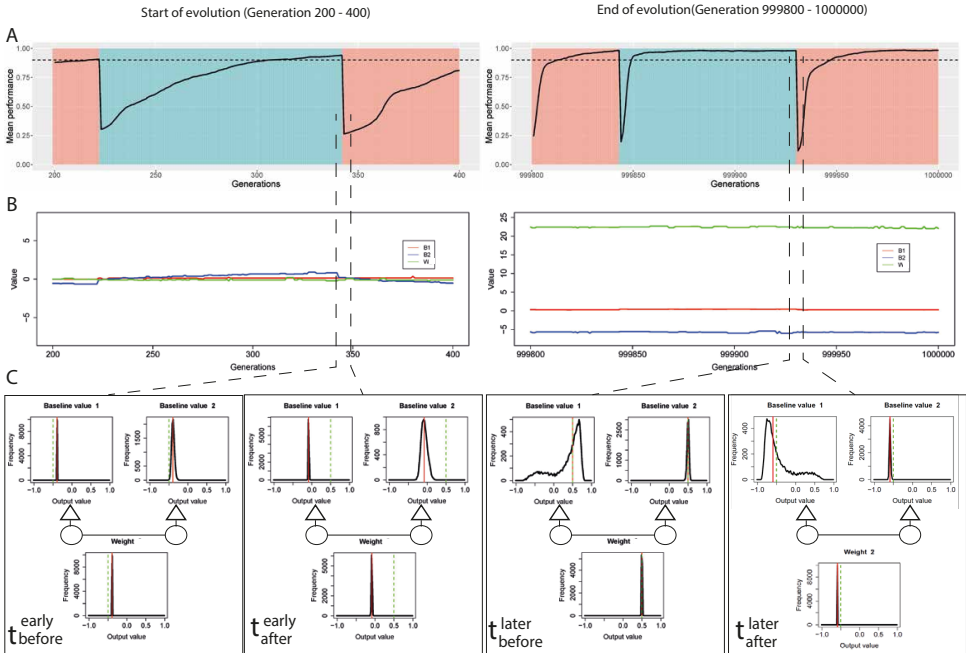

Figure 5 | Evolution of a refined mutational transformer. The figure shows a representative simulation of the evolution of a 1-1 GRN with sigmoidal transfer function. **A.** Evolutionary trajectory of mean population fitness at the start (generations 200 to 400, left panel) and the end (final 200 generations, right panel) of the simulation. The state of the environment is indicated by the background colour (blue:$y_{opt} = 0.5$, red:$y_{opt} = -0.5$). **B.** Evolved allelic values at the three network loci at the start (left panel) and the end (right panel) of the simulation. **C.** Distributions of the phenotypic effects of mutations at four different time points during the simulation. The first two time points are early in the simulation, shortly before (t^{early}_{before}) and after (t^{early}_{after}) an environmental change. The other time points are from a later stage in the simulation, shortly before (t^{later}_{before}) and after (t^{later}_{after}) an environmental change. A comparison of the phenotypic-effect distributions at the early time points with those at the later time points reveals that for two of the GRN loci genetic mutations have a small effect on the phenotype, while the GRN locus encoding $b1$ is "sensitive": mutations at this locus produce a broad spectrum of phenotypes. Moreover, the distribution of phenotypic effects at the sensitive locus is strongly asymmetric and bimodal, with a sharp peak close to the current environmental optimum and a second maximum close to the phenotypic optimum at the other environmental state.

distribution of phenotypes (output values). As can be seen in Figure 6 A, the ability of node 2 to transform the asymmetrical distribution of outputs of node 1 into a bimodal distribution of phenotypes relies on the presence of a non-linear (sigmoidal) transform function. This shows why in our minimal model the ability to canalise mutations towards particular phenotypic values is contingent on the presence of a non-linear transfer function. As we previously noted the mechanism of a mutational canaliser seems to be symmetrical, after mutation to the other environmental optimum a bimodal distribution of phenotypes is maintained. Figure 6 B shows that this symmetry is a consequence of the sigmoid transfer function. If we apply the average mutation to the sensitive locus ($b1$ =+ 0.1 in Figure 6 B) this causes the bimodal distribution to "flip". The main phenotypic peak now sits on the alternative optimum and the smaller peak on the current optimum. This, therefore, shows that canalisers not only have evolved to easily move to an alternative adaptive phenotype but that they have also evolved an architecture that allows them to easily revert back to the previous adaptive phenotype as well.

3.4. DISCUSSION

We have demonstrated that even in a very simple model of a GRN the genotype-to-phenotype map can evolve to facilitate rapid adaptation to fluctuating environments. Even with such a simple genotype-to-phenotype map consisting of just three loci, evolution can lead to a mapping, such that adaptation to an environmental change occurs very fast and through a single mutation. Building on the work of those who studied this phenomenon before us [10, 22], we term this phenomenon a mutational transformer, where the phenotypic state rapidly changes between alternative adaptive states through mutation. Mutational transformers evolve in both GRNs with both linear and non-linear gene interactions. Whilst both types of mutational transformers facilitate rapid adaptation, the time to adaptation is shorter for non-linear GRNs. This points to the fact that these two different types of GRN lead to the evolution of mutational transformers that function through two distinct mechanisms. Both mechanisms enhance the evolvability of GRNs. However they do so in two distinct ways. Linear GRNs rapidly switch phenotype by amplifying the phenotypic effects of small mutations on a specific locus. Thus these GRNs evolve to reach a relatively wide range of phenotypes through a single mutation; we term this type of mechanism a mutation amplifier. A mutational amplifier increases the amount of phenotypic variation that is created through mutation, providing a wider range of phenotypic variance on which selection can act. We show that the range of phenotypic variation is tuned to the specific distance between the two adaptive phenotypes encountered during evolution. Whilst the range of phenotypic variation is tuned, the phenotypes generated through mutation are not biassed towards the particular adaptive values. This also highlights a key drawback of mutation amplifiers; whilst they accelerate evolution when a population is maladapted (not on a fitness peak), they lead to the production of a lot of deleterious phenotypes when a population is well adapted (on a fitness peak). These properties of mutation amplifiers are reminiscent of results obtained by those studying the evolution of mutation rates, here it has been shown that high mutation rates are advantageous only when a population is maladapted [5]. On the other hand, non-linear GRNs (sigmoid transfer function) evolve a more refined mutational transformer.

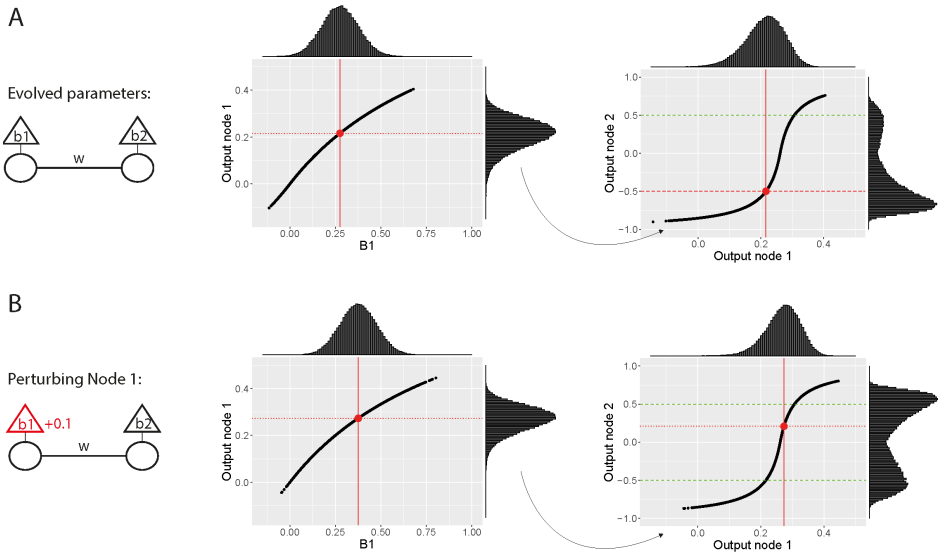

Figure 6 | Transformation of normally distributed genetic mutations into a bimodal distribution of phenotypic effects. The plots in each panel (A, B) show how different 1-1 networks transform a normal distribution of mutations on the sensitive locus ($b1$ in this case) into a (different) distribution of phenotypes. **A** represents an evolved and adapted GRN, and **B** shows the same GRN but now with a mutated/perturbed value of its sensitive locus, The values of the sensitive locus are on the x-axis of the plots on the left column, while the values of the phenotype are projected on the y-axis of the plots on the right column. The plots on the left show the mapping of mutations on $b1$ to outputs of node 1, the solid red line corresponds with the non-mutated original value of $b1$, while the dashed red line shows the value of the corresponding original output. The plots on the right show how the distribution of outputs of node 1 (now on the x-axis) is transformed into a distribution of phenotypes (outputs of node 2, y-axis). Also in this case the red and dashed line mark the non-mutated input and output values, while the dashed green lines show the values of the two environmental optima. **A:** The mutations-to-phenotype mapping of an evolved network. The distribution of outputs of node 1 produced by mutations is skewed but not bimodal. The distribution of phenotypic values in node 2 instead is clearly bimodal, with two peaks corresponding to the two optimal phenotypic values of the two environmental states. Therefore, mutations on node 1 of an evolved network will preferentially produce adaptive outcomes. In the evolved network we can see how node 1, although being the one sensitive to mutations that cause the mutational transformer (see Figure 5 C), does not create the bimodal distribution of phenotypes. It instead skews the distribution of mutations. The asymmetry in these outputs is then used in node 2 to create a bimodal distribution of phenotypes. **B:** The mutations-to-phenotype mapping of a network for where the sensitive locus ($b1$) is perturbed (adding the average mutation step = 0.1 to its evolved value). Node 1 still skews the distribution of outputs but towards slightly lower values. This causes the creation of a bimodal distribution of outputs in node 2 but now with the two peaks inverted. If before the main peak of the bimodal distribution sat on the environmental optimum value of 0.5, now it sits on the other optimum value -0.5. At the same time, the smaller peak now sits at 0.5 instead of -0.5. This shows that the architectures of these GRNs evolved to be able to match the environmental optimum and, as well, rapidly switch to the other environmental optimum via a single mutation.

In these GRNs, the distribution of phenotypes generated through mutation is targeted towards the two adaptive phenotypes encountered during evolution. The non-linear interactions are essential to this strategy: they allow normally distributed mutations to create a bimodal distribution of phenotypes. In other words, the genotype-to-phenotype map evolves to canalise the effects of mutations towards adaptive outcomes; we term this mechanism a mutation canaliser. A mutational canaliser, biases variation towards particular adaptive outcomes, shaping the way mutations affect fitness. This more refined mechanism explains why non-linear GRNs adapt faster in response to a change in environment than their linear counterparts. Interestingly the mechanism behind the mutation canaliser seems to closely resemble the phenomenon described by Crombach and Hogeweg [10], where the evolution of the genotype-to-phenotype map increased the frequency of beneficial mutations. When comparing the mutation amplifier and the mutation canaliser two key differences are apparent. Since the mutation canaliser preferentially produces adaptive outcomes it has a much lower production of deleterious phenotypes when the population is well-adapted (on a fitness peak) compared to the mutation amplifier. However, this lower production of deleterious phenotypes comes at a cost: mutation canalisers are only expected to enhance adaptation when environmental fluctuations are repetitive (i.e. previous adaptive challenges are representative of future adaptive challenges), whereas a mutation amplifier is also expected to enhance adaptation in more irregularly fluctuating environments. In other words, mutational amplifiers have a broader scope than mutational canalisers (see also [1]). Whilst above we compare and contrast the two caricatures of the mutation amplifier and the mutation canaliser, it is worth noting that in our opinion mutation amplification and mutation canalization are not mutually exclusive, it is possible to amplify the phenotypic effects of mutations whilst also somewhat biassing the effects of mutation towards particular adaptive outcomes. In our model the environmental fluctuations are repetitive, switching between two different adaptive values, therefore previous adaptive challenges are representative of future adaptive challenges. It is clear that mutation amplifiers can enhance adaptation in such repetitively fluctuating environments. However, given that the phenotypic variation generated by a mutation amplifier is not directed towards a particular phenotype, mutation amplifiers are also expected to enhance adaptation in environments that fluctuate in a more irregular manner. We think that a mechanism similar to the mutational amplifier and the mutational canaliser might be responsible for the pattern we observed in some empirical examples described above, such as heteroresistance, yeast thermal adaptation, and evolution of lactose metabolism. The rapid switching to an adaptive phenotype using a single or very few mutational steps closely resembles the behaviour of mutational transformers in our model. In each of these three empirical cases it is very likely that populations have previously repeatedly faced the relevant environmental change (antibiotics presence/absence, different growth temperatures, loss of lactose metabolising plasmid). Therefore we strongly suspect that in these cases the structure of the underlying GRN is not a fluke, it is instead the product of the evolution of evolvability, which has led to the creation of a mutational transformer. Furthermore, the mutational canaliser is reminiscent of many of the examples of developmental bias described in the literature. Consider for example how the structure of the GRN shapes the available variation in Bicyclus anynana [24], where the structure of the underlying GRN seems to

favour the production of particular phenotypic variants over others. Our results are largely aligned with those obtained by Crombach and Hogeweg [10], and Cuypers et al. [22], who studied similar questions using a different set of models. Their models used more complex GRNs and explicitly modelled a wide set of biological mechanisms, aiming to more closely mimic biological complexity. We instead set out to create a minimal model of a mutational transformer, which allowed us to uncover in detail the evolution of the underlying mechanisms. We have demonstrated the evolution of mutational transformers across a wide range of different network architectures. It can be concluded that a minimal complexity of two nodes (three loci) is required, a network with a single node is not able to evolve a mutational transformer. Surprisingly, complexity beyond two nodes does not seem to improve the functioning (i.e. time to adaptation) of the mutational transformer. We demonstrate that mechanisms that shape the effect of mutations on the phenotype can readily evolve in very simple systems. The fact that mutational transformers readily evolve in our extremely simple GRN model leads us to expect they may be a rather widespread phenomenon, in organisms facing fluctuating environments. Mutational transformers might be especially prevalent when organisms face repetitively fluctuating environments, where past adaptive challenges are likely to be similar to future adaptive challenges. Future work should explore exactly if and how mutational transformers can evolve under more diverse and complex environmental regimes (e.g.: more than two alternating environmental optima) or environments where the phenotype is represented by more than one dimension). In a more general sense, our results indicate that the genotype-to-phenotype map can easily evolve to shape the phenotypic effects of mutation towards adaptive outcomes, even with minimal complexity. This outcome seems to contradict the assumptions of standard evolutionary theory, which assigns little relevance to the exact nature of the genotype-to-phenotype map. However, recent work has shown that explicitly considering the structure of the genotype-to-phenotype map greatly influences the outcomes of evolutionary models [23, 25]. Our results are in line with these findings, we demonstrate that even a simple model of a genotype-to-phenotype map produces dynamics rarely observed in more classical models i.e. the evolution of evolvability. All in all we show in detail how mutational transformers evolve and function. We argue that mutational transformers can greatly impact evolutionary dynamics and that their emergence represents a clear example of the evolution of evolvability.

3.5. ACKNOWLEDGEMENTS

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I-1

Intermezzo 1: Towards a mechanistic model of Non-genetic inheritance

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Published as part of:1. Adrian-Kalchhauser I, Sultan SE, Shama LNS, Spence-Jones H, Tiso S, Keller Valsecchi CI, Weissing FJ. Understanding "Non-genetic" Inheritance: Insights from Molecular-Evolutionary Crosstalk. *Trends in Ecology & Evolution* 2020;35(12):1078-1089. doi:10.1016/j.tree.2020.08.011

During the initial months of my Ph.D. program, I had the opportunity to participate in a workshop that aimed to foster collaboration between evolutionary biologists and molecular biologists, with a specific focus on the topic of non-genetic inheritance. The workshop aimed to cross-fertilize each other's perspectives via the exchange of expertise between these two fields. The molecular biologists in the workshop provided a comprehensive understanding of the various non-genetic mechanisms that operate across the tree of life, thereby enriching the toolkit available to evolutionary biologists for studying the significance and implications of non-genetic inheritance in the process of evolution. Evolutionary biologists, on the other hand, would help ground molecular mechanisms in the broader context of the theory of evolution and adaptation. This helped molecular biologists gain a more comprehensive understanding of the theoretical underpinnings of evolution and identify meaningful and relevant cases of non-genetic inheritance which would be worth further investigation and attention.

This fruitful cross-talk and collaboration ultimately led to the publication of a review paper:

Understanding 'Non-genetic' Inheritance: Insights from Molecular-Evolutionary Crosstalk. Adrian-Kalchhauser, I., Sultan, S.E., Shama, L.N.S., Spence-Jones, H., Tiso, S., Keller Valsecchi, C.I., Weissing, F.J. *Trends in Ecology and Evolution* 35(12), 1078–1089 (2020)

For this publication, we critically evaluated the strengths and weaknesses of previous theoretical approaches and proposed a novel modeling framework that leveraged the mechanistic insights from molecular biology to overcome some of the limitations in the field.

This interlude consists of three short sections. The first summarizes the main insights of the cross talk. The second reviews existing evolutionary models of non-genetic inheritance. The third uses the insights obtained from the crosstalk to sketch a more mechanistic modelling approach to non-genetic inheritance.

I-1.1. FOUR KEY INSIGHTS OF A MOLECULAR-EVOLUTIONARY CROSSTALK ON NON-GENETIC INHERITANCE

A brief review of the molecular mechanisms underlying 'non-genetic' inheritance (NGI) reveals a daunting diversity and complexity of epigenetic, cytoplasmic, and other mechanisms. Evolutionary ecologists interested in NGI may profit from the following insights (articulated in 1):

1. Most forms of NGI correspond to inherited gene regulation (IGR). IGR provides a unifying concept for the diverse heritable factors that may alter offspring gene expression.

2. In IGR systems, genetic and non-genetic factors are strongly interdependent. IGR systems are functionally intertwined with DNA sequences rather than existing as separate entities. Studies of NGI must take this interdependence into consideration.

3. IGR mechanisms exhibit enormous phylogenetic and operational variation. The

precise mechanisms underlying IGR vary across taxa and can exhibit diverse operational characteristics. Therefore, considerable caution is required when generalising findings across taxa or contexts.

4. Epigenetic elements are of a probabilistic nature. Epigenetic factors act as probabilistic and interactive regulatory elements, challenging the traditional view of deterministic "epialleles" with defined genomic locations and effects.

These features can inform choices for future theoretical IGR research that implements an approach with a focus on regulation mechanisms. 'Non-genetic' and genetic aspects of inheritance are often treated as separate streams of information, both conceptually and experimentally. Accordingly, statistical and modeling approaches often rely on the (linear) decomposition into 'genetic' and 'non-genetic' effects, and on the independent quantification of these effects. Yet, gene sequence variants and heritable factors that regulate their activity are, in fact, deeply interwoven, suggesting that an interaction-based perspective may be more fruitful. How can the interplay of genetic and 'non-genetic' factors be incorporated in eco-evolutionary models in a realistic manner? The close interaction of genetic and 'non-genetic' regulatory elements calls for the development of regulatory network models, together with new tools for deriving overarching principles from such mechanistic models.

I-1.2. EXISTING MODELS OF NON-GENETIC INHERITANCE

Mathematical and computational models play a crucial role in mapping the implications of epigenetic modifications, maternal effects, and other aspects of 'non-genetic' inheritance for ecology and evolution. Even the relatively simple initial models (e.g. 2) reveal that adding these additional modes of inheritance to genetic models can systematically and strongly affect the dynamics and outcome of adaptive evolution. At present, there are two dominant modeling approaches.

1. Extensions of population genetic and quantitative genetic models: Here, classic population genetics and quantitative genetics models are expanded by including 'non-genetic' inheritance to study possible effects on evolutionary dynamics. These models are further used for estimating genetic parameters such as heritabilities and the phenotypic resemblance of relatives (e.g. 3, 4).

    1.1 Population genetics models tend to focus on the special case of epialleles at a single locus (e.g. 5, 6). Even for simple scenarios, such models are very complex, making them mathematically intractable. Accordingly, they are generally studied numerically or by means of computer simulations. Some population genetics models do not model the evolutionary dynamics directly, but instead, assume that evolution corresponds to an adaptive walk on a fitness landscape [7]. Such models can be applied to a broad class of 'non-genetic' mechanisms [8], but have often entailed somewhat unrealistic assumptions regarding how fitness reflects the interplay of genetic and 'non-genetic' factors (e.g. that fitness can be split into separate genetic and epigenetic portions).

1.2 Quantitative genetics models (e.g. 2, 9, 10) are technically more tractable, but they are based on strong and empirically-untested assumptions (such as a normal distribution of genetic and 'non genetic' effects, with stable variances and covariances). Perhaps most importantly, quantitative genetics models tend to assume that genetic and 'non-genetic' effects are additive (or that selection is very weak, implying that non-additive effects are negligible). Such additivity assumptions are not supported by the available data (e.g. 11) and do not align with the view that 'non-genetic' inheritance is best understood as inherited gene regulatory information.

1.3 By means of a Price equation approach, population genetics and quantitative genetics models can be viewed from a unified perspective [12]. This provides useful insights such as the result that 'non-genetic' inheritance can foster rapid adaptation when the population is far from a fitness peak, while it will often lead to a fitness reduction in an already well-adapted population [13]. To date, however, applications of the Price equation (e.g. 12) have also relied on simplifying and potentially misleading assumptions such as the additivity of genetic and 'non-genetic' effects.

2. Conceptual models based on the interplay of 'information channels': Here, genetic and 'non-genetic' effects are viewed as cues providing potentially adaptive information about the state of the environment (e.g. 14, 15). These models seek to ask what kinds of cues (for instance, inherited parental effects versus an individual's current information) will evolve to be used in a given scenario (depending on such factors as temporal versus spatial environmental fluctuation and transgenerational correlation). In contrast to most population genetics and quantitative genetics models, the information channel approach explicitly models the machinery integrating and interpreting different kinds of information. This allows an important additional question to be addressed: how do these information-integrating systems themselves evolve? At present, however, information channel models reflect highly simplifying assumptions regarding the nature of genetic and 'non-genetic' cues and the way these cues are processed: the phenotype results from the weighted summation of different cues, and the information-processing machinery is represented by the weighing factors.

I-1.3. A GENE REGULATORY NETWORK MODEL INCORPORATING NON-GENETIC INHERITANCE

To our knowledge, a mechanistic model for the evolutionary causes and consequences of 'non-genetic' inheritance that reflects the three key features of these systems (as explained in Section I-1.1) has not yet been proposed. Figure 1 illustrates a possible structure for such a model. The 'interpretive machinery' of a cell [12] is represented by a regulatory network where genetic and non-genetic factors interact in a variety of ways. Such a flexible network model could do justice to the various interactions among genetic, epigenetic, and environmental factors, providing for an inclusive understanding of inherited gene regulatory information, and might serve as a useful check of the robustness of the pre-

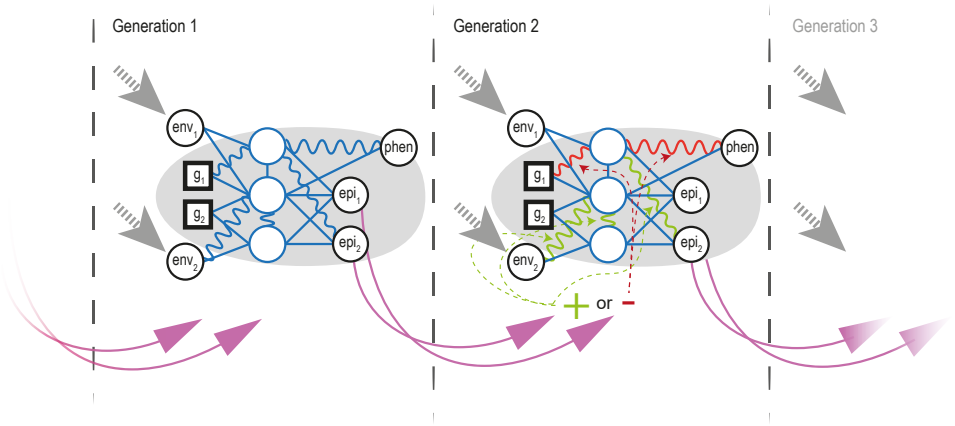

Figure 1 | *Proposed mechanistic modelling framework for studying the evolution of IGR* The model is based on a gene regulatory network (GRN), which integrates environmental (env_1,env_2) and genetic ($g_{1,2}$) information to produce a phenotype (phen). To account for IGR, we assume that in addition to the phenotype, epigenetic factors/signals are produced(epi_1 epi_2, which can affect the functioning of the GRN of the offspring. The connection weights of the GRN represent the influence of gene products (like transcription factors) on the expression of other genes. These connection weights are inheritable, but some of them (wavy connections) can be modified by epigenetic factors/effects. The graph in the picture illustrates that epigenetic factors can either down (epi_1) or up (epi_2) regulate the expression of genes in the next generation. The effects of these factors can be either unspecific or specific, e.g. in the figure, it is shown how epigenetic factors are targeted to specific (evolvable) gene sequences. Note that epigenetic factors can influence the connections controlling the expression of the factor themselves in future generations.

dictions made by the approaches discussed above. Figure 1 illustrates the transmission of epigenetic information from a parent in generation 1 to an offspring in generation 2. Both individuals harbor a gene regulatory network (GRN, blue lines) that determines the phenotype in response to genetic and environmental information. As in standard GRN models (e.g. 16), the nodes (blue circles) represent regulatory genes, and the connections between nodes represent the influence of transcription factors (or other regulatory elements) on the expression of other genes. The GRN integrates environmental (env_i) and genetic (g_j) information to produce the phenotype (phen). To study the effects of inherited gene regulation, we propose to expand the standard GRN models in two ways. First, as illustrated in the parent, the GRN not only mediates the expression of the phenotype; it also induces the production of epigenetic factors (epi_k) that are transmitted to the offspring. Which epigenetic factors are produced depends on the genotype and the environment of the parent. Second, as illustrated in the offspring, the connections between the nodes of the GRN are not solely genetically determined. Some connections (indicated by waves) can be up (+) or down (-) regulated by inherited epigenetic factors. The effects of these factors can be either unspecific (big + or – signs) or targeted to specific evolvable gene sequences (green and red arrows). Notice that in the proposed model the production of epigenetic factors in the offspring is partly determined by the epigenetic factors inherited from its parent, potentially resulting in a 'relay race' mode of regulation [1].

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I-2

INTERMEZZO 2: THE EVOLUTIONARY PERSISTENCE OF A PLASTIC PHENOTYPIC SWITCH IN THE ABSENCE OF SELECTION

Tiso Stefano, Riederer Jana M., Eldijk Timo J.B., Franz J. Weissing

This chapter has been published as part of"Phenotypic plasticity explains apparent reverse evolution of fat synthesis in parasitic wasps. Visser, B., Alborn, H.T., Rondeaux, S., Haillot, M., Hance, T., Rebar, D., Riederer, J.M., Tiso, S., Eldijk, T.J.B., Weissing, F.J., Nieberding, C.M. *Scientific Reports* 11(1), 1–13 (2021) [1]".

During my Ph.D. we collaborated with researchers from Louvain University (BE) on a study on the apparent reverse evolution of lipogenesis in parasitic wasps [1]. we contributed by providing the theoretical framework to contextualize and corroborate the empirical section of this project. Together with my colleagues, I developed an individual-based model that showed how adaptive plastic responses can maintain functionality against deleterious mutation for very long periods of time. Our conclusions from this model helped to explain the *apparent* disappearance and re-evolution of complex traits (such as lipogenesis) and resolve the *apparent* conflict of these observations with Dollo's law of irreversibility [2, 3].

Caroline Nieberding, Bertanne Visser, and their collaborators detected the presence of fatty acid synthesis (lipogenesis) in the parasitic wasp of the species *Leptopilina heterotoma*. These parasitic wasps were never recorded to express lipogenic activity before and were thought to have lost this complex metabolic trait 200 million years previous as Ellers and Visser pointed out in a previous publication [4]. The overall absence of this trait in parasitoid Hymenoptera is explained by the fact that parasitic wasps' development takes place inside fat-rich insect larvae [4]. Therefore, these wasps do not need to produce their own fat as they can easily gain this resource from their host; and since lipogenesis is a costly trait to maintain, they have gradually lost it. In the same publication, however, the authors remark that in some cases host-generalist species seemed to have re-evolved lipogenesis from scratch. This discovery seemed to contradict one of the few law-like principles in evolutionary biology: Dollo's law of irreversibility[2]. This principle articulated by 19th-century Belgian paleontologist Louis Dollo states that: "an organism never returns exactly to a former state, even if it finds itself placed in conditions of existence identical to those in which it has previously lived."[3], meaning that an organism will never re-evolve the same complex adaptation again, once they have lost it. To reconcile Dollo's law of irreversibility and the reappearance of the apparently lost lipogenic metabolism, Ellers, Nieberding and Visser speculated that lipogenesis was never actually lost in parasitic wasps, but instead kept dormant in the vast majority of cases and plastically reactivated only when strictly necessary. In [1], and later Nieberding, Visser et al. it was indeed shown that lipogenesis reappears when these wasps are raised in larval hosts under starvation. These starved hosts have very low body-fat percentages and thus do not present the parasitic wasps with the fat-rich environment in which they normally develop, forcing them to re-express lipogenesis. This explanation, however, requires that a plastic response can maintain functionality over long periods of time even if selection is not acting on it. One would normally expect that, in the absence of selection deleterious mutations would accumulate and gradually deteriorate the expressed trait. However, we argued that when considering a network of genes and their non-linear interactions, instead of single genes that only interact linearly, the effects of deleterious mutations would be greatly reduced. Interconnected and non-linear genomes are able to evolve mutational robustness [5–7] allowing unselected traits to remain functional for much longer periods of time. To corroborate this argument, we designed an individual-based simulation to test if indeed it was possible for a plastic phenotype-switch to maintain correct functionality (i.e. **on** in *rare* fat-poor environments, **off** in *frequent* fat-rich environments). The results from this model show that the on–off switch underlying plasticity can evolve mutational robustness and be maintained in the genome for hundreds to thousands of generations.

This helped to solidify the conceptual background necessary to support the claims and explanation of the empirical findings. This work resulted in a publication:

Phenotypic plasticity explains apparent reverse evolution of fat synthesis in parasitic wasps. Visser, B., Alborn, H.T., Rondeaux, S., Haillot, M., Hance, T., Rebar, D., Riederer, J.M., Tiso, S., Eldijk, T.J.B., Weissing, F.J., Nieberding, C.M. *Scientific Reports* 11(1), 1–13 (2021) [1]

Below, I give an account of the modelling aspects of the article. This part is relevant for my thesis, as it inspired my work on sporadic selection in Chapter 4.

I-2.1. THE MODEL

We consider the general situation where phenotypic plasticity is only sporadically adaptive and ask the question of whether and under what circumstances plasticity can remain functional over long evolutionary time periods when the regulatory processes underlying plasticity are gradually broken down by mutations. We consider a regulatory mechanism that switches on or off a pathway (like fat synthesis) in response to environmental conditions (e.g., host fat content).

I-2.1.1. FITNESS CONSIDERATIONS

We assume that the local environment of an individual is characterized by two factors: fat content F and nutrient content N, where nutrients represent sugars and other carbohydrates that can be used to synthesize fat. Nutrients are measured in units corresponding to the amount of fat that can be synthesized from them. We assume that fitness (viability and/or fecundity) is directly proportional to the amount of fat stored by the individual. When fat synthesis is switched off, this amount is equal to F, the amount of fat in the environment. When fat synthesis is switched on, the amount of fat stored is assumed to be $N - c + (1 - k)F$. This expression reflects the following assumptions: *(i)* fat is synthesized from the available nutrients, but this comes at a fitness cost c; *(ii)* fat can still be absorbed from the environment but at a reduced rate *(1-k)*. It is adaptive to switch on fat synthesis if $N - c + (1 - k)F$ is larger than F, or equivalently if $F < (N - c)/k$. The right-hand side of this inequality is a straight line, which is illustrated by the blue line in Fig. 1. The three boxes in Fig. 1 illustrate three types of environmental conditions.

- Red box: low-fat environments. Here, $F < (N - c)/k$ is always satisfied, implying that fat synthesis should be switched on constitutively.

- Yellow box: high-fat environments. Here, $F > (N - c)/k$, implying that fat synthesis should be switched off constitutively.

- Orange box: intermediate-fat environments. Here, fat synthesis should be plastic and switched on if for the given environment *(N, F)* the fat content is below the blue line and switched off otherwise.

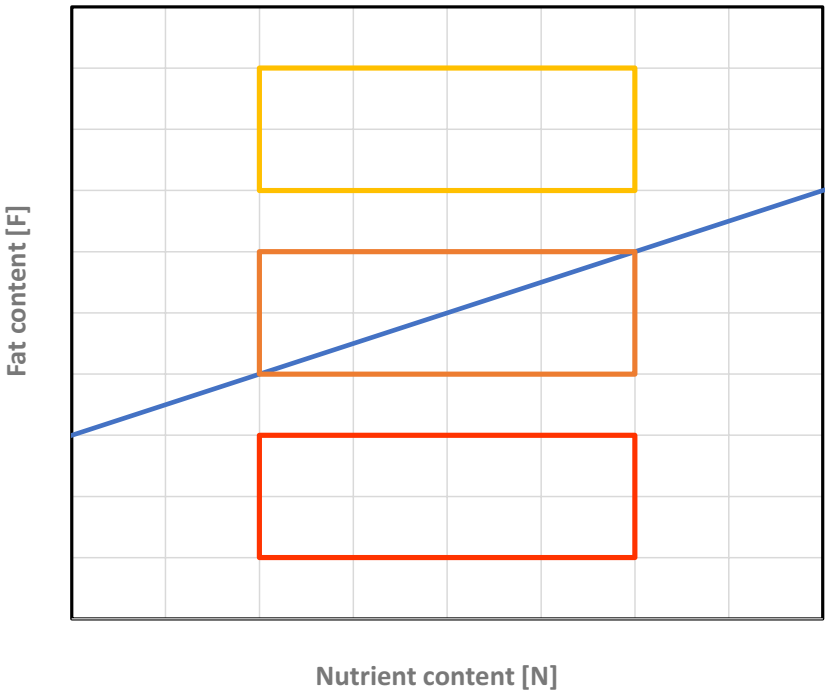

Figure 1 | Environmental conditions encountered by the model organisms. For a given combination of environmental nutrient content N and environmental fat content F, it is adaptive to switch on fat synthesis if (N,F) is below the blue line (corresponding to $F < (N-c)/k$) and to switch it off otherwise. The three boxes illustrate three types of environment: a low-fat environment (red) where fat synthesis should be switched on constitutively; a high-fat environment (yellow) where fat synthesis should be switched off constitutively; and an intermediate-fat environment (orange) where a plastic switch is selectively favored.

The simulations reported here were all run for the parameters $k = \frac{1}{2}$ and $c = \frac{1}{4}$. We also investigated many other combinations of these parameters; in all cases, the results were very similar to those reported here.

I-2.1.2. GENE REGULATORY NETWORKS (GRN)

In our model, the switching device was implemented by an evolving gene regulatory network (GRN, as in 8). As shown in Fig. 2, the networks considered had two receptor (or input) nodes, several (levels of) processor nodes, and an effector node. The two receptor nodes sense the fat and nutrient content in the local environment, respectively. The effector node switches on fat synthesis if the combined weighted inputs from all nodes connected to the effector node exceeds a threshold value T and switches it off otherwise. We ran simulations for GRNs of varying complexity, but for simplicity, we here focus on the simplest possible network, consisting of just two receptor nodes and an effector node. If F and N are the local fat and nutrient content and w_F and w_N are the corresponding weighing factors, fat synthesis is switched on if $w_F F + w_N N > T$ (and off otherwise). Hence, this simple GRN is characterised by the weighing factors w_F and w_N and the threshold T.

Simulations (shown in Fig. 3) are based on the most straightforward possible network, consisting of two receptor nodes (sensing the fat and the nutrient content in the local environment, respectively) and an effector node that switches on fat synthesis if the combined weighted input of the two receptor nodes exceeds a threshold value T and switches it off otherwise. Hence, fat synthesis is switched on if $w_F F + w_N N > T$ (and off otherwise). The GRN is characterized by the weighing factors w_F and w_N and the threshold T. These parameters are transmitted from parents to offspring, and they evolve subject to mutation and selection. For the simple GRN described above, the switching device is 100% adaptive when the switch is on (i.e., $w_F F + w_N N > T$) if $F < (N - c)/k$ and off otherwise. A simple calculation yields that this is the case if: $w_N > 0, w_F = -k\, w_N$ and $T = c\, w_N$.

I-2.1.3. EVOLUTION OF THE GRN

For simplicity, we consider an asexual haploid population with discrete, non-overlapping generations and fixed population size N=10,000. Each individual has several gene loci, each locus encoding one parameter of the GRN. In the case of the simple network described above, there are three gene loci, each with infinitely many alleles. Each individual harbors three alleles, which correspond to the GRN parameters w_F, w_N, and T, and hence determine the functioning of the genetic switch. In the simulations, each individual encounters a randomly chosen environment *(N, F)*. Based on its (genetically encoded) GRN, the individual decides on whether to switch on or off fat synthesis. If the synthesis is switched on, the individual's fitness is given by $N - c + (1 - k)F$; otherwise, its fitness is given by F. Subsequently, the individuals produce offspring, where the number of offspring produced is proportional to the individual's fitness. Each offspring inherits the genetic parameters of its parent, subject to mutation. With probability μ (per locus) a mutation occurs. In such a case the parental value (in the case of a simple network: the parent's allelic value w_F, w_N, or T) is changed to a mutated value ($w_F + \delta$, $w_N + \delta$, or $T + \delta$),

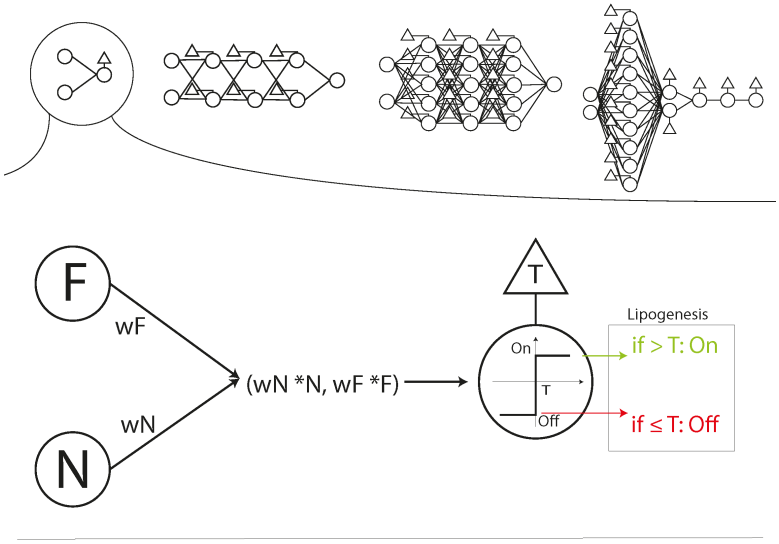

Figure 2 | Top: GRNs of varying complexity were simulated. The results reported here refer to the simplest GRN considered (encircled). Bottom: In-detail illustration of the functioning of the simplest GRN. Two local concentrations of fat and nutrients F and N feed into two separate connections and are multiplied by the respective connections weights: w_F w_N. The values of the two weighted inputs are then summed together. If the sum is > then the threshold value T the switch for plastic lipogenesis is switched on, otherwise it is kept off.

where the mutational step size δ is drawn from a normal distribution with mean zero and standard deviation σ. In the reported simulations, we chose $\mu = 0.001$ and $\sigma = 0.1$. The speed of evolution is proportional to $\mu \cdot \sigma^2$, implying that the rate of change in Fig. 3 (both the decay of plasticity and the rate of regaining adaptive plasticity) are positively related to μ and σ.

I-2.1.4. PREADAPTATION OF THE GRNS

Starting with a population with randomly initialized alleles for the GRN parameters, we first let the population evolve for 10,000 generations in the intermediate-fat environment (the orange box in Fig. 1). In all replicate simulations, a "perfectly adapted switch" (corresponding to $w_N > 0$ and $F = -kw_N$ and $T = cw_N$) evolved, typically within 1,000 generations. Still, the evolved GRNs differed across replicates, as they evolved different values of $_{wN} > 0$. These evolved networks were used to seed the populations in the subsequent "decay" simulations.

I-2.1.5. EVOLUTIONARY DECAY OF THE GRNS

For the decay experiments reported in Fig. 3, we initiated a large number of monomorphic replicate populations with one of the perfectly adapted GRNs from the preadaptation phase. These populations were exposed for an extended period of time (1,000,000 generations) to a high-fat environment (the yellow box in Fig. 1), where all preadapted GRNs

switched off fat synthesis. However, in some scenarios, the environmental conditions changed back sporadically (with probability q) to the intermediate-fat environment (the orange box in Fig. 1), where it is adaptive to switch on lipogenesis in 50% of the environmental conditions (when (N, F) is below the blue line in Fig. 1). In Fig. 3, we report on the changing rates $q = 0.0$ (no changing back; red), $q = 0.001$ (changing back once every 1,000 generations; purple), and $q = 0.01$ (changing back once every 100 generations; pink). When such a change occurred, the population was exposed to the intermediate-fat environment for t generations (Fig. 3 is based on $t = 3$).

Throughout the simulation, the performance of the network was monitored every 100 generations as follows: 100 GRNs were chosen at random from the population, and each of these GRNs was exposed to 100 randomly chosen environmental conditions from the intermediate-fat environment (orange box in Fig. 1). From this, we could determine the average percentage of "correct" decisions (where the network should be switched on if and only if $F < (N - c)/k$. 1.0 means that the GRN is still making 100% adaptive decisions; 0.5 means that the GRN only makes 50% adaptive decisions, as would be expected by a random GRN or a GRN that switches the pathway constitutively on or off. This measure for performance in the "old" intermediate-fat environment was determined for 100 replicate simulations per scenario and plotted in Fig. 3 (mean ± standard deviation).

I-2.2. SIMULATION RESULTS: ROBUSTNESS ALLOWS FOR A PLASTIC ADAPTIVE SWITCH TO BE MAINTAINED EVEN WHEN RARELY USED

If plasticity of fat synthesis arose in the common ancestor of parasitic wasps, and wasps are generally exposed to lipid-rich hosts, the question arises whether a switching device that is not used for extensive periods of time should be lost during the course of evolution. To investigate this, we ran individual-based simulations that monitored the sustained functionality of a switching device (a gene regulatory network that could decay by mutation) that is only sporadically used in evolutionary time. Figure 3 shows that the switching device rapidly disintegrates (red simulations) if it is never used (see the methods section for model assumptions, modeling details, and simulation settings). However, even very infrequent use (pink: every 100 generations; purple: every 1000 generations) suffices to keep the switching device largely intact. Interestingly, the switching device does not erode gradually, but instead slowly evolves an improved performance over evolutionary time (i.e., the percentage of correct decisions increases with the increasing number of generations). An inspection of the evolving gene regulatory networks (GRNs) reveals that they become more and more robust (i.e., less and less affected by mutational decay), in line with earlier findings on network evolution [9].

The simulations in Fig. 3 are representative of all networks and parameters considered. Whenever $q = 0.0$, the performance of the regulatory switch eroded in evolutionary time, but typically at a much lower rate in the case of the more complex GRNs. Whenever $q = 0.01$, the performance of the switch went back to levels above 90% and even above 95% for the more complex GRNs. Even for $q = 0.001$, a sustained performance level above

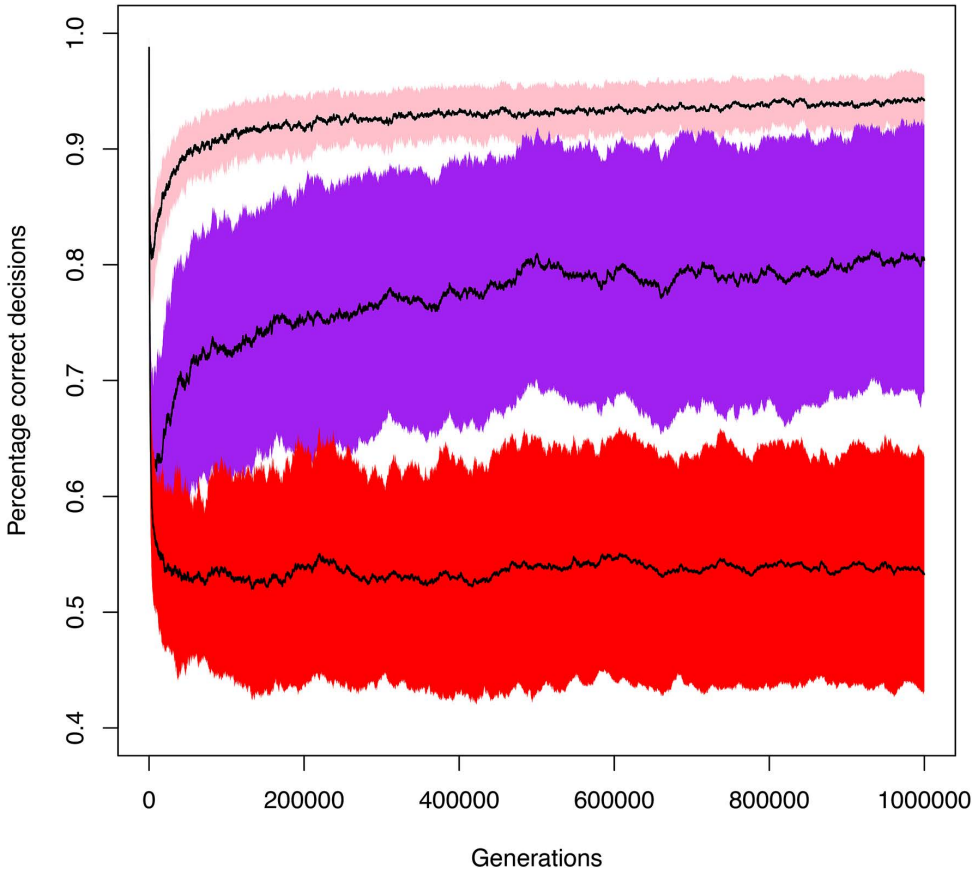

Figure 3 | We first evolved replicate GRNs in a variable environment where it is adaptive to switch on a metabolic pathway (fat synthesis) under low-fat conditions and to switch it off under high-fat conditions. In generation 0, a monomorphic population was established, where all N = 10,000 individuals were endowed with the same well-performing GRN (different across replicates). Subsequently, the population evolved subject to selection, mutation (μ = 0.001 per gene locus), and genetic drift in a fat-rich environment, where it is adaptive to constitutively switch off the metabolic pathway. Every 100 generations, we monitored the performance of a sample of GRNs (percentage correct decisions) in the original (fat-variable) environment: 1.0 means that the GRN is still making 100% adaptive decisions; 0.5 means that the GRN only makes 50% adaptive decisions, as would be expected by a random GRN or a GRN that switches the pathway constitutively on or off. The colored graphs show the average performance (± standard deviation) of the GRNs for three scenarios (100 replicates per simulation). Red: the population never again encounters the fat-variable environment; performance converges to 0.5, corresponding to constitutively switching off fat synthesis and hence the loss of adaptive plasticity. Pink: the individuals encounter a fat-variable environment on average every 100 generations; after an initial rapid drop in performance, a sustained high performance (> 90% correct decisions) of the GRNs is regained after about 100,000 generations. Purple: the individuals encounter a fat-variable environment on average every 1000 generations; after an initial rapid drop in performance, an intermediate performance (> 75% correct decisions) is regained gradually.

75% was obtained in all cases. Intriguingly, in the last two scenarios the performance level first drops rapidly (from 1.0 to a much lower level, although this drop is less pronounced in the more complex GRNs) and subsequently recovers to reach high levels again. Apparently, the GRNs have evolved a higher level of robustness, a property that seems to be typical for evolving networks [6, 7, 10]. For the simple GRN studied in Fig. 3, this outcome can be explained as follows. The initial network was characterized by the genetic parameters $w_N > 0, w_F = -k \cdot w_N$ and $T = c \cdot w_N$ (see above), where w_N was typically a small positive number. In the course of evolutionary time, the relation between the three evolving parameters remained approximately the same, but w_N (and with it the other parameters) evolved to much larger values. This automatically resulted in an increasingly robust network, since mutations with a given step size distribution affect the performance of a network much less when the corresponding parameter is large in absolute value. We also considered alternative network structures (see Fig. 2), and obtained very similar results.

I-2.3. DISCUSSION

We showed that a switch underlying plastic responses evolves mutational robustness and can withstand decay if it remains unused for extended periods of time. Another modeling study found that adaptive plasticity will be maintained in the genome for 10^8 generations [11]. Our simulation study shows that non-switching rapidly evolves in a fat-rich environment (leading to the loss of plasticity), but once the device has evolved mutational robustness, only incidental 'switching on' of the trait is sufficient for plasticity to be maintained within the genome. Plasticity itself can thus be highly robust to mutational change, which can apply also to other traits and systems. Our results further revealed large differences in the slopes of reaction norms between families, suggesting that there is genetic variation for plastic expression of fat synthesis. The plasticity of fat synthesis itself may thus evolve according to the local fat availability of host populations in the wild.

Phenotypically plastic organisms can incur different types of costs [12]. In our simple model, we only consider the cost of phenotype-environment mismatching, that is, the costs of expressing the 'wrong' phenotype in a given environment. When placed in a high-fat environment, the preadapted GRNs in our simulations take the 'right' decision to switch off lipogenesis. Accordingly, they do not face any costs of mismatching. Yet, the genetic switch rapidly decays (as indicated in Fig. 3 by the rapid drop in performance when tested in an intermediate-fat environment), due to the accumulation of mutations. It is not unlikely that there are additional fitness costs of plasticity, such as the costs for the production and maintenance of the machinery underlying plasticity [12]. In the presence of such constitutive costs, plasticity will be selected against when organisms are living in an environment where only one phenotype is optimal (as in the high- and low-fat environments in Fig. 1). This would obviously affect the evolutionary dynamics in Fig. 3, but the size of the effect is difficult to judge, as the constitutive costs of plasticity are notoriously difficult to quantify. In the case of the simple switching device considered in

our model, we consider the constitutive costs of plasticity as marginal, but these costs might be substantial in other scenarios.

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4

The Evolution and Maintenance of Complex Adaptations under Sporadic Selection: A Gene Regulatory Network Approach

Stefano Tiso, Franz J. Weissing

ABSTRACT

Many organisms exhibit complex adaptations that are only beneficial sporadically, once every few generations. How can such adaptations evolve and how can they be maintained when being eroded by genetic drift and mutation accumulation? Theoretical work on these questions has focused on simple traits, but many persisting adaptations are complex. Here, we take a simulation approach, based on evolving gene regulatory networks (GRNs), to study the evolutionary emergence and maintenance of a complex trait under sporadic selection. We systematically change the sporadicity of selection, the intricacy of the selective task, and the complexity of the network. The simulations reveal that complex adaptations readily emerge and are stably maintained at high quality over thousands of generations if the sporadicity of selection is moderate (selection taking place once every 5 or 10 generations). In the case of more extreme sporadicity (selection once every 100 generations), the adaptation still emerges and remains visible, but the performance of the evolving networks is of lower quality. In our model, the genome-wide mutation rate increases with network complexity. Despite the larger mutational load, more complex network architectures maintained a higher fitness, especially when the selective task is intricate. Robustness evolved through stabilizing selection on canalizing mutations. In conclusion, our study demonstrates that complex adaptations can arise and persist across extended time spans, even they are honed only sporadically by selection.

4.1. INTRODUCTION

How do organisms cope with sporadic events that significantly impact their survival and fitness? While much of the existing research has focused on adaptation to frequent or cyclical events, such as seasonal changes or other periodic phenomena, little attention has been paid to understanding how organisms cope and respond to rare but impactful events such as wildfires, floods, or the advent of novel pathogens [1–4]. These events are often happen so sporadically on a human timescale that it becomes empirically challenging to study their evolutionary consequences. Nonetheless, on an evolutionary timescale, they could still be happening regularly enough to force organisms to maintain (or even evolve) adaptive mechanisms to deal with them [5]. Empirical evidence suggests that there is an abundance of traits maintained over long periods of time, which serve their adaptive purpose only on rare occasions. *Escherichia coli* populations can revert immediately to their original anaerobic metabolism even after 30 years of being cultured in oxygen-rich environments [6]. *Caenorhabditis elegans* can maintain resistance to a bacterial pathogen even when exposed to it only sporadically[7]. Parasitic wasps can activate their dormant fat metabolism in response to extreme starvation conditions that occur very infrequently [8]. Semi-blind cave-fish still retain visual preferences for partners [9], and a vast literature indicates that complex vestigial behaviours can be maintained for long periods even when not being selected for [10–17] and could potentially serve as facilitators of innovation [18]. These observations are interesting and somehow counter-intuitive, as one would expect unselected traits to be lost due to the accumulation of deleterious mutations. However, despite the many empirical studies providing insights on this topic directly or indirectly, to our knowledge, little theoretical work is being conducted [19]. Some theoretical studies exist [20–22], however, these studies mainly focus on simple adaptations. For instance, [23, 24], considers the effect of mutations on a single gene that correlates linearly with fitness. Little is known about more intricate scenarios, where traits are multidimensional and determined by multiple genes, with complex non-linear genotype-to-phenotype (G-P) mappings. It is this type of complex trait, however, that most of the above-mentioned observations in nature seem to refer to. Theoretical knowledge is of fundamental importance, as studying how long-term sporadic selective events affect evolutionary outcomes in the wild is almost impossible. Understanding how organisms cope with sporadic selective events can have significant implications for hard-pressing matters such as rare-pathogen resistance, species preservation, and adaptation to the increase in extreme weather events due to climate change. Additionally, when considering the evolutionary implications of sporadic selective events a second question ought to be addressed: their emergence. Understanding how and if adaptations can emerge through sporadic selection is as relevant as determining the mechanisms of their maintenance. By means of a simulation study, we, therefore, address two questions: can complex adaptations to sporadic events be maintained against the accumulation of deleterious mutations? And can these adaptations emerge in the first place when they are so rarely selected for?

Theoretical studies on the maintenance and decay of a trait in the absence of selection have been conducted as far back as the founding of the Modern Synthesis, in the

framework of population and quantitative genetics [25–29]. Some of these studies provide the verbal arguments on how traits could be lost in the absence of selection [25–27], while other also provide analytical predictions [28, 29]. However, these studies consider simple traits encoded by single loci which directly correlate with fitness. There seems to be a common agreement early on that complex gene interactions (as well as other factors) must be considered to paint the full picture [27, 28].

Other lines of research also focus on the effect of unselected mutations on the outcomes of evolution such as research on mutational meltdown and the Neutral Theory of Evolution [21, 23, 24]. Mutational meltdown studies focus on providing predictions on how fast adaptations can be irreparably lost due to the accumulation of deleterious mutations in finite asexual and sexual populations. Lynch's work in particular [23, 24], through both analytical and computational models explains how the loss of adaptation is dependent on mutation rates, population demographics, and mutational effects. In particular mutation rate exponentially correlates with the time needed to lose adaptation (named extinction in the original papers as organism fitness in their models is based on a single trait).

All the aforementioned studies operate within the theoretical framework of population and quantitative genetics, which, has significant limitations in its ability to represent complex traits and G-P mappings. Nonetheless, theoretical literature coming from these approaches is well accepted, leading, in more recent decades, to little expansion on the topic from other theoretical perspectives [8, 20, 30], despite a relative abundance of empirical studies [15, 31–35].

Masel et al. [20] extend such theory by using mathematical models to delineate conditions under which rarely utilized adaptive plastic traits can persist over evolutionary timescales. Their key finding is that sufficiently frequent environmental fluctuations must reverse the degradation of plastic traits, in order to counteract ongoing mutation and selection pressures. However, this relies on rather strict binary assumptions, including that individuals either fully possess the plastic trait or completely lack it, and that any improper trait expression is instantaneously lethal. In Visser et al. [8] we tackle a similar question, by implementing individual-based simulations of slightly more complex, but yet relatively simple (one-dimensional phenotype controlled by three loci), gene regulatory switches underlying plasticity. We observe that such switches can evolve increasing robustness to mutation during extended periods of disuse, thereby allowing long-term trait retention even with only sporadic activation. While both papers emphasize the primacy of environmental change frequencies relative to trait-eroding pressures, Masel's et al. model requires the former to regularly override the latter, whereas Visser's et al. model shows that intrinsic evolution of the switch to resist mutation can also suffice.

The research on GRN evolution [36–38], addresses the issues with the simplicity of the G-P representation mentioned above, and provides interesting insights. GRNs often exhibit a property that is not observed in more traditional models where genes are considered disconnected entities: robustness [39, 40]. This refers to their ability to stably maintain a trait despite perturbations such as mutations or changes in the environment [41], which is also what we observed in [8]. Robustness is typically studied in the context of its impact on the evolution of novel traits and the evolvability of a system, with little attention given to its role in sporadic selection events, robustness likely also can enable

the maintenance complex adaptations through prolonged mutation accumulation punctuated by periodic selection. While robustness may help preserve adaptations during long periods of accumulation of deleterious mutations, it may also limit variation for selection to act upon during sporadic instances of selection. Some research, however, suggests that robustness can protect against long periods of mutation accumulation while still facilitating rapid adaptation and diversity [42].

In this paper, we will investigate a model to study persistence, loss, or even emergence of complex traits when selection only happens sporadically. To do so, we use an individual-based model for the evolution of an adaptive reaction norm. Adaptive reaction norms need to capture phenotype changes across an environmental gradient, representing thus a complex adaptive challenge. Within each individual, the reaction norm is produced by an underlying gene regulatory network (GRN). Thus the reaction norms provide a defined target for a complex phenotype, while the GRNs allow complexity in the genotype-phenotype map. Finally, we implement sporadic selection by alternating brief periods of selection with longer intervals without selection. When selection is applied, an individual's chance of reproducing is proportional to how well their phenotype matches the target environment, otherwise, when selection is absent all individuals are equally likely to reproduce. The absence of selection for long time spans allows mutations to neutrally accumulate across the population. By varying the length of these intervals, we can examine how the frequency of selection affects the maintenance and emergence of complex traits. Overall, with this modeling framework, we are able to enquire about the effects on the evolution of adaptations to sporadic selective events of selection frequency, complexity of the selective challenge, and G-P architecture. We conduct two types of experiments: maintenance experiments, where we allow the reaction norm to evolve under constant selection before exposing it to sporadic selection, and emergence experiments, where selection is sporadic from the start. For each experiment type, we run simulations for different lengths of mutation accumulation periods, as well as controls to compare with the outcomes of evolution in pure mutation accumulation or constant selection. Finally, we test the effects of evolution on GRNs with varying degrees of architectural complexity faced with different selective challenges .

We, therefore, investigate the following questions: can complex adaptations be maintained or even arise when selected only sporadically? At what frequency does sporadic selection cause complex adaptations to decay due to mutation accumulation? How does the complexity of the GRN and selective challenges affect complex adaptations under sporadic selection?

4.2. THE MODEL

4.2.1. OVERVIEW

The model structure is illustrated in Figure 1. Each simulation follows an evolving population of 1000 individuals over 100'000 generations. Generations are discrete and non-overlapping and reproduction is asexual. Each individual harbors a (heritable) gene-

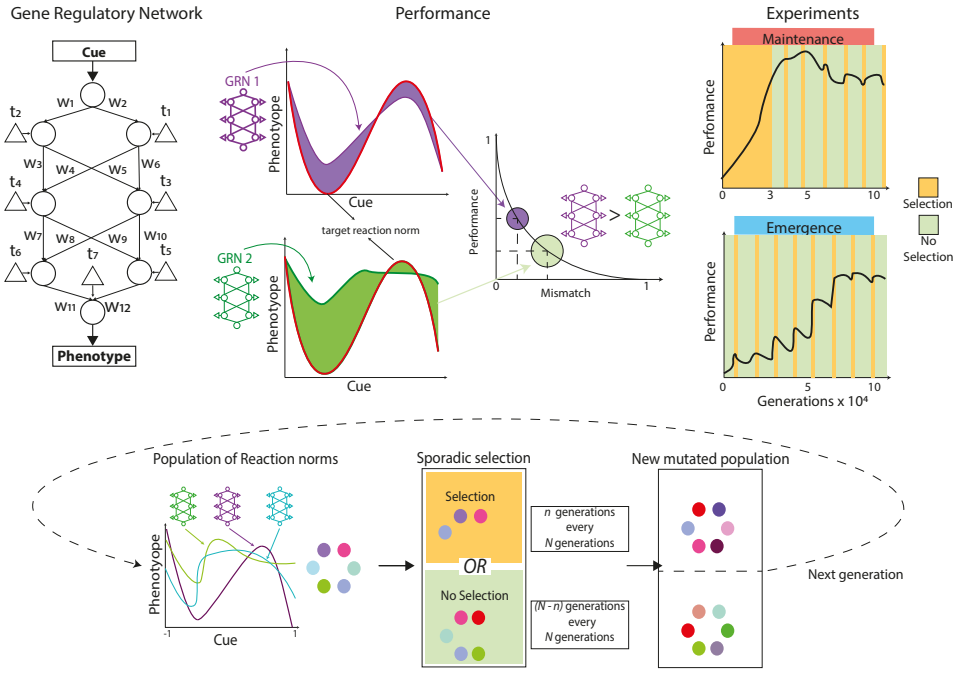

Figure 1 | Model structure. (A) We consider a population where each individual harbors a gene regulatory network (GRN). Each GRN is characterized by weighing factors w_i and baseline activities t_j that are genetically encoded (and evolving), constant throughout an individual lifetime, and transmitted from parent to offspring (subject to some mutation). For a given set of weights and baseline activities, a GRN processes cues (inputs), resulting in a cue-specific phenotypic response (outputs). This input-output relationship is the 'reaction norm' encoded by the GRN. (B) Individuals are selected based on how well the reaction norm encoded by their GRN matches a target function (red curve). The area between the GRN-encoded reaction norm and the target function indicates the 'mismatch' between GRN and the target. We assume that the 'performance' of a GRN (which is proportional to the lifetime reproductive success of its bearers) decreases exponentially with the mismatch. (C) Individuals are selected only sporadically (for n generations, followed by N-n generations without selection). We conducted two types of simulation experiments. In the 'maintenance' experiments, we studied the build-up of adaptation in the first period of 3,3 x 10^4 generations of continual selection (every generation) and the subsequent decay of adaptation in the remainder of 7,7 x 10^4 generations of sporadic selection. In the 'emergence' experiments, we studied the build-up of adaptation in a period of 10^5 generations of sporadic selection. (D) This panel indicates how, over the generations, the GRNs evolve by the interplay of performance-based selection, its absence, and mutation.

regulatory network (GRN) that processes environmental inputs and produces an output y for each input x (Fig. 1 A). The input-output relationship created by the GRN of individual i is described by a function $y = R_i(x)$, which we call the "reaction norm" of individual i (Fig. 1 B). Selection acts on these reaction norms and, hence, on the underlying GRNs. However, selection only occurs sporadically (Fig. 1 C - D)- in many generations, selection does not occur, and all GRNs have the same expected reproductive success. In those generations where selection occurs, the task is to match an environmental function $y = E(x)$, where E remains the same throughout the whole simulation. When selection occurs, those individuals whose reaction norm R_i matches the target function E most closely have the highest expected reproductive success. More specifically, the expected reproductive success of an individual i is proportional to $e^{(-\Delta_i(R_i,E))}$, where $\Delta_i(R_i,E) = \int_{-1}^{1} |R_i(x) - E(x)|dx$ is the average distance between the functions R_i and E (Fig. 1 B). For convenience, we approximated $\Delta_i(R_i,E)$ by the distance $|R_i(x_j) - E(x_j)|$ for 40 randomly chosen values $x_j \in [-1,1]$. At the end of each generation, 1,000 offspring are produced (by a weighted lottery) in such a way that the expected number of offspring of individual i is either 1 (in generations without selection) or $e^{(-\Delta_i(R_i,E))}$ (in generations with selection). Each offspring inherits the GRN of its parent, subject to mutation (as specified in more detail below). We record how well populations adapt to match a target reaction norm function E despite the long period of mutation accumulation. We run simulations with different E functions, different GRNs architectures, and different levels of sporadicity of selection. Finally, we test for two different scenarios, one in which first populations evolve in constant selection and are only subsequently exposed to sporadic selection (maintenance of adaptation) and one in which sporadic selection goes on from the start (emergence of adaptation) (Fig. 1 C).

4.2.2. PERFORMANCE AND SELECTION

The performance of an individual with a reaction norm R_i is negatively related to the mismatch $\Delta(R_i,E)$ between R_i and the optimal reaction norm E. To evaluate the mismatch we take the distance $\Delta_i(R_i,E) = \frac{1}{n} \cdot \sum_{j=1}^{n} |R_i(x_j) - E(x_j)|$ averaged over all of the 40 environmental inputs $x_j \in \vec{x}\{x_1,...,x_{40}\} \in [-1,1]$ individuals are exposed to (Δ_i). We then use a Gaussian function to quantify and rescale performance between 0 and 1: $P_i = exp(-\Delta_i)$ In those generations where selection is acting, performance is a proxy for Darwinian fitness: the expected number of offspring that an individual i contributes to the next generation is proportional to the individual's performance P_i. In generations without selection the number of offspring per individual is Poisson distributed with a mean value of 1. In both cases, the offspring inherits the parent's regulatory network, after mutation (see below). Through evolutionary time, the task of the network can be thought of as the approximation of functions rn_{opt}: populations are selected to "encode" the complex relationship between environmental cues and optimal phenotype.

Performance-based selection only happens sporadically. It will take place for brief intervals of time across longer stretches of relaxed selection, while mutations will keep accumulating. The notation we use to indicate different regimes is n/N. n indicates the number of generations where performance-based selection will take place at the start of an N generations-long period, for the remainder of N relaxed selection will be

acting. Once N generations have passed, the cycle will repeat, and performance-based selection will act again. We run simulations for a total of 6 different regimes of sporadic selection: 1/1 or constant selection, 1/5, 1/10, 10/100, 1/100, and 0/1 or no selection. From now onward in this text, when we will refer to the term selection it will signify performance-based selection, unless explicitly specified otherwise.

4.2.3. SIMULATION SCENARIOS

We design two different types of simulations: maintenance and emergence. During maintenance experiments, populations will undergo a first period (1/3 of the 100'000 total generations of the simulations) of constant selection, to ensure that a functioning adaptation is evolved, and only later will start being exposed to sporadic selection. In emergence experiments instead, populations will be exposed to sporadic selection from the beginning. We run these two types of experiments for all 6 regimes of sporadic selection listed above.

4.2.4. THE NETWORKS

The reaction norm R_i of each individual i is encoded by a heritable multi-layered, feed-forward, regulatory network. It consists of one single input, one to three layers of internal nodes, and one output node. The input is contributed by the environment and is not part of the network itself. The following notation will be used to represent the size of the networks: 1-n_1-n_2-n_3-o, 1 represents the single input/environmental cue, n_k is the number of nodes in internal layer k, and o is the number of output values. For instance, 1-2-2-2-1 refers to a network with one input, 3 internal layers with two nodes per internal layer, and one output node. In this investigation, all networks will receive only one input/environmental cue and will only have one output/phenotypic response. We vary the number of internal layers and of nodes per layer for the different scenarios we study. Therefore all networks in this experiment have a 1-*-[...]-*-1 structure. Each node has a baseline value and a series of incoming connections: the ith weight is the weight of the connection to the ith node in the previous layer. Baseline values and weights are unbounded real numbers. Given a series of inputs, nodes generate an output. In the first layer of internal nodes, the environmental cue x acts as an input. In the next layers, the output of each node in the previous layer becomes one input. This operation is repeated until the final layer is reached, resulting in the output of the network. Given a series of inputs, the output of a node is calculated with the following formula:

$$output = f(b + \sum_{i=1}^{n_{inputs}} o_i * w_i)$$

, where b = baseline value, o_i = output of the previous node i, w_i = weight of the connection to previous node i, and $f(z)$

$$f(z) = (\frac{z}{1+|z|})$$

The activation function $f(z)$, is a monotonic sigmoidal function, bounded between -1 and 1. Hence, usage of this function ensures that all node values, including that of the output

node, are in the interval [-1,1]. It also allows non-linear interactions within the network, which is important for its functioning [43]. The value of nodes in the first internal layer is calculated with the environmental input, then gets passed on as input to the second layer, etc, until the output is obtained in the final layer.

4.2.5. REPRODUCTION AND INHERITANCE

Networks are encoded by a number of heritable loci proportional to the number of nodes in the network (input node excluded). For each node, there is:

1. One baseline value locus, an infinite-allele locus (represented by an unbounded real number) encoding the baseline value of the node
2. As many weight loci as there are nodes in the previous layers. They are infinite-allele loci (represented by unbounded real numbers) encoding the connection weight between each node in the previous layer and the current node

Different elements of the network can mutate:

1. Baseline node value. With a rate of 0.01, the baseline value locus of a node changes to another allele: the previous value changes by an amount drawn from a normal distribution $N(\mu,\sigma)$, where $\mu = 0$ and $\sigma = 0.1$.
2. Connection weights. With a rate of 0.01, a weight locus changes to another allele: the previous value changes by an amount drawn from a normal distribution $N(\mu,\sigma)$, where $\mu = 0$ and $\sigma = 0.1$.

4.2.6. ENVIRONMENTAL TARGET FUNCTIONS

All individuals are presented with the same series of 40 environmental cues (x) uniformly sampled across the range of possible environmental values $\mathbb{R} \in [-1,1]$. We run simulations for three different environmental target functions that are presented y polynomials of increasing complexity. The three functions, which are illustrated in Figure 4 are:

1. *type* 1 (simple):
$$E_1(x) = x^3$$
, is monotonically increasing with an inflection point at x=0.
2. *type* 2 (intermediate):
$$E_2(x) = 3x - 4x^3$$
, is U-shaped for $x < 0$, with a minimum at $x = -0.5$., and hump-shaped for $x > 0$, with a maximum at $x = 0.5$.
3. *type* 3 (complex):
$$E_3(x) = -0.8 + 9.32x^2 - 15.84x^4 + 6.33x^6$$
, with maxima at $x = -0.5$ and $x = 0.5$ and a minimum at $x = 0$.

4.3. RESULTS

We first study how in both maintenance and emergence experiments (see Fig. 1C) the evolutionary outcome depends on the 'sporadicity' of selection. We look for distinguishing features between the two types of experiments in the evolution of complex traits by both looking at the final results of evolution and in-detail evolutionary trajectories of single replicate simulations. These results are based on our default setting: a *type* 2 target reaction norm and a *type* 1-2-2-2-1 GRN (as in Fig. 1). In the final part of the Results section, we show how the evolutionary outcome is affected by the complexity of the target function and the complexity of the evolving GRNs.

4.3.1. EFFECT OF SPORADICITY ON THE EMERGENCE AND MAINTENANCE OF A COMPLEX ADAPTATION

Figure 2 summarizes the outcome of 600 long-term simulations run for six increasingly sporadic regimes of selection. We compare the average performance for the last 10'000 generations of 50 replicate simulations across 6 increasingly sporadic regimes of selection (constant selection, selection for 1 in 5 generations, 1 in 10, 10 in 100, 1 in 100, and no selection) for both maintenance and emergence experiments. When selection is constant (1/1) the two types of experiments are the same, the final level of adaptation is highest (same for both experiments, average performance = 0.72, Fig. 2 left plot), and the best replicates match the target almost perfectly (Fig. 2 top panels on the right). When no selection takes place instead (No_selection), performance levels are at a low baseline level(Fig. 2 left-side average performance $\approx$ 0.4, Fig. 2 left plot) and adaptation gets completely lost (Fig 2 bottom panels on the right). Performance decreases as selection becomes increasingly sparse. Within these extremes, the performance of the evolved GRNs decreases. Nonetheless, the average performance remains quite high unless selection is very infrequent. Populations from 1/5 experiments have on average the same level of performance as populations from 10/100 experiments. There is a significant drop only when selection becomes extremely sporadic (1/100). Performance stays the same for the two regimes 1 in 10 and 10 in 100, suggesting that the relative frequency of generations with selection on mutation accumulation (n/N) is more important than the spacing of selective events. Looking at in-detail snapshots of different evolved replicates (Fig. 2 panels on the right), a common pattern emerges across different regimes. Reaction norms are generally S-shaped, with different degrees of matching to the target reaction norm (*type* 2 in this case, a function with one local minimum, one local maximum, and an inflection point in-between). Even at low levels of adaptation evolved reaction norms can match the an inflection point between the local minimum and maximum precisely, while their extremities often remain flat and do not match the curve around the minimum and maximum. The matching increases for more frequent selection and reaction norms are also able to more closely match the minimum and maximum, usually by stretching their S shape and eventually adding one or two extra curvatures (see Fig. 2 panel on the right for the regime 10/100). However, not even the top-performing GRNs (1/1 regimes) manage to match the target reaction norm perfectly, despite being very close. The detailed snapshots also show that variation in the population changes along regimes: for 1/1 regimes almost

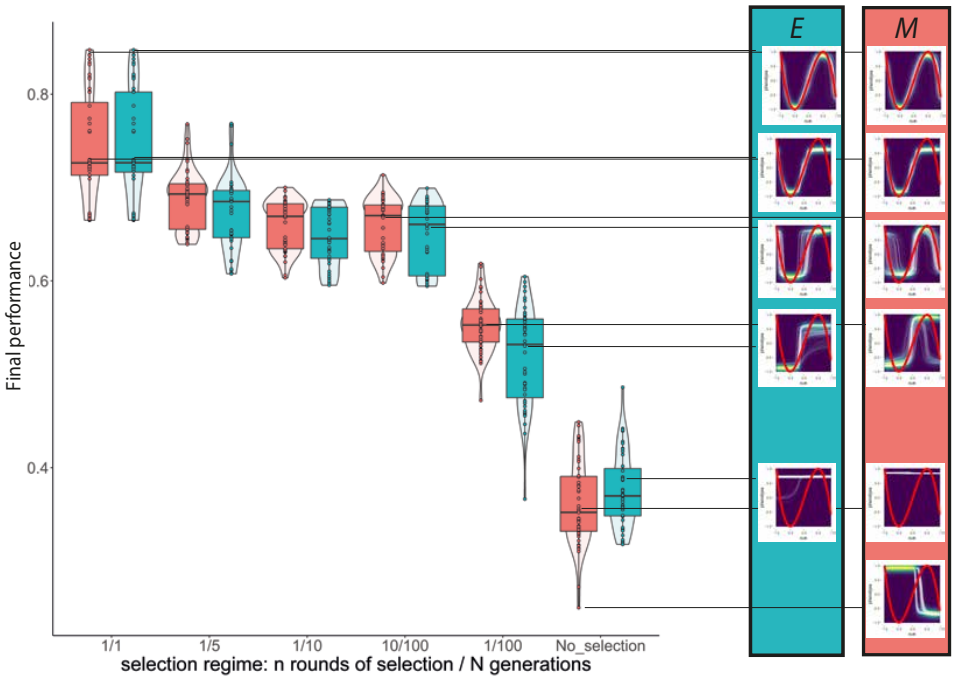

Figure 2 | Final GRN performance in the emergence and maintenance scenario for different degrees of sporadicity. For six different selection regimes n/N (n rounds of selection followed by $N-n$ rounds without selection), ranging from constant selection (1/1) to highly sporadic selection (1/100) and no selection (0/1) as a control, 50 replicate simulations were run for both the emergence ('E', blue) and the maintenance ('M', red) scenario. The violin plots show the average performance of these replicates over the final 10^4 (of in total 10^5) generations. For both scenarios, the panels to the right illustrate the evolutionary outcome in specific replicates differing in average performance. Each sub-panel shows the target function (thick red curve) and the evolved GRN reaction norms of the 1,000 individuals in the population (thin white curves). These panels, therefore, exhibit the degree of variation within a population and visualize the mismatch between the target function and the evolved reaction norms. These simulations were run for networks with three inner layers with 2 nodes each (1-2-2-2-1), and a target environmental function of *type2* with 1 minimum and 1 maximum (intermediate complexity).

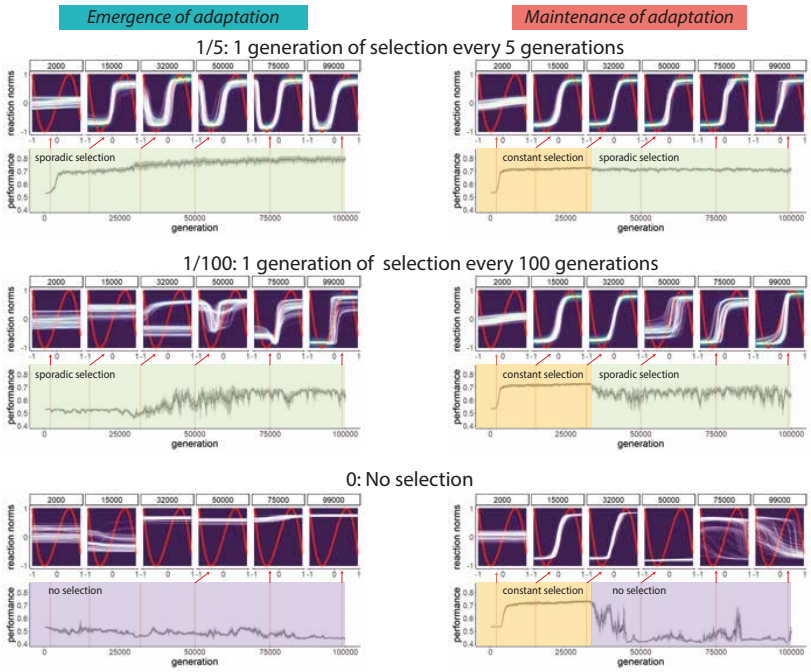

Figure 3 | Examples of evolutionary trajectories in the emergence (left) and maintenance (right) scenario for (A) low sporadicity (n/N = 1/5), (B) high sporadicity (n/N = 1/100), and (C) in the absence of selection. In each of the six cases, one representative simulation is shown. For each simulation, the lower plot shows how the average population performance (± one standard deviation) changes over the course of the generations. The thumbnail graphs on top of these plots illustrate the state of the population in six different generations (t = 2,000, 15,000, 32,000, 50,000, 75,000, and 99,000). Each of the thumbnails shows the target function (thick red curve) and the reaction norm of the GRNs of the 1,000 population members in the corresponding generation. These simulations were run for networks with three inner layers with 2 nodes each (1-2-2-2-1), and a target reaction norm with 1 minimum and 1 maximum (intermediate complexity).

no variation is observable, with almost all reaction norms largely overlapping on the entire environmental range. However, lower regimes show a much higher variation (see 10/100) and in some cases even polymorphism (see 1/100). Finally, emergence and maintenance experiments reach comparable levels of adaptation. One might have expected that having an initial kick-start via constant selection before being exposed to substantial amounts of mutation accumulation/unselected mutations (maintenance experiments, Fig. 2 red color) would confer an advantage in obtaining higher performance levels. Instead, an equally high level of performance can be obtained by evolving adaptation from scratch, having to contend with long periods of mutation accumulation from the start (emergence experiments, Fig. 2 blue color).

4.3.2. A MORE DETAILED LOOK AT THE EVOLUTIONARY TRAJECTORIES

Figure 3 shows the evolutionary dynamics of replicates for different sporadic selection regimes in greater detail, comparing side-by-side simulations in the same selection regime but from emergence and maintenance experiments. Despite achieving similar final results, emergence and maintenance experiments follow different evolutionary trajectories. In maintenance experiments (Fig. 3 plots on the right column), adapted reaction norms manage to evolve during the period of constant selection to which the population is subjected. Performance plateaus at a high level rapidly (in the first ≈ 4000 to 6000 generations). Afterward, when sporadic selection starts (generation 33'000), performance drops and begins to oscillate, with the reaction norms increasing in diversity. Although the drop and oscillations eventually stabilise, a full recovery of the original level of performance is never achieved. The opposite trend is observed for emergence experiments. Performance grows more slowly (usually performance plateaus around ≈33'000 to ≈ 40' 000 generations), and both oscillations in performance and diversity in the reaction norms decrease over time. The evolutionary dynamics also vary depending on the frequency of selection events. As selection becomes more sporadic, maintenance experiments exhibit a more pronounced drop in performance and increased oscillations while diversity of reaction norms grows. Emergence experiments take gradually longer for their performance to plateau, the sparser selection is. In a mildly sporadic selection regime (1/5, Fig 3A), performance in the emergence and maintenance experiments reaches a similar level in a comparably short amount of time (≈4000 generations). Reaction norms in the emergence experiment are more diverse and performance undergoes a second increase around generation 32'000, surpassing the maintenance experiment. This shows that in some emergence experiments, performance can increase even past the level obtained by the populations subjected to constant selection in maintenance experiments. However this is not always the case, and maintenance experiments can achieve as well higher performance than their emergence counterpart (as shown by the violin plots in Fig. 2). In the case of very sporadic selection (1/100, Fig. 3B), performance plateaus more slowly in the emergence experiments and diversity is higher. It is possible to observe a clear-cut case of polymorphism in the population around generation 32 '000. Nonetheless, the population gradually converges to one phenotype, and performance increases and plateaus also in this regime, despite more slowly. For maintenance experiments, there is a significant drop in performance and the reaction norms become more and more heterogeneous after sporadic selection starts. Finally, for pure mutation accumulation regimes (No_Selection, Fig. 3C), adaptation either never develops (emergence experiments) or is very rapidly lost (maintenance experiments). These latter simulations were run in order to confirm that in other regimes, even when selection is very rare, performance is not maintained just because GRNs produce adapted reaction norms by default but because selection is able to drive their evolution.

4.3.3. EFFECTS OF THE TARGET FUNCTION AND NETWORK ARCHITECTURE ON THE EVOLUTIONARY OUTCOME

Figure 4 shows that the results listed above hold for a wide range of different target reaction norms and genetic architectures. We ran simulations for simple, intermediate,

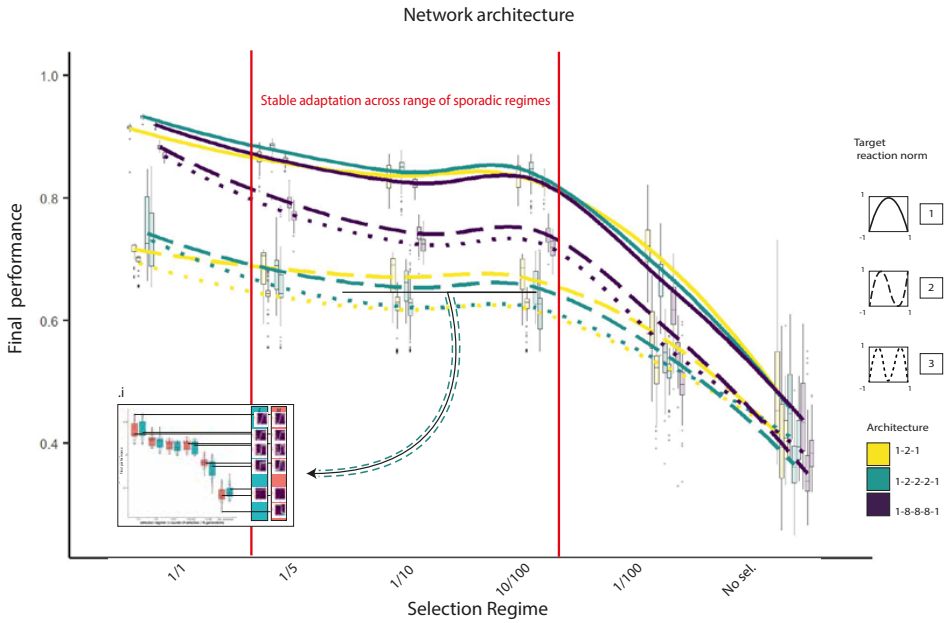

Figure 4 | Effect of target reaction norm and network architecture on the relationship between the sporadicity of selection and final population performance. Figure 2 (inserted as a thumbnail) shows how the final population performance depends on the sporadicity of selection for one target reaction norm (type 2) and one network architecture (type 1-2-2-2-1). Here, this figure is generalized by considering all combinations of three target reaction norms (types 1, 2, and 3; solid, dashed, and dotted lines respectively) and three network architectures (types 1-2-1, 1-2-2-2-1, and 1-8-8-8-1; yellow, blue, and purple colors) for a total of 5400 simulations. Emergence and maintenance results are aggregated in this plot as no substantial difference appears. Colored lines in the plot represent the trend in performance of a specific architecture x target reaction norm combination (e.g dashed blue represents the combination performance trend for the combination shown in Fig. 2). The two vertical red lines delimit the interval of selection regimes where performance is maintained stably.

and complex target reaction norms and GRN architectures (9 possible reaction norm and architecture combinations, for a total of 5400 simulations, see Methods for a more detailed explanation). Each architecture can have different numbers of layers and nodes per layer; the more genetic loci and layers compose it the better one architecture will be able to produce complex reaction norms. It is important to notice that the per-locus mutation rate is kept constant across architectures, meaning that more complex architectures are subjected to a higher per-genome mutational load. Fig. 2 and 3 showcase results only for a combination of target reaction norm and complexity both with intermediate complexity (*type* 1-2-2-2-1 GRN and *type* 2 target reaction norm). However, the same results are observed in all the possible permutations of reaction norms and architectures we explored. Performance is highest when selection is constant, is stably maintained across a wide range of sporadic selection regimes, starts to decrease only in extreme conditions, and is completely lost only when no selection takes place. The simpler the reaction norm the higher performance will be in all regimes (except for No_selection, where of course the target reaction norm is not relevant). For the simplest reaction norm (1 single local maximum) performance is the highest as expected, with all three architectures performing comparatively well. The most complex architecture (1-8-8-8-1) performs significantly better for more complex reaction norms. This is interesting, since, despite a higher mutational load, the most complex architectures not only maintain functionality across sporadic selection but are also able to better adapt to complex selective tasks. The 1-2-1 and 1-2-2-2-1 architectures have similar performances with the exception of 1-2-2-2-1 performing better in constant selection. One interesting observation is that when selection becomes extremely sporadic (1/100) the 1-8-8-8-1 architecture has a more pronounced drop in performance and has a similar level to the two other smaller architectures. The results shown in Fig. 2 represent a scenario of intermediate complexity, where the outcome is actually not as good as the best possible scenario achieved by more complex GRNs. The fact that sub-optimal scenarios such as the ones in Fig.2 still show the capability to maintain adaptation in sporadic selection suggests that our results are indeed general over a wide range of parameters.

4.4. DISCUSSION

Our study investigates the impact of sporadic selection on the evolution of complex adaptations in gene regulatory networks (GRNs). We report three main findings: 1) sporadic selection can preserve complex adaptations even across extended periods of mutation accumulation; 2) complex adaptations can emerge as readily as they are maintained; and 3) the complexity of the genome and the selective challenge does not affect the general trend in adaptation, but complex genomes can better adapt to complex challenges despite an increased mutational load. In the following discussion, we delve deeper into these results and their implications.

Longer periods of mutation accumulation have a detrimental impact on the adaptation level of a trait. However, mutation accumulation has a lesser effect than anticipated. Selection directs evolution toward adaptive outcomes even when it is two orders of magnitude shorter than mutation accumulation periods (i.e., 1 generation of selection for every 100). Performance remains consistent across a broad range of regimes, with fitness

decreasing in a step-wise manner rather than linearly. Furthermore, the relative frequency of selection against accumulation periods is more relevant in determining the quality of adaptation than the total amount of mutation accumulation. When comparing selection 1 generation for every 10 and every 10 for every 100, one might expect that deleterious variants would fixate more frequently if mutations accumulate for 90 generations instead of 9. Surprisingly, the final performance does not differ between the two regimes.

Complex adaptations can emerge as readily as they can be maintained during sporadic selection. Populations achieve the same level of performance if they are allowed to evolve a complex adaptation in constant selection beforehand (maintenance experiments) or if they evolve in sporadic selection from the beginning (emergence experiments). This finding can help explain how organisms can be adapted to challenges they do not usually encounter , as they can acquire adaptations to new environmental conditions as long as they face them sporadically. This result holds even when selection is extremely rare (1 generation for every 100). However, maintenance and emergence experiments reach the same level of adaptation through different evolutionary trajectories. Maintenance experiments evolve adaptations early on; when sporadic selection begins, they can maintain them, although their performance gradually declines. In contrast, emergence experiments take longer to discover adaptations but continue improving despite sporadic selection. In some instances (but not all), emergence experiments lead to even better adaptations than maintenance experiments.

Adaptation of GRNs in sporadic selection maintains the same general pattern across various degrees of complexity. All network architectures achieve similar (and high) performance for simple selective challenges, but complex architectures perform better in complex challenges. We did not expect this outcome for two reasons. First, the per-gene mutation rate is fixed across architectures. Thus, larger architectures are subject to a higher mutational load (e.g., a reproducing *type* 1-8-8-8-1 GRN, which is encoded by 170 gene loci, has an average of 1.7 mutations per genome, while a *type* 1-2-1 GRN, which is encoded by 9 gene loci has ten times fewer chances). Second, more interacting genes require more time to co-evolve the adaptive expression of a trait. These factors should make the performance of a more complex GRN more susceptible to extended periods of mutation accumulation. Yet, complex GRNs significantly outperform simpler ones in complex selective challenges and perform equally well in simple ones across regimes of sporadic selection. This suggests that larger GRNs can evolve configurations that readily fit complex reaction norms without incurring a substantial cost in mutational fragility or speed of evolution. However, this ability has limits: for extremely rare selection regimes (1/100), complex architectures experience a more significant drop in performance, equalizing all architectures' performance.

Comparing our results with those from other studies with similar goals [8, 20, 25, 28–30, 33, 44] we find a number of aligned conclusions, as well as relevant differences that highlight the merits and limitations of our modeling approach. Our findings indicate that GRNs can evolve configurations that buffer the effects of mutations on their phenotypes and fitness. Previous theoretical models, mainly coming from classic population and quantitative genetics, have suggested that traits can be preserved for a relatively long time if they are not directly selected against [20, 25, 29, 33, 44]. We concur to some extent, but our conclusions only partially align with these predictions. While we observe that

complex traits can be maintained for extended periods even when selection is extremely rare, complex traits in our model rapidly degrade under pure mutation accumulation (see Fig. 3 bottom-right plot). This discrepancy with previous studies can be attributed to our model specifically representing complex adaptations, in contrast to most other models, which study simple adaptations encoded by simple genomes. In our model, the target adaptation is complex, encoded by a GRN with genes interacting non-linearly to express a range of phenotypes. Consequently, mutations have a more significant impact, and uncontrolled mutation accumulation can degrade adaptations more rapidly, as some of the studies we cited predict [26, 28]. Nonetheless, even in this scenario, sporadic pulses of selection (even when much shorter in duration than the periods of accumulation) can exponentially increase the length of time for which an adaptation is maintained, to the point that they can prevent its loss or enable its emergence.

These outcomes underscore the importance of considering multiple approaches when modeling evolutionary phenomena and demonstrate that mechanistic models that explicitly describe regulatory mechanisms can yield new insights. We argue that considering regulation is crucial not only because it leads to more nuanced outcomes but also because it is a realistic assumption. Regulatory processes are involved in the expression of almost any trait, even in cases we generally consider to be constitutively expressed, such as the evolution of agouti fur coloration for mimetism [45].

One aspect we would like to expand on in future work is implementing the possibility of extinction, which is currently absent. In our model, the population size remains constant, irrespective of selection occurs or not. Accordingly, we have considered a scenario of 'soft selection', where reproductive success is determined by the relative performance of individuals. In case of 'hard selection', maladapted populations could decline, making it more and more difficult to regain adaptive mutations. Nevertheless, our model could be a good approximation for scenarios where sporadic selection does not act on the survival component but the reproductive component of fitness. For instance this would be the case in a scenario where a resource appears sporadically and is accessible only through a particular adaptation (e.g. an abundance of seeds that require a specific beak shape, favorable temperatures that allow redirecting fat reserves into the mating ornaments, etc.). These scenarios where adequately responding provides a selective advantage rather than preventing a disadvantage are common, and the most likely to lead to the emergence of new traits via sporadic selection. Scenarios where not responding reduces survival or reproduction could be more restrictive for "sporadic adaptations" to persist. One important factor that is often taken into consideration when studying the decay of traits in the absence of selection is the cost of maintaining the trait itself (this is especially true for plastic response traits [6]). Results of various models already show that traits decay much faster when there is a cost to the maintenance of the trait, [20, 29, 30]. However, our model does not explicitly consider fitness costs for maintaining complex adaptations. If it did, its outcomes would likely be different, especially across GRN architectures. Most likely, more complex architectures would not do better. Considering the expression of complex traits to be costly in terms of fitness has a solid empirical and logical basis, and we acknowledge that our model does not take this into account. Nonetheless, this is intentional, a more important but indirect cost is the focus of this study: the cost of having a large and fragile architecture. To avoid confounding effects,

we decided to not implement explicit costs. Examples include the display of singing behavior (leg motions) in mute crickets and the preservation of anaerobic metabolism in E.coli after 30 years of oxygen-rich selection. It is not always the case that there is a cost to maintaining complex traits, or that this cost cannot be offset. For instance, in parasitoid wasps, most enzymes for complex fat synthesis also play roles in other processes, offsetting costs when not producing fat. Another limitation of this model is that reproduction is asexual. In biological populations, sexual reproduction is ubiquitous and leads to recombination of alleles within populations. This could speed up both the generation of complex traits, as well as their decay in the absence of selection. Future models should implement sexual reproduction to capture these effects.

Future work should focus on implementing some of the additions mentioned above, such as the possibility of extinction, costs for maintaining complex traits, and sexual reproduction. These additions would likely modify the outcomes, potentially restricting the parameter space in which sporadic selection leads to the emergence and maintenance of complex traits. However, implementing these factors may also reveal new insights into the relationships between complexity, robustness, and evolvability.

Despite its limitations, this model provides insights into how complexity can arise and be maintained in biological systems, even in the absence of consistent selection pressures. The results suggest that complex traits and architectures may be more evolvable and robust to mutation than traditionally thought. This has implications for understanding the prevalence of complex adaptations in nature, as well as for theories of major evolutionary transitions and the origins of complexity. In summary, while limited in scope, our model provides evidence that sporadic selection can lead to the emergence and maintenance of complex traits, especially when the underlying architecture is robust to mutations. These insights contribute to a more nuanced understanding of how complexity arises and persists in evolving systems. With further development, this model system has the potential to yield additional insights into the evolution of complexity.

4.5. ACKNOWLEDGMENTS

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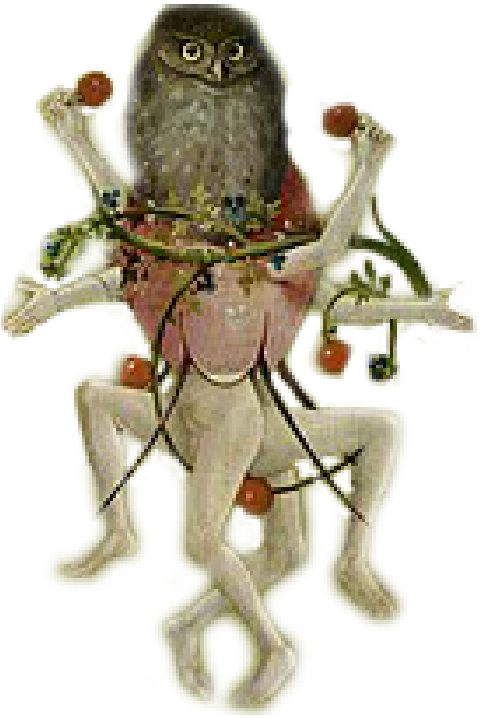

5

BIOLOGICAL INSIGHTS ON GRAMMAR-STRUCTURED MUTATIONS IMPROVE FITNESS AND DIVERSITY

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ABSTRACT

Grammar-Guided Genetic Programming (GGGP) employs a variety of concepts from evolutionary theory to autonomously design solutions for a given task. Recent insights from evolutionary biology can lead to further improvements in GGGP algorithms. In this paper, we propose a new mutation approach called Facilitated Mutation (FM) that is based on the theory of Facilitated Variation. We evaluate the performance of FM on the evolution of neural network optimizers for image classification, a relevant task in Evolutionary Computation, with important implications for the field of Machine Learning. We compare FM and FM combined with crossover (FMX) against a typical mutation approach to assess the benefits of the approach. We find that FMX provides statistical improvements in key metrics, creating a superior optimizer overall (+0.5% average test accuracy), improving the average quality of solutions (+53% average population fitness), and discovering more diverse high-quality behaviours (+523 high-quality solutions discovered on average). Additionally, FM and FMX reduce the number of fitness evaluations in an evolutionary run, reducing computational costs. FM's implementation cost is minimal and the approach is theoretically applicable to any algorithm where genes are associated with a grammar non-terminal, making this approach applicable in many existing GGGP systems.

5.1. INTRODUCTION

Genetic Programming (GP) is an Evolutionary Algorithm (EA) that evolves programs to solve a given task. A relevant branch of GP is Grammar-Guided Genetic Programming (GGGP) which uses a grammar to translate the representation (i.e., the genotype, a data structure representing the program) into an executable program (i.e., the phenotype or solution). In GGGP, practitioners can include domain knowledge by designing the grammar to bias the evolutionary search toward certain solutions. This advantage has led to successful applications of GGGP in many problem domains [1–3].

Despite these successes, GGGP can face difficulties exploring challenging solution spaces. Some GGGP approaches are prone to poor locality [4], meaning that small changes in the genotype lead to drastic changes in the phenotype. These problems are evident in tasks like the evolution of optimizers for neural network training. Carvalho et al. [5] use GGGP to evolve optimizers that compete with state-of-the-art human-designed solutions. While the result is favorable, the low average fitness of the population and the scarcity of reasonable solutions suggest that evolution is not exploring the solution space efficiently. Evolution appears to be randomly sampling the solution space (occasionally stumbling on promising genotypes) [6], rather than systematically improving upon incrementally fit solutions. Locality is often regarded as a matter of representation [4], but insights from evolutionary biology hint that variation operators may be part of the problem.

In nature, different parts of the genome display different amounts of susceptibility to mutation [7]. Core genes that have a vital function tend to mutate less [8], while genes with minor effects tend to mutate more often [9]. GGGP problems may share some of these dynamics with natural systems. Nevertheless, GGGP traditionally applies variation operators (mutation and crossover) uniformly across all genes without differentiating their functional role [10]. By not discriminating among the different types of genes, researchers have little control over the effect of variation on the phenotype and, thus, on the evolutionary trajectory of the system.

In this work, we propose a method to overcome the uncontrolled effects of mutations and allow a more faithful inheritance of traits by mimicking biological systems. We call our method Facilitated Mutation (FM), as it, in part, takes inspiration from the theory of Facilitated variation proposed by Kirschner and Gerhart in [11]. In FM, each grammar non-terminal uses a different mutation rate according to its predicted influence on the phenotype: low-impact mutations are frequent, while high-impact mutations are rare. We expect FM to enable the evolutionary algorithm to explore the solution space in a more structured way, reducing reliance on random sampling. Note that FM is not a mutation operator, it is simply a novel way to apply existing mutation operators. Consequently, the implementation cost of FM is minimal and the efficacy is related to the mutation operator used.

We validate FM by evolving optimizers to train neural networks for image-recognition tasks. We compare our new mutational approach with the one used in past works [5]. Our contributions are as follows: 1) The best optimizers found by FM are statistically superior to the best optimizers found by competing approaches. 2) The average fitness of the population is statistically superior using FM. 3) FM statistically increases the diversity of discovered viable solutions. 4) FM reduces the computational cost of experiments.

The remainder of this manuscript will be structured as follows: In the Background, we provide the rationale from evolutionary biology guiding our design choices, as well as the current work in evolving optimizers and how it relates to larger GP and GGGP challenges. In the Methods, we describe the implementation of FM and how it applies to optimizer evolution. In the Experimental Setup, we describe our validation experiments and the metrics we use to compare FM against more traditional mutational approaches. In the Results, we compare results from two versions of FM against two versions of traditional mutation approaches on four metrics: performance of the best solution, average performance of the evolved solutions, diversity, and computational cost. Finally, in the Conclusions section, we summarize our findings and extend our reasoning to general consideration and future directions.

5.2. BACKGROUND

5.2.1. INSIGHTS FROM BIOLOGICAL EVOLUTIONARY THEORY

Significant advancements in genomics, cell physiology, and developmental biology allowed evolutionary biology to gain an unprecedented understanding of the role of mutations and their effects on evolution. Of particular interest are insights on **heterogeneous mutation rates** and **heterogeneous mutation effects**.

Mutations that lead to phenotypic change do not happen homogeneously across the genome [12, 13]. There are conserved genes that code for fundamental *core processes*, that only mutate their function rarely, being more *robust* to mutation.[14, 15]. Many cellular and developmental processes are fundamentally unchanged since their emergence, such as the formation of micro-tubule structures [16, 17], cell adhesion processes [18–20], and anteroposterior axis formation [21]. Most mutations, instead, affect the phenotype by changing *regulatory elements* [22]. Changes in these elements do not alter the function of the core processes but regulate their activities and how they combine [23].

Not only do mutations not happen randomly across the genome, but they also have non-random effects on the phenotype depending on where they happen. If a mutation alters the functioning of a core process, it will fundamentally change the inner workings of the organism, most likely leading to a non-viable phenotype. Instead, mutations on regulatory sequences change the levels of expression of core processes and their interactions, producing functional phenotypes that are variations on the original theme.

Like biological systems, in evolutionary computation (EC) different genes play different roles in determining the final phenotype. However, unlike biological systems, EC often implements only a single mutation rate for all genes. By taking heterogeneous mutation rates and heterogeneous mutation effects into account EC systems might be able to display other interesting dynamics discussed by evolutionary biology: **developmental biases** and **facilitated variation**.

Empirical and theoretical research in evolutionary-developmental biology suggests that evolution has led to (and might have selected for) organisms whose core processes bias random mutations to produce a non-random subset of phenotypes. In the literature, this phenomenon is called developmental bias [24, 25]. Examples of developmental biases are: the number and distribution of digits, limbs, and segments in tetrapods [26],

the structural and pigment coloration of insect wing [27], and flower morphology [28].

In line with the observations illustrated so far, the Theory of Facilitated Variation by Gerhart and Kirschner [11] postulates that organisms are structured to produce variation easily and that this variation is biased towards adaptive phenotypes. Organisms can do this because they contain an archive of evolved core processes that rarely change due to mutations and maintain functionality independently of context. Core processes can easily combine their activities through mutations on regulatory sequences and, since they maintain functionality across contexts, the outcome of these combinations is biased to be neutral or beneficial.

The predictions of this theory have profound implications for evolutionary dynamics. Facilitated variation can explain how evolution is dramatically faster than we expect since it selects core processes that favor the rapid discovery of functional solutions, instead of proceeding randomly [29]. By applying heterogeneous mutation rates and considering the heterogeneous effects of mutations, EC systems could behave in similar ways to what Facilitated variation predicts, possibly improving their efficiency and effectiveness. By slowing the mutation rate of core genes and increasing the rate of regulatory ones, evolution would explore only a few core processes at the time (low mutation rate), but in different combinations (high mutation rate). This would steer the search trajectory towards a reduced subset of all the possible solutions, giving time to select and improve the most successful ones before new ones are introduced. Thus, EC systems could incrementally build an archive of good solutions that can recombine together effectively, granting a better exploration of the solution space.

5.2.2. MUTATION AND CROSSOVER IN GGGP

In GGGP the genome of individuals encodes for a set of derived rules, defined by how its genome is translated by a grammar. These production rules can either be non-terminals (invoking more production rules) or terminals (constants or operations). Mutations in GGGP change the genome, potentially changing one or more of the chained production rules.

A change in a production rule can lead to an important reshaping of the resulting behavior. Mutating a terminal into a non-terminal (which will invoke more production rules) will expand the number of executed operations. Mutating a non-terminal into a terminal instead will cancel a series of operations, preventing them from ever being called. Thus, it is important to realize that, independently of which representation is used (e.g. Grammatical Evolution [GE], Structured Grammatical Evolution [SGE], etc.), GGGP mutations can cause different effects of different magnitudes depending on where they happen. GGGP often uses mutations in tandem with another variation operator: crossover. This is done to improve the exploration of the solution space. During crossover, a region of the genotype of one individual is swapped with a region from another one. The resulting individual is therefore a novel combination of the two parent genotypes. However, there is no guarantee that this new combination will resemble a combination of the two parents. Traditionally in GGGP, crossover methods are operated not by identifying functionally equivalent regions of the genome, but by simply exchanging non-terminals. Therefore, crossover in GGGP leads to highly diverse, but very unstructured variation.

As biology suggests, if core components are not protected, arbitrarily changing the genome structure (both through mutations and crossover) will likely lead to system-wide deleterious effects. Nonetheless, studies in GGGP normally use a uniform rate of mutation over the entire genome and rely heavily on crossover.

5.2.3. EVOLUTION OF OPTIMIZERS SHOWCASES BOTH POTENTIAL AND ISSUES OF GGGP

Optimizers are a relevant research topic in computer science as their application domains like Machine Learning, have deep practical implications.

These algorithms can train Artificial Neural Networks to solve a target task using back-propagation [30]. Recent research shows that GP is a viable way to design competitive optimizers. GP systems such as PushGP[31–33] and AutoML-Zero[34] both have notable results in evolving optimizers.

GGGP also achieves important results through the AutoLR framework [5], which is based on the sub-branch of GGGP known as Structured Grammatical Evolution (SGE) [35]. AutoLR is capable of evolving human-competitive optimizers from scratch without prior knowledge of the components used by human-made ones. However, despite the good results, some problems are evident in the way that AutoLR navigates the solution space. Very few solutions obtain a high fitness and most discovered solutions are non-functional. Moreover, high fitness solutions emerge mainly during early evolution and do not improve any further, suggesting that the evolutionary algorithm may be behaving similarly to random search[6, 36, 37]. Random search can be helpful to explore a solution space [38–40], but it does not fit the goals of Evolutionary Computation to incrementally produce diverse and competitive solutions.

The shortcomings of AutoLR are likely caused by the fragility of the solutions to homogeneous mutation rates and indiscriminate use of crossover. Much like natural systems have core processes necessary for viable phenotypes, optimizers have core components (e.g., the gradient) that are likely to generate non-viable phenotypes when changed. But unlike natural systems, current optimizer evolution in GGGP does not consider heterogeneous mutation rates and mutation effects. These issues are not exclusive to AutoLR and GGGP. In fact, this is a well-known problem in GP [6, 36, 37], where algorithms often display low locality (changes in the genotype are disproportionally reflected on the phenotype) [35, 41].

Some researchers have already worked to mitigate this issue. Whigham [42] already in 1996 proposed the fine-tuning of heterogeneous mutation rates in GGGP. However, this is a small part of broader work from Whigham at the time, and only covers this idea briefly, not considering how biological evolution insights can inform design decisions in this subject. There is also work that explores forms of heterogeneous mutation outside of GP. Evolution Strategies (ES) are an entire type of EA that focuses heavily on adaptive control of parameters [43]. These systems adapt mutation rates and mutation steps for more successful mutation outcomes. However, these systems are commonly used for continuous search problems and do not address the same tasks as GGGP. Additionally, ES do not have grammars that can easily link different genes to different mutation rates in a structured, expertly informed way. As such, the possible benefits of a solution that mimics

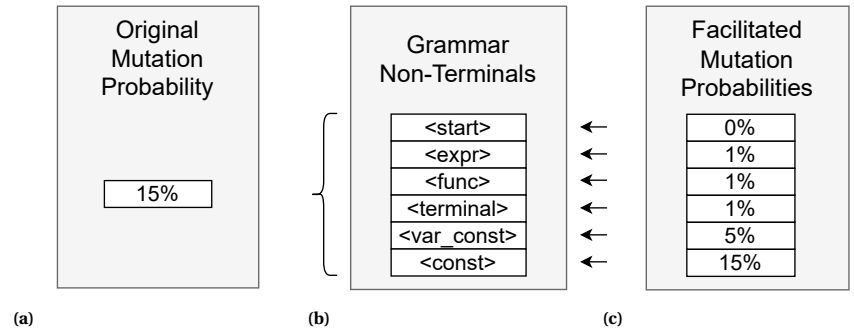


Figure 1 | Traditionally, mutation operators use a single mutation rate (1a) for all grammar non-terminals (1b). In FM, there is a separate mutation rate for each non-terminal (1c)

the patterns of natural mutations to address poor locality remains largely unexplored.

5.3. METHODS

5.3.1. FACILITATED MUTATION

The traditional GGGP approach applies mutationsuniformly across the genome not exploiting more fine-tuned mutations as natural systems do. In this work, we propose a new mutation approach for GGGP, Facilitated Mutation (FM), to replicate natural mutations.

Like in nature, mutations on different genes in GP produce different effects. FM uses heterogeneous mutation rates to support these differences.

The goal of this endeavor is to lead to an efficient, incremental search of the solution space, bringing tangible benefits.

Specifically, FM leverages the structure of the existing grammar to create several tiers of mutations. Each grammar non-terminal uses a different mutation rate: lower for more impactful non-terminals, higher for less influential ones, as shown in Figure 1c. Separate mutation rates allow practitioners to use their expert knowledge to tune the rate of different mutation effects optimally.

It is important to notice that FM is not a mutation operator, the approach only regulates the application of the ones already in use.FM is consequently widely applicable since it does not require the implementation of new complex algorithms or operators. Instead, it only requires small adjustments to the way that mutation is applied in the system.

Moreover, biological insights suggest that swapping arbitrary regions of the genotype via crossover will more often than not disrupt the coordinated activity of genes, causing high-impact deleterious variation. Thus FM, when it employs it, sets crossover at low rates to allow time for new combinations of genes to fine-tune their concerted action.

5.3.2. FACILITATED MUTATION IN OPTIMIZER EVOLUTION

In this work, we apply the ideas of FM to AutoLR [5], a framework for the evolution of neural network optimizers based on a GGGP engine called SGE [35]. The evolution of optimizers is an excellent task to evaluate FM. Optimizers have clearly distinguishable core components (e.g., gradient) crucial to their functioning, and regulatory components (e.g., tuning parameter values) dedicate to their fine-tuning. These distinct components would benefit from different mutation rates.

However, the original AutoLR grammar combines some crucial and tuning components in the same non-terminals. Specifically, the gradient shares a non-terminal with other, less important ones. The gradient is the most important component of an optimizer, any change to the gradient in an active gene will likely result in a complete overhaul of the solutions' behavior. To improve the efficacy of FM, we create a new grammar that separates components with different roles in different non-terminals. The new grammar still maintains the original grammar's functionality, as it uses the same components. In Figure 2, we show the two grammars, side-by-side. The complete grammars can be found at [44, 45]We refer the reader to the AutoLR paper [5] for details on the design decisions, operations, and variables of the original grammar.

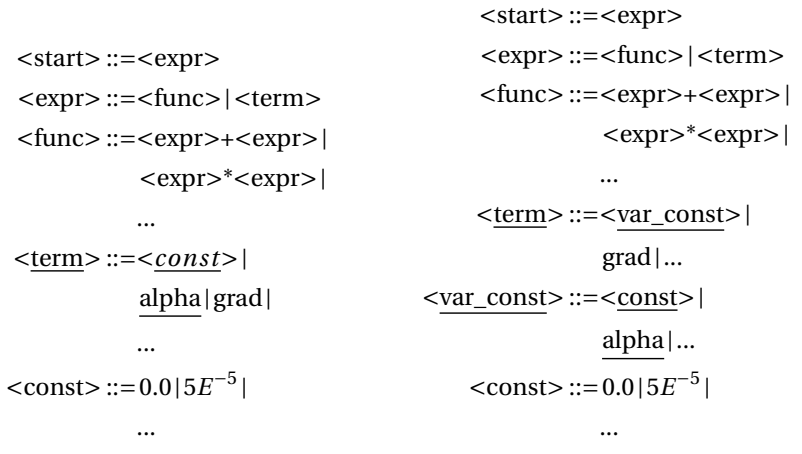
Leveraging these new non-terminals, we set specific mutation rates based on expert knowledge. We assign a 0.15 mutation rate to constants, allowing easy tuning of parameters. We assign a 0.05 mutation rate for changes between constants and other variables. We assign a 0.01 mutation rate to all other non-terminals. This 0.01 mutation rate affects changes that influence the behavior of the optimizer the most i.e.: changes from the gradient to anything else, as well as changes in the operations between variables. Figure 1c presents the complete set of parameters. Finally, we allow FM to make use of crossover (FM with crossover: FMX), but at a rate that is significantly smaller than previous setups (FMX crossover rate = 0.01 << original crossover rate = 0.9). We chose this crossover rate to be just high enough to avoid stagnation on local minima but not high enough to disrupt solutions that emerge from mutation.

5.4. EXPERIMENTAL SETUP

We validate Facilitated Mutation by using AutoLR to evolve optimizers that train a Convolutional Neural Network for image classification. Optimizers are assigned a fitness value based on the classification accuracy achieved by the network they trainand undergo tournament selection of size two.Winners of the tournament reproduce with a probability of undergoing mutation and crossover.This procedure is repeated until the population for the next generation is filled up. The population size is 100 individuals, and evolution runs for 200 generations. The best solution of each generation is preserved via elitism . We run 30 independent simulations for four different mutation approaches:

1. **Facilitated Mutation with rare crossover (FMX)**: our novel solution implementing both changes to mutation rates and to crossover (*low rate*).

2. **Facilitated Mutation (FM)**: our novel solution in isolation to investigate its effectiveness in the absence of crossover.



(a) Original Grammar. **(b)** Grammar for Facilitated Mutation.

Figure 2 | The original grammar used in AutoLR (2a) and the adapted version for Facilitated Mutation (2b). Non-terminals that have been added or changed are underlined.

3. **Original mutation without crossover (OM)**: the original solution without crossover, for direct comparisons with FM.

4. **Original mutation with crossover (OMX)**: the original setup used in past works [5].

Table 5.1 describes the evolution parameters for each approach. We use four metrics to analyze and compare the results from the different mutational approaches (FMX, FM, OM, OMX):

- **Best Fitness**: The *fitness of the best evolved solution* in a simulation.

- **Population Fitness**: The *average fitness of the population.* We also consider the *distribution of fitness in the population.*

- **Population Diversity**: The *number of different viable and distinct solutions in a population.* We distinguish solutions by the equation encoding the optimizer. We set a threshold of accuracyto distinguish non-viable (< 0.5) and viable (> 0.5) solutions.

- **Computational Cost**: *Number of training events over the evolutionary run.* Most computational costs in AutoLR come from the training necessary for the fitness evaluation.AutoLR avoids re-evaluating solutions it has already encountered (see 5.4.2); thus, algorithms that bias variation can reduce computational costs.

Table 5.1 | Evolutionary parameters for the four different setups

Parameters	Value			
	FMX	FM	OM	OMX
Crossover Rate	0.01	0.0	0.0	0.9
Mutation Rate	Heterogeneous		Homogeneous (0.15)	
Population Size	100			
Generations	200			
Elitism	1%			
Tournament Size	2			
Max Depth	17			

5.4.1. TASK, TRAINING AND FITNESS EVALUATION

We evolve the optimizers to train a simple convolutional neural network [46] for image classification on Fashion-MNIST [47]. Fashion-MNIST is a data set with 60000 28x28 gray-scale training images. Each image depicts an article of clothing belonging to one of ten categories (e.g., T-shirt/top, trousers, pullover). The optimizer trains the network to label these images correctly. Training has two stop conditions. First, the procedure terminates when a maximum number of epochs is reached. Second, an early stop mechanism terminates the procedure if there is no progress for a set number of consecutive epochs. To ensure a fair fitness assessment, we split Fashion-MNIST into three data sets: Training Data - During training, the network assigns labels to this data and the optimizer changes the network's weights based on the errors of the assigned label. The optimizer directly interacts with this data. Validation Data - During training, validation metrics are calculated on this data to monitor progress. The early stop mechanism monitors these validation metrics to terminate training when no progress is made. The optimizer does not interact directly with this data. Fitness Data - After training concludes, accuracy is calculated using this data and used as the optimizer's fitness. The optimizer does not directly interact with this data, ensuring an unbiased fitness assessment.

A table summarizing the parameter values used in our Fashion-MNIST experiments is presented in the supplementary material (Supplementary Material Table 1).

5.4.2. SOLUTION ARCHIVE AND VIABLE SOLUTION PRE-SELECTION

Once fitness is calculated, the optimizer and its fitness are recorded in an archive. Before evaluating any optimizer, the system will check the archive for an existing record to avoid re-evaluation, since multiple genomes can encode for the same optimizer. Additionally, the system can detect dysfunctional optimizers before evaluation: an optimizer that does not use the gradient cannot train a neural network, so AutoLR assigns them a poor fitness value (0.1) and skips evaluationThese two procedures together save considerable computational resources.

5.4.3. POST-HOC ANALYSIS

While the fitness function provides a quick assessment of the quality of an optimizer, it is ultimately an abridged procedure that focuses on efficiency. We use limited training size, few training epochs, and an early stop mechanism to speed up computation during the evolutionary simulations. However, these expedients limit the thoroughness of the training and thus hinder the assessment of the full capabilities of an optimizer. We perform a post-hoc analysis on the best evolved solution for each approach to assess their quality more accurately. The procedure for post-hoc analysis is similar to the fitness evaluation, with a few key differences. Training uses 56500 examples instead of 6500 and continues for 1000 epochs instead of 100. Moreover, the early stop mechanism is removed. After training, the weights that achieved the best validation-accuracy are restored for a final test-accuracy assessment. The system calculates the test-accuracy using 10000 images from the Fashion-MNIST test data. This test data is a separate data set, never used during evolution, thus ensuring an accurate assessment of the optimizers' ability to generalize to new data. We repeat the entire procedure 15 times, training the network from scratch and recalculating the test accuracy in each repetition. After all these steps, the average test-accuracy of an optimizer is the best measure of its actual quality.

5.5. RESULTS

In this section, we report and analyze the results obtained from the 30 runs of each of the four mutation approaches (FMX, FM, OMX, OM). For a summary of the results, see Table 5.2, best results for each metric are highlighted in bold. We conduct a statistical comparison between the different mutational approaches using the t-test (significance level set to $\alpha < 0.05$) and calculate the effect size and p-value [48]. A full overview can be found in the supplementary material (Supplementary Material Tables 2 and 3). In this section's figures, if there are statistically significant differences displayed, we use asterisks to show the significance level and omit the full p-values ($0.05 > p > 0.01$ = *, $0.01 \geq p \geq 0.001$ = **, $0.01 \geq p \geq 0.001$ = ***, $p < 0.0001$ = ****). The four different metrics: **Best fitness**, **Population fitness**, **Population diversity** and **Computational cost** are discussed separately in different subsections.

5.5.1. BEST FITNESS

We perform post-hoc analysis on the single best optimizer out of the 30 different runs from each approach and compare their final quality. The result of the test and validation accuracy assessments, as well as the p-value results from pairwise optimizer comparisons, are shown in Figure 3. Looking at the results, one can see that the approaches that rely on FM produce the best results overall.

After post-hoc analysis, FMX produced the most effective optimizer, classifying 93.3% of test images correctly on average. FMX's optimizer is statistically superior to all other approaches in test and validation-accuracy. The FM and OMX optimizers are comparable in test-accuracy, despite FM being superior in validation-accuracy. OM evolved the weakest optimizer. These results confirm that FM aids evolution in producing superior

Table 5.2 | Results summary

Approach	Metrics			
	Best Fitness	Population Fitness	Population Diversity	Computational Cost
FMX	**0.933[L]**	**0.625[L]**	**599[M]**	2915
FM	0.931	**0.608[L]**	323	**2137[M]**
OM	0.927	0.281	122	2978
OMX	0.928	0.093	73	10933

Values for the four metrics across approaches. Best results are in bold with Cohen's d [48] effect size as "S": small; "M":medium; and "L":large

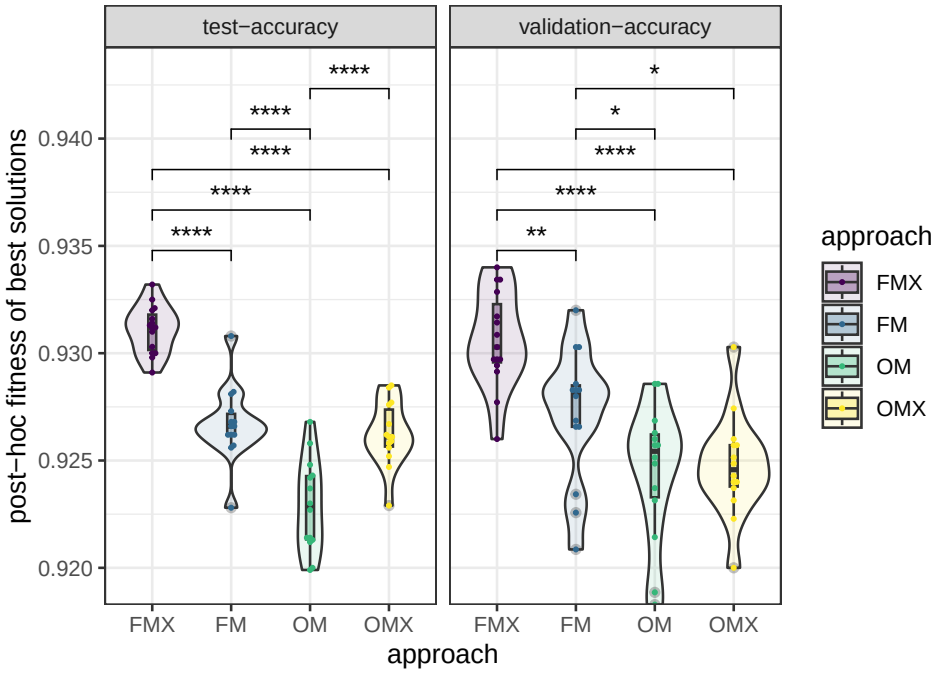


Figure 3 | Test and validation accuracies of the best optimizer for each setup in post-hoc analysis.

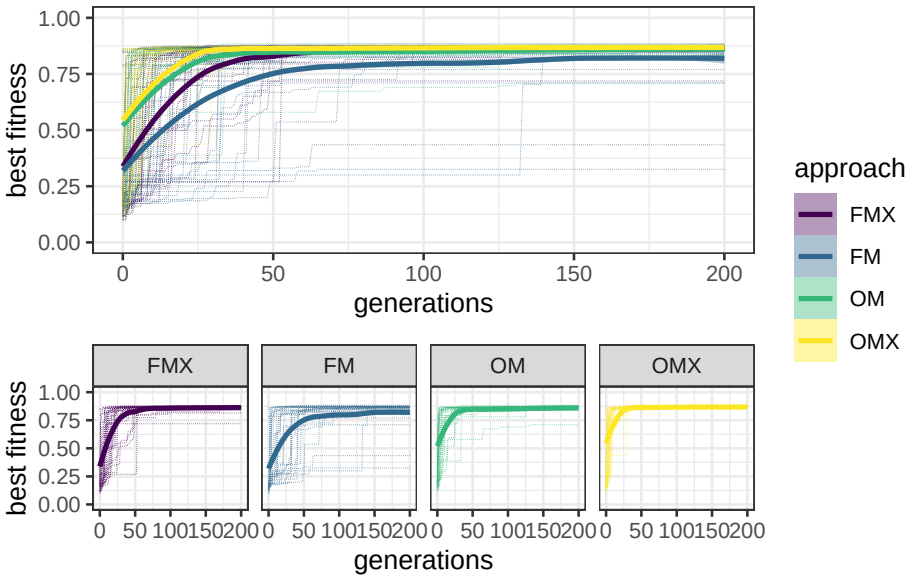


Figure 4 | Fitness of the best individual of the 30 populations over time for the four different approaches. Bold lines show mean values in each approach.

optimizers. Both OM and FM improve when combined with crossover, suggesting that this operator contributes to discovering the best solutions despite its apparent destructive effects.

We also analyzed the consistency of the approaches, comparing the best solutions found during evolution by all 30 runs of each approach (shown in Figure 4). FMX, OM, and OMX have comparable consistency in discovering high-fitness solutions. OM and OMX methods plateau rapidly as their mutational approaches explore the solution space very fast but impair incremental change, meanwhile FMX reaches a comparable level more gradually by steadily improving upon discovered solutions. FM is the most inconsistent, with a substantial number of runs below 0.5 fitness for the best solution.

This is due to the absence of crossover combined with the prevalence of neutral mutations, and the use of a solution archive. Without crossing over, FM mutation can only navigate the solution space via frequent regulatory mutations that do not affect the solution i.e.: neutral mutations. This evolutionary pattern is called neutral search, and it allows solutions to move across the solution space while being shielded from selection. Neutral search is computationally efficient in AutoLR because the system only spends time evaluating new solutions and not new genotypes. However, since runs for all mutational approaches terminate after a fixed number of generations, FM will explore fewer solutions than the others. Therefore, FM is penalized by AutoLR as it has fewer chances of discovering promising optimizers and is more prone to get stuck into sub-optimal regions of the solution space. On the other hand, FMX can discover higher-performance solutions thanks to the low rate of crossing over, which nudges the

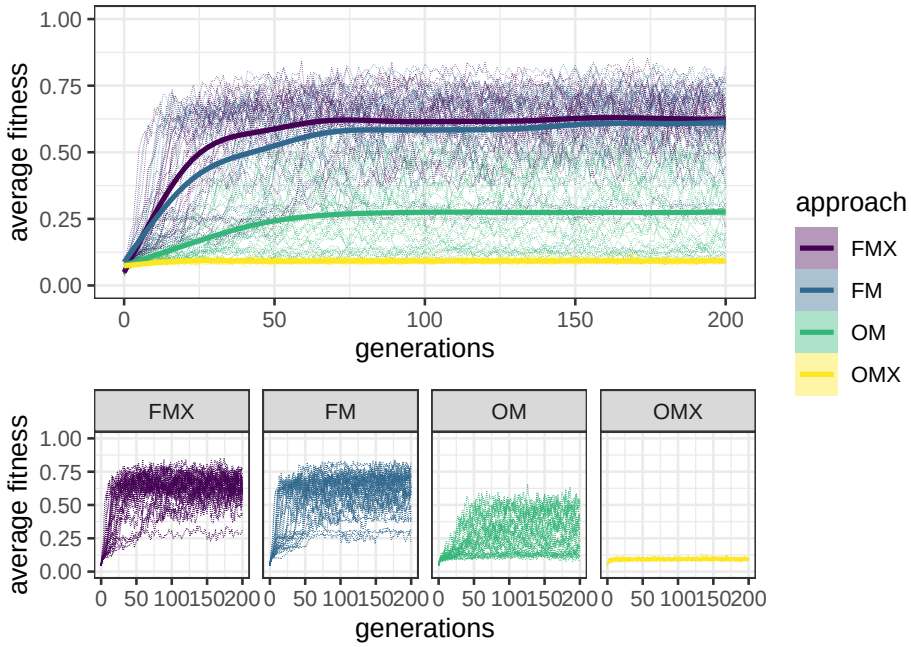


Figure 5 | Average fitness of the 30 populations over time for the four different approaches.

population into unexplored regions of the solution space, where new solutions can be evaluated. OMX and OM need not rely on the crossover as their highly disruptive mutation approach is sufficient to move them to new regions of the solution space.

5.5.2. POPULATION FITNESS

FM and FMX substantially improve the average solution fitness in the population. At any point in time, FM and FMX populations have higher average fitness than OM/OMX (Figure 5). FMX and FM have an average population fitness of ≈ 0.6, OM and OMX have average fitnesses of ≈ 0.3 and ≈ 0.1, respectively, as Figure 6 shows. We find that this difference is statistically significant at the end of evolution.

In OMX and OM, average solutions in a population have a fitness one order of magnitude smaller than the best one (OMX and OM average solution fitness ≈ 0.1, OMX and OM best solution fitness ≈ 0.9). This is in accordance with results from previous studies[5]. In contrast to FMX, OMX proceeds by randomly sampling good solutions from a vast solution space thanks to its uniform mutation rate and high crossover rate (0.9) but does not traverse the search space efficiently, inhibiting fit solutions from producing incrementally fitter offspring. These results demonstrate the delicate role of crossover rate in balancing exploration and exploitation and the importance of tuning this parameter correctly. A high crossover rate has a highly negative impact on population fitness. Population fitness in OMX (crossover rate = 0.9) is significantly lower than its crossover-free

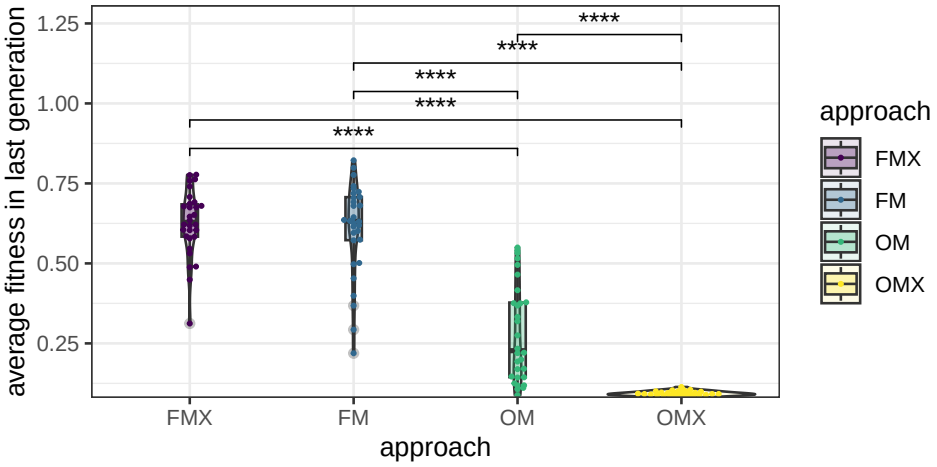


Figure 6 | Average fitness in the last generation of each run for all approaches.

counterpart OM (crossover rate = 0). This difference contrasts with the positive effect that small crossover rates can have, as observed in Section 5.5.1. FMX and FM population fitnesses do not show the same drop. The difference in the response to crossover may caused by the different crossover rates (FMX crossover rate = 0.01 << OMX crossover rate = 0.9). Further study into how each approach reacts to changes in crossover rate is necessary to confirm this idea since such experiments are outside the scope of this work.

Improved population fitness supports the hypothesis that FM and FMX approaches can improve the navigation of the solution space. However, analyzing the population's fitness alone is not sufficient. Many identical copies with high fitness could lead to the same result. It is essential to analyze the diversity of solutions discovered by each approach to asses if FM and FMX approaches can explore the solution space better.

5.5.3. POPULATION DIVERSITY

We measure population diversity as the *number of viable unique behaviors in the population.* The metric we use is a modified version of the *variety* measure proposed by Koza [49]. This measure is defined as "the percentage of individuals for which no exact duplicate exists elsewhere in the population" we adapt this to work on *number of unique behaviors* rather than percentages but retain the focus on "individuals for which no exact duplicate exists elsewhere in the population" which we abbreviate to "unique behaviors in the population". We could use an edit-distance based measure instead, but we find that a simple, behavior focused measure is the best match for this study.

Optimizers are fragile and non-viable solutions are frequent, thus including non-viable solutions in our analysis would confound information about the diversity of optimizers that actually contribute to evolution. We find "the number of *viable* unique behaviors" by considering solutions with a fitness above the threshold of 0.5, that are able to train the network in some capacity. We statistically compare the total number of

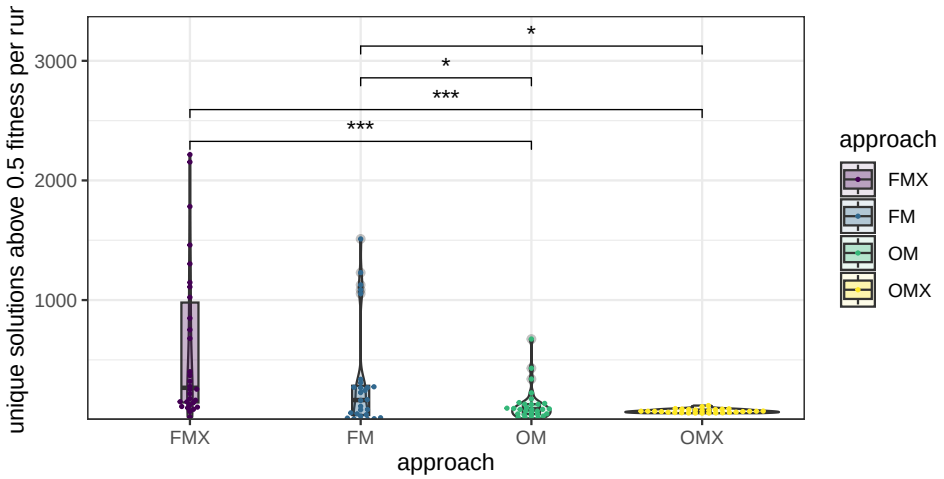


Figure 7 | Unique behaviours above 0.5 fitness discovered per run. Higher values mean more diversity.

unique viable behaviors discovered per run (Figure 7, for the number of unique behaviors regardless of fitness see Supplementary Material Figure 1).

FMX produces a significantly higher diversity of viable solutions than all other approaches (on average 600 viable unique solutions per run). FM produces on average 300 and OM 120, although this difference is not statistically significant, while OMX is statistically worse than all other approaches with just 70 unique viable solutions per run on average (Figure 7).

The lack of diversity in OMX clues us into the failures of this approach and confirms our hypothesis that FMs and FMX can explore the solution space more efficiently. Despite the vast amount of new behaviours that crossover introduces into the population, OMX cannot convert this into functioning behaviours that aid evolution. The combined insights about diversity and population fitness suggest that FMX and FM can keep a diverse, viable population longer than OM and OMX. In sum, these experiments suggest that FM and FMX succeed in the stated goal of improving the search for the solution space, possibly allowing them to avoid stagnation.

However, the results raise questions about performance. We previously hypothesized that FM might be computationally cheaper by evaluating fewer solutions thanks to an increase in neutral search. This analysis of diversity suggests that FM evaluates more viable solutions. One possible explanation for this result is that FM is saving resources on evaluations that are not reflected in our measure of diversity. Specifically, FM may reduce the number of evaluations below the threshold used to analyze diversity. To clarify this aspect, we analyze the computational cost of the experiments directly.

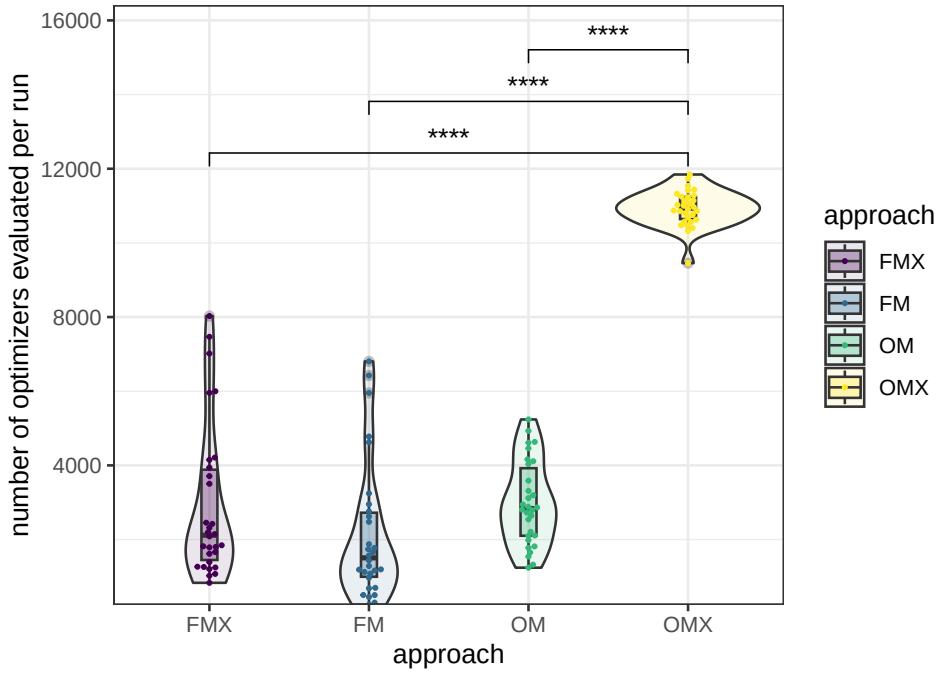


Figure 8 | Total number of evaluated optimizers, for all approaches. Lower values mean less computational costs.

5.5.4. COMPUTATIONAL COSTS

Most of the computational costs of AutoLR come from the neural network training necessary for the fitness evaluation. In Figure 8, we statistically compare the number of training and evaluations (the main computational cost) executed in all approaches. AutoLR uses a solution archive and pre-selects only for functional optimizers to avoid redundant and irrelevant evaluations. Due to these mechanisms, the number of optimizers evaluated in a run is the number of unique behaviours that include at least one gradient component. All approaches we have implemented for the first time in this work (FMX, FM, OM) are statistically cheaper in terms of computational cost than previously implemented ones (OMX), see Figure 8). There is no statistically significant difference between FMX, FM, and OM (although FM sits just outside the established significance threshold when compared to OM, with a p-value = 0.057, see Supplementary Material Table 3). Moreover, the results seem to indicate that FM is empirically cheaper than all the other approaches, but this difference is not statistically significant. FM thus seems to manage to be cheaper while at the same time having a high diversity of good optimizers (see Section 5.5.3). This suggests that FM performs neutral search (saving computational resources) in high-fitness regions of the problem space.

5.6. CONCLUSIONS

This paper proposes Facilitated Mutation, a mutational approach for GGGP inspired by biological evolution. Facilitated mutation splits the single mutation rate commonly used in these approaches into different values for each grammar non-terminal. We compare Facilitated Mutation approaches with (FMX) and without crossover (FM) against more traditional mutational approaches with (OMX) and without (OM) crossover. We validate these approaches on optimizer evolution, an evolutionary machine learning task with a challenging solution space, and real-world applications, and compare them based on the best optimizer discovered, average population fitness, population diversity , and computational cost .

FMX is comparable or better to original mutation approaches in all aspects. When compared to the best OM-based alternative: FMX evolved a better optimizer (+0.5% network test accuracy than OMX), and on average: produced better and discovered more solutions (+34% population fitness, +477 viable solutions) than OM These benefits come with no significant downsides, the approach has similar computational costs to OM and is significantly cheaper than OMX (saving $\approx$ 118000 fitness evaluations).

Comparing the two mutation approaches without crossover (FM vs. OM), we find that FM discovered a better optimizer (+0.4% network test accuracy), improved population fitness by +32%, discovered 200 additional viable individuals and decreased computational costs by 850 fitness evaluations on average.

While we only study its benefits in the context of an SGE-based framework, FM is applicable to any GGGP where each gene is directly associated with a grammar non-terminal. FM is simple in implementation and applicable to any GP problem, but the effectiveness depends on the type of problem and the choice of proper grammar and mutation rates.

5.6.1. FUTURE WORK

FM and FMX achieve several remarkable results in this work, but these mutational approaches can still be pushed further.

In this work, the rates of mutation and crossover have not been further fine-tuned after their initial choice. There are many ways to further optimize mutation rates for FM; most notably, we think a promising direction is to let the mutation rates themselves evolve. Since the submission of this work, we have created a new version of FM with adaptive mutation rates and outlined a grammar design procedure to amplify the benefits of this type of mutation [50].

Our analysis of crossover in this work can also be expanded. We focused on assessing the benefits of our solution through an informed decision of crossover rate (FMX crossover rate = 0.01) and drawing comparisons with another work with a comparable setup (OMX crossover rate = 0.9). However, we do not investigate how the different mutation approaches respond to different crossover rates. Crossover significantly improved the best fitness of both approaches so it is essential to study how these methods respond to varying crossover rates.

The analysis of the different mutational approaches can also be expanded to under-

stand all their implications better. While we found viable measures for all aspects of the evolution we studied, technical limitations stopped us from looking at other metrics, such as solution complexity, the phenotypic distance between solutions, and the total run time of experiments.

Other aspects of evolution could also be studied, such as detecting optimizers that evolution discovers repeatedly and how these vary depending on the approach. The improved diversity and population fitness of FM approaches also warrant further research into whether the approach can also improve the system's evolvability and adaptation to different networks and tasks through transfer learning. In particular we find important to evaluate the performance of our approach in more challenging image recognition data sets (e.g., CIFAR-10). From our first analyses it appears that the performance for the Fashion-MNIST data set is saturated (see 4). FM methods may show an even larger margin of improvement in more complex data sets, where simpler mutational regimes (OM) could not navigate the higher complexity of the search space.

FM may improve performance even further for structured problems (e.g., robot control, path finding) where there is a limited set of possible actions that can be fully defined by the grammar. This contrasts with optimizer evolution, where the set of variables and operations used for evolution is arbitrarily restricted, infinitely varied and endlessly debatable.

Finally, previous works compared the evolved optimizers with human-made solutions. We did not perform this type of analysis since the focus of this work was on improving evolution, but such insights would be interesting in an extended version of this paper.

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6

Conclusion

There will be time, there will be time
To prepare a face to meet the faces that you meet;
There will be time to murder and create,
And time for all the works and days of hands
That lift and drop a question on your plate;
Time for you and time for me,
And time yet for a hundred indecisions,
And for a hundred visions and revisions
Before the taking of a toast and tea.
...
Do I dare
Disturb the universe?
In a minute there is time
For decisions and revisions which a minute will reverse.

TS Eliot, The Love Song of J. Alfred Prufrock

In this final chapter of my thesis, I will try to convey the scientific insights I have gleaned during my trajectory as a PhD candidate. However, first, I think it is important to acknowledge that research is conducted by people (me in this case) and that people, willing or not, have personal and biased opinions. This chapter is written with a philosophical view in mind. To promote a clearer understanding of my conclusions, or at least a clearer perspective on the importance I attribute to them, I think it is necessary to articulate this view. To this end, I will divide this chapter into two parts: In the first part, I will try my hand at some philosophical considerations, to provide clarity about what my perspective is. In the second part, I will summarise my findings and the conclusions I draw, as well as the limitations and future directions I see for this line of research.

6.1. PART 1: A POST-MODERN SYNTHESIS

6.1.1. A POST-MODERN PUN

The section title "A Post-Modern Synthesis", is obviously a pun, playing on the name of one of the bulwarks of the evolutionary theory: the Modern Synthesis; and the now so popular, although not universally liked, philosophical term: post-modern. The aim of the pun is to show what my (somehow post-modern) philosophical standpoint is concerning the study of evolution. I think that we should abandon some modernist categorical concepts of evolutionary thinking (which I may unjustly associate with the Modern Synthesis here) in favour of a more supple, more post-modern, demeanour. The Modern Synthesis of Evolutionary Biology refers to an attempt at a comprehensive explanation of how evolution works developed in the early 20th century. It tries to reconcile Darwin's theory of natural selection with the principles of a population-oriented view of Mendelian genetics emphasising that: (1) Mendelian genetics can be reconciled with quantitative genetics (at that time called Biometry [1, 2]), and (2) the laws underlying both mendelian genetics and quantitative genetics are both compatible with evolution by natural selection (i.e. improved adaptation over the generations). The latter is not self-evident, as it had been shown by Weismann that Darwin's own theory of inheritance was incompatible with his ideas on adaptive evolution (see [3] for an in-depth rundown). On its ideas, the standard evolutionary theory was constructed, with all its advantages and downfalls (phenomenological, short-term, gene-centric; as already discussed in **Chapter 1**). The standard theory has been helming research for almost a century now. Nonetheless, in recent times some evolutionary biologists, counting me amongst their ranks, say that the standard theory is too restrictive to paint the full picture. Many have called for expansions to the paradigm, but it seems that it is extremely difficult to come up with a general evolutionary "synthesis". And here enters the post-modern part of this pun. I argue that if we are ever going to have a synthesis in Evolutionary biology this is going to be a post-modern one, a pluralistic one. Post-modernism arose from the rubble of the Second World War which was, for many, the end of the Modern Era. It ushered in a cohort of ideas that made Modernity tremble, such as there is no universal truth, progress, or good, matter comes before meaning, and human language is inadequate to communicate beyond our basic needs. Amid this philosophical storm of "slings and arrows", nonetheless sat a smouldering ember of hope. A hope that decided not to ignore

or justify our incomprehensible and contradictory nature, but to embrace it in all its facets. A hope which still dared to say: there is something worth it, but it is not that easy! This is what I see in post-modernism, and this is why I find myself adhering to some of its views. It is from the philosophy of post-modernism that I take some ideas I would like to apply to evolutionary biology, in particular, that of pluralism.

6.1.2. PLURALISM IN POST-MODERNISM

The idea of pluralism is best embodied, in my opinion, by Simone de Beauvoir in: "The Ethics of Ambiguity". The message from Beauvoir's work is that we should do away with a logic made of dichotomies. Things are neither black nor white, instead, they often are points in the middle of one, or even many, spectrums. One can only hope to see the world as completely as possible by looking through as many of its facets as possible. It is important, however, to note that pluralism should not be confused with relativism. Pluralism does not imply abandoning basic rules of argumentation: arguments need to be logically correct, and sound and can, and should, be evaluated against each other. However, many arguments can coexist to explain the same phenomenon. In sum, I use post-modern in my title to refer to pluralism as one of the ways to obtain an actual evolutionary "synthesis". Advocating for the idea that in evolutionary studies, and science in general, many views should not entail conflict per se, but instead, they allow us to appreciate evolution in a richer way. Pluralism does not only entail different views. You could also have pluralism in terms of explanation or methods. Different approaches can suit different purposes: e.g. population genetics to predict the changes in frequency of beneficial alleles in the upcoming generations of artificially selected crops, and evo -devo to discover new evolutionary mechanisms these alleles allow.

6.1.3. PLURALISM IN EVOLUTIONARY BIOLOGY

I argue that this pluralistic view has its uses in evolutionary biology (and science in general) and that it can help us advance our understanding of the natural world. The relation of pluralism to scientific research is metaphorically similar to the relation of a multidimensional fitness landscape to the study of adaptive trajectories [4] or the relation of a complex Gene Regulatory Network (GRN) to the study of evolvability and robustness [5] (see **Chapter 1**). By adding dimensions, and therefore degrees of freedom, to evolutionary models (e.g. looking at fitness landscapes with more than three dimensions [4], or considering more realistic, and therefore more intricate, Genotype-Phenotype maps [5]) evolution has many more paths to achieve a certain adaptation than previously expected. In the same fashion if we, through pluralism, enlarge the dimensions in which we can approach evolutionary questions via different perspectives (e.g. via a population genetics approach, a mechanistic GRN approach, or seeing evolution as a learning process) we can increase the number of research lines we can leverage to explain evolutionary phenomena.

Some lines of research not only serve as a metaphor for the usefulness of pluralism but are in and of themselves an example of this pluralism in action. What I mean by pluralism in this case, is the willingness of certain scientists to detach themselves from the traditional paradigm with which some natural phenomena are interpreted, and approach

them from a completely different angle. In a certain sense, I equate pluralism to a very strong form of multi-disciplinarity, where not only findings from one field can be used to enhance research in another (see for instance the recent improvements in protein-folding prediction thanks to artificial intelligence), but the entire frame of reference is used. This attitude in my opinion can lead to significant leaps forward in our scientific understanding with relatively little effort.

I will quickly discuss my interpretation of the three examples already mentioned in this thesis: the Modern Synthesis, the work concept of bioinformatics, and the interpretation of evolutionary processes as a form of learning. The Modern Synthesis is perhaps the most famous and important example of pluralism applied to evolutionary studies. It is common knowledge that Darwin left this world with one regret: his inability to explain the mechanism of inheritance. He knew that this missing explanation could undermine its entire theory. It was the work of Gregor Mendel and his discovery of the gene that solved this conundrum (alas! a bit too late for Darwin). However, initially, Mendel's work, by proving the existence of discrete units of inheritance (the genes), did seem to put Darwin's theory in jeopardy as it could not explain the presence of continuous traits in nature. The work of Fisher, Haldane, Wright, Huxley, and many other scientists who contributed to the Modern Synthesis at the time allowed us to reconcile this seemingly fatal flaw of evolutionary theory and push it forward. They did so by bringing into the fold of this discipline the language and ideas of statistics and physics. This allowed them to explain the complexity and continuity of the natural world through its discrete components. If not for the work of these scientists, and the research that followed from it, the research I conduct on evolvability would most likely not exist. Another important example that also played a role in my research is that of bioinformatics by Pauline Hogeweg. As Hogeweg explains in detail in her opinion paper [6], she and Hesper started using this term in the 1970s. By choosing the name bioinformatics, they starkly outlined their research against other fields such as biochemistry or biophysics, making it clear and immediate that t he focus of their research was the way information is transmitted and transformed, and that they considered this aspect a key feature of life. By explicitly looking at how information flows in biological systems, their intent was to bridge the gap between phenomena at different organisational scales via emergent properties. This information-focused computational approach has arguably given birth to a whole branch of more mechanistic, individual-based, and emergent models. These types of models would later be adopted by evolutionary biologists (such as Gunther Wagner, Andreas Wagner, and Hogeweg herself) to study aspects of evolution normally inaccessible to standard theory. Most of the research on evolvability comes from this branch, and it is in its spirit that I conduct mine as well.

Finally, also connected to ideas from informatics, the work of Richard Watson is, as well, an excellent example of a pluralistic approach in evolutionary biology. Richard Watson, a computational scientist by formation, takes ideas from the field of Machine Learning and applies them to interpret evolutionary processes. In two seminal papers [7, 8] Watson traces the interesting parallel between evolution and reinforcement learning, one form of learning in Artificial Intelligence, used to solve complex problems for which the solution is unknown. By unveiling this similitude, Watson brings a new perspective to explain evolvability, where each past evolutionary success is imprinted and stored in

the organism's "memory". A process that gradually leads to the "generalisation" of a set of selective problems, allowing organisms to deploy/evolve efficient solutions more and more easily. It is in the light of these insights that I developed my ideas and defined my focus in the vast field of evolvability research. I did this though with the additional insight, coming from Hogeweg's research, that it is in the representation (i.e. the architecture) that one can observe how this process takes place.

In conclusion, pluralism in these different approaches does not only benefit the individual research lines. Evolutionary biology as a whole can, in the light of pluralism, benefit from their coexistence. The study of evolution is a young science when compared to physics, chemistry, or even natural biology, with just a little over a century of research to its credit. Yet evolutionary biology poses some of the most complex questions about the most complex forms of matter in the known universe: life. No star nor galaxy holds a candle in terms of complexity to the metabolism of even the smallest bacterium (its biology), even less so to its interactions with other species (its ecology), not to mention its changes through time (its evolution). For this reason, we are far from having clear answers to most of these questions, and sometimes there is even much debate on their validity in the first place. A pluralistic approach, with its richness of views and interpretations, can help us chart this unknown territory that is evolution. In this endeavour, different types of research can occupy different niches, pushing forward the overall progress. Scientists such as Watson and Hogeweg propose new and explorative theoretical concepts, others work to incorporate these insights into existing frameworks and generalise them, and others still strive to apply them in real-world contexts. In this pluralistic "research ecosystem", I would like my niche to be one that stimulates research by creating a new lexicon of evolutionary dynamics. Via the use of conceptual models that strive to push the boundaries of current theory, I aim not to describe or predict the natural world directly, but instead to tease the attention of the community towards new interesting phenomena.

6.2. PART 2: INSIGHTS ON EVOLVABILITY AND ARCHITECTURE

The body of my insights can be divided into two types: those I gained from reasoning and researching broad topics related to evolvability aiming to combine and connect ideas in new ways (Chapters 1, 2, and Intermezzo 1), and those which I derived from analysing computational models, often involving a mechanistic comprehension of the role of architecture in evolvability (Chapters 3, 4, 5, and Intermezzo 2). When talking about the "architecture" of a GRN in my models, I typically refer not to different topologies (number of nodes and the way the nodes are connected), but to the specific configuration of the strengths of the gene-to-gene interactions. I also conducted some research on evolving GRN topology together with a Master's student (Clem Carle), and as a follow-up to **Chapter 5** but this work is not included in this PhD thesis. Each insight relates to one research chapter and possibly one intermezzo. I will discuss my insights in the order they appear in this thesis.

In **Chapter 1** I argue that an architectural perspective provides a cohesive framework for understanding evolvability across levels of biological organisation. Organismal architecture: the structural relationships between the elements of an organism, is the

feature that ultimately allows evolution to act not only as a “blind watchmaker” but also as a "reminiscing architect". Selection blindly shapes organisms to overcome selective challenges, but these adaptations become ingrained in their architecture facilitating the persistence of adaptations. This way evolution can store and re-deploy adaptations to build upon what has come before. My architectural perspective on evolvability stems from a broader idea that a mechanistic approach (i.e. paying attention to explicitly consider the mechanisms underlying biological phenomena) is key to highlighting important evolutionary dynamics. A mechanistic perspective can help reconcile the plurality of concepts around evolvability [9]. In **Chapter 2**, I argue that it is possible to categorise different determinants for evolvability on a mechanistic basis. I identify three categories of ways in which biological mechanisms can influence evolvability: by providing variation (e.g. mutation rate), by biassing the effects of variation on fitness (e.g. developmental bias), and finally by shaping the selection process (e.g. of the effect of generation time on the speed of adaptive evolution). By thinking in terms of mechanisms and how they act it becomes evident that the same mechanism can often influence evolvability in multiple ways - for example, an organism’s plastic response to the environment can affect both variation and the biassing of variation effects. **Chapter 2** also shows how the timescale as well as the type of scientific questions/approaches themselves can heavily influence the conclusions one could draw from the same data. These different conclusions, although coming from different presuppositions, are not mutually exclusive and can concur with a general explanation. I argue, therefore, that rather than attempting to subsume all observations under a single conceptual framework, it is important to clearly state the framework being used to avoid misunderstanding.

In summary, my research suggests a mechanistic and architectural framework provides a productive perspective for studying evolvability. Within this view, evolvability in all its manifestations arises from the structural relationships that connect the components of living systems across levels of organisation. An appreciation of architecture is key to developing a cohesive understanding of how evolvability contributes to the adaptive success of life on Earth. This concept helps to better appreciate the results of the simulation studies I conducted.

In **Chapter 3** I indeed observe that when interconnected together, even simple ensembles of inheritable regulatory units (genes), can easily display potential for the evolution of evolvability through natural selection. This finding is in line with pre-existing literature on Gene Regulatory Network studies that show how by accounting for complex networks of interactions between genes, evolvability can be easily observed evolving [10, 11]. However, in this case, my research brings the additional insight that architectures need not be complex to achieve this. As we show, even simple GRN systems with less than five genes and simple linear interactions can structure themselves to increase their ability to adapt to varying selective challenges. In this study, individuals achieved this result by evolving an architecture that biases the effects of random mutations toward adaptive outcomes. Small mutations can cause substantial changes in the phenotype, allowing it to “switch” from one adaptive state to another rapidly. This expedites adaptation after an environmental shift, thus increasing evolvability. Contrary to expectations, non-linear gene interactions are not required to evolve such a switch and an increase in evolvability. Thus, even architectures entailing only linear interactions can integrate

information about the selective challenge into their architecture. This finding highlights how for evolvability, the existence of structural relationships between the elements of an organism may matter more than their nature (linear vs. non-linear). In **Chapter 4** I show that adaptations can evolve and endure even when selection occurs infrequently. This has two important implications. Once adaptations are obtained, they can stay ingrained in organisms for an extended period without being repeatedly selected, serving as evolutionary "memory" that can be recalled again if need be. Secondly, organisms can evolve this type of adaptation, and consequently acquire evolutionary "memory," even by just being exposed to selection rarely. This finding can help explain how organisms often display complex adaptations to environments to which apparently they have never or only very sporadically exposed (as discussed as well in Intermezzo 2). These findings in my model hold for a wide range of GRN architectures, selection event rarity, and selective challenges. They are also partially in line with previous theoretical models, which show that adaptations can be maintained for long periods in infinite populations with simple binary phenotypes. However, by including finite populations, complex multidimensional phenotypes, and more complex genomes (e.g., GRNs instead of simple genotype-to-phenotype mappings), my model yields different and new results from the previous ones as well. Despite being capable of being maintained for long periods of time in rare selection, in my model, adaptations decay very rapidly when selection is completely absent. This does not happen in previous models, where adaptations can resist for extremely long periods of time (tens of thousands of generations) under pure mutational drift. The fact that in my model adaptations decay rapidly when only mutations take place and instead persist when selection happens infrequently, shows that individuals evolve a form of robustness to mutations in response to rare selective events. The fact that in previous models individuals can simply maintain adaptation for long periods without any selective incentive, suggests that in their case robustness was innate and not evolved. Therefore once again, considering architecture (GRN vs. simple genotype-to-phenotype mappings) leads to interesting and different results than more standard modelling would predict. The evolution of robustness in response to rare selection can be interpreted as the ability of GRNs to integrate past selective events in their architecture (in this case understood as the strength of their gene-to-gene interaction) through evolution, similar to the findings in **Chapter 3**. Even very simple GRN architectures (less than 10 genes) display this ability. However, I also found that GRNs of varying sizes have different evolutionary properties. When selected against more complicated selective challenges, larger architectures show a higher level of adaptation. This happens despite these larger architectures possessing more mutable loci, and therefore being more prone to mutations that might disrupt their functioning. Our model suggests that bigger and potentially more fragile architectures are not necessarily an impairment or a cost. Evolution seems able to compensate for their potential fragility by more easily tuning them to complex tasks and making them robust thanks to the many degrees of freedom they offer with their high number of genes. Overall, these findings (chapters 3 and 4 and Intermezzo 2) highlight the importance of the structural relationships between elements of an organism in improving its ability to adapt.

In the cross-over research in Evolutionary Computation that I conducted in **Chapter 5**, I show how ideas from evolutionary biology can support research in other evolutionary

fields, particularly that of Grammar Guided Genetic Programming (GGGP). In this project, I show how biasing mutation rates and effects can improve efficiency when evolution searches for adaptations. These ideas are partially inspired by the theory of Facilitated Variation from Kirschner and Gerhart [12], whose work on evolvability is influential [13] in evolutionary biology but not very well known in the field of evolutionary computation. My modelling choices in this chapter adhere to a more phenomenological approach. I do not let biased mutation effects emerge spontaneously, rather, I explicitly implement different mutation rates for tiers of genes with different effects. However, this phenomenological implementation is only possible thanks to the focus on the structural relationships of the genotype-to-phenotype map used in GGGP. This representation is embodied by a grammar that, by definition, describes the relationships between the components that participate in phenotype formation. By taking advantage of the architectural features of this grammar it is possible to efficiently assign appropriate mutation rates that allow my method to beat other state-of-the-art solutions. This shows yet again that organismal architecture is a key aspect in understanding and predicting evolutionary dynamics.

GGGP has numerous intriguing properties that make it a promising candidate for future evolutionary studies as well. The amount of potential grammar that can be designed is virtually limitless, and more importantly, the elements of the grammar itself, as well as its associated mutation rates can be readily evolved too. This opens up a wide range of applications, both practical and theoretical.

In particular, the study of evolvability can benefit from this modelling framework. Individuals can be evolved against many different well-characterised tasks (i.e. selective challenges) used in Artificial Intelligence and their phenotype can be easily interpreted thanks to the grammar determining its genotype-phenotype map. Moreover, I find the utilisation of image recognition as a selective task particularly interesting, as it can be calibrated to various degrees of complexity. This could allow researchers to study evolvability on a fine-grained scale. In a project not shown in this thesis, I (and my collaborator Pedro Carvalho) extend the study presented in **Chapter 5** to determine if our method improves adaptation not only for one but for multiple image recognition tasks. The idea is that by starting from simple image-recognition tasks and progressively increasing their complexity the biased mutation rates will allow the preservation of functional modules. Thanks to the grammar, these modules are easily identifiable in the genome, and it is possible to observe if they are redeployed for more complex challenges. Preliminary results suggest that solutions evolved with our method adapt more rapidly to new challenges (have higher evolvability) and display more streamlined and coherent phenotypes. This solution maintains high performance not only for the present selective challenge to which they are exposed but also for past ones.

In summary, my research highlights how an architectural perspective could be crucial for developing a comprehensive understanding of evolvability. The structural relationships between components at all levels of biological organisation fundamentally shape how evolvability arises and contributes to adaptive evolution. Whether considering insights on defining and reconciling evolvability, or insights derived from computational models of regulatory networks, architecture lies at the heart of evolvability. Simple network architectures can evolve complex mechanisms for biasing mutations and adapting to

infrequent selection pressures. And ideas inspired by biological systems architectures can even benefit other evolutionary methods like evolutionary computation. Organismal architecture is the key to comprehending evolvability in all its diverse manifestations, from genetic evolution to non-genetic inheritance and beyond. By adopting an architectural vantage point, we gain a cohesive framework for exploring evolvability as a fundamental property of life.

6.2.1. FUTURE DIRECTIONS: STUDYING EVOLVABILITY AND NOVELTY, OPEN-ENDED EVOLUTION

The GRN models I discuss in this thesis have an important limitation when it comes to the study of evolvability: they can provide only limited insights into evolvability intended as the ability to produce evolutionary novelty. GRN models are an appropriate tool for studying "fine-tuning evolvability," or how variation can be shaped by adjusting architecture. However, these models tell us little about what might have underlined high-level evolutionary phenomena, such as the major evolutionary transitions (such as aerobic metabolism, sexual reproduction, multicellularity, bilateral symmetry, etc.). Despite allowing for unpredictable and counterintuitive outcomes, these models have a pre-established, unchangeable set of behaviours, thus making it quite impossible for them to come up with game-changing solutions. It is extremely difficult to design a simulation where GRNs that, as in **Chapter 3**, are selected to adapt to different environments, end up discovering multicellularity. Behaviours in these systems need to be pre-programmed and cannot be altered, so evolution can only fine-tune and complexify the dynamics, not revolutionise them. If one wants to observe the emergence of such phenomena the simulation needs to be appropriately “seeded” for those. I think that understanding how evolution can produce novel traits is one of the most fascinating enigmas of evolutionary studies.Understanding major transitions in evolution is a challenge for all evolutionary scientists and not only biologists. To this day, we have not yet been able to produce a model where evolution does not eventually stagnate and instead demonstrates the “open-endedness” we observe in life’s historical record.

This is one of the main interests of the research field of Artificial Life, in particular of that sub-group of scientists who are striving to obtain or detect evidence of “Open-ended evolution” [14–16]. A debate is ongoing within this multi-disciplinary community as to what is “Open-ended evolution”, how one can measure it and most importantly how one can obtain it. This debate is unknown to most biologists, as the scientists involved often hail from computational backgrounds. However, interesting insights have been garnered, and rigorous metrics of “open-endedness” are under constant development and review [17]. I think evolutionary biology should step in more decisively and bring its contribution to this discussion.

Harnessing insights from other disciplines and potentially aiding them in their endeavours could create a fertile ecosystem. Different evolutionary disciplines could help each other in speeding up progress along multiple lines of research. For instance, I think that many of the models adopted by artificial life scientists could be of great interest to biologists [18, 19]. The field of Artificial Life is a very creative modelling field, where few modelling paradigms are established, and uniqueness in mechanisms and dynamics is

valued. This attitude leads to a wide variety of models, which are substantially different. Thus, when general insights are drawn from this varied bouquet of representations of evolution it makes a strong argument for their soundness. At the same time, providing more accurate insights into how biological organisms work could offer better guidance for artificial life modelling choices.

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About the Author

I was born in Padova (Camposampiero), Italy in 1992, and from a young age, I developed a deep fascination with evolution, thanks to my father's captivating tales about nature and biology. In 2012, I embarked on my Bachelor's degree in Biology at the University of Padova, with a specific focus on evolutionary theory. It was during this time that I delved into the intriguing realm of the Extended Evolutionary Synthesis, an experience that solidified my passion for exploring cutting-edge theories in evolutionary studies. During my MEME Erasmus Mundus Master in Ecology and Evolution, I conducted a research project at the University of Montpelier modeling the evolution of Papillomavirus infection strategy, analyzed behavioral patterns of wild cricket populations at the University of Munich to understand the link between movement and reproductive success, and explored multidisciplinary approaches in robotic evolution at the CRN in Rome, evolving adaptive robots based on their surrounding environment. Throughout these formative experiences, my fascination with evolvability and evolutionary memory grew stronger. Recognizing the need for expert guidance, I sought the mentorship of Franjo, a renowned researcher in the field. Serendipitously, Franjo proved to be the ideal mentor, providing invaluable insights and direction for my academic journey. Looking ahead, I am now poised to embark on a Ph.D., building upon my prior research and seeking to make significant contributions to the field of evolutionary studies. Following the completion of my doctoral studies, I aspire to relocate to Paris, where I aim to secure a position in research and development. My goal is to continue pursuing my passion for multidisciplinary research at the interface of evolutionary studies and AI, leveraging my enthusiasm and expertise to drive innovative advancements in these domains.

LIST OF PUBLICATIONS

6. Adrian-Kalchhauser, I., Sultan, S. E., Shama, L. N. S., Spence-Jones, H., **Tiso, S.**, Keller Valsecchi, C. I., & Weissing, F. J. (2020). Understanding "Non-genetic" Inheritance: Insights from Molecular-Evolutionary Crosstalk. Trends in Ecology and Evolution, **35(12)**, 1078–1089.

5. Adrian-Kalchhauser, I., Sultan, S. E., Shama, L., Spence-Jones, H., **Tiso, S.**, Keller Valsecchi, C. I., & Weissing, F. J. (2021). Inherited Gene Regulation Unifies Molecular Approaches to Nongenetic Inheritance: Response to Edelaar et al. Trends in Ecology and Evolution, **36(6)**, 477.

4. Niemelä, P. T., **Tiso, S.**, & Dingemanse, N. J. (2021). Density-dependent individual variation in male attractiveness in a wild field cricket. Behavioral Ecology, **32(4)**, 707–716.

3. Visser, B., Alborn, H. T., Rondeaux, S., Haillot, M., Hance, T., Rebar, D., Riederer, J. M., **Tiso, S.**, van Eldijk, T. J. B. B., Weissing, F. J., & Nieberding, C. M. (2021). Phenotypic plasticity explains apparent reverse evolution of fat synthesis in parasitic wasps. Scientific Reports, **11(1)**, 1–13.

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1. **Tiso, S.**, Carvalho, P., Lourenço, N., & Machado, P. (2023). Biological insights on grammar-structured mutations improve fitness and diversity. GECCO '23: Proceedings of the Genetic and Evolutionary Computation Conference.