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THE POLYSEMY OF GENES: ANALOGICAL AND AGENT-BASED MODELS INVESTIGATION OF PHENOTYPIC PLASTICITY

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Abstract

The aim of this thesis is to investigate phenotypic plasticity and its evolution from two different, yet complementary angles: while the first part is theoretical and philosophical, the second is based on a computational approach. In the first part, I try to develop a conceptual framework which is alternative, but not necessarily in conflict with the so-called “gene’s eye view”, that considers genes as the fundamental units of biological evolution, continuously competing to ensure survival, in the form of copies, in the next generation. To expand this narrow perspective, I start my analysis by isolating the main structural aspects shared by phenotypic plasticity, that is, the ability of a given genotype to produce alternative phenotypes in response to environmental conditions, and polysemy, that is, the ability of a word or expression to generate alternative meanings in response to contextual factors. Based on this linguistic analogy, I introduce a broader conceptual model which could combine the centrality of genes and the outstanding importance of their interaction with the environment and with other regions of the genome, also including the pivotal role of developmental processes in the equation. Besides its limitations, this alternative view may serve as an updated heuristic tool to better understand and communicate biological evolution, or even to suggest some new hypotheses. The second part of the thesis involves a specific gene that is known to enable/disable this “biological polysemy”. In this section, I introduce agent-based modelling, that is, programming the behaviour of individual computational entities and letting them interact to produce emerging patterns spontaneously. In some contexts, this modelling technique may be suitable for investigating evolutionary processes in a much faster and controllable way than laboratory or field research. Using the modelling environment NetLogo, I implement an agent-based model that simulates the competition between

plastic and non-plastic strains of the nematode worm *Pristionchus pacificus*, which is an exemplary organism in the field of evolutionary developmental biology. The global behaviour of the system is monitored in terms of frequency of fixation and mean time to fixation of two alleles of the developmental switch gene *eud-1*, one enabling mouth-form dimorphism and predation (+), the other leading to the constitutive expression of a single morph (*e*, which stands for *eud-1*). The model includes benefits in fecundity for each phenotype, costs in fecundity due to starvation or to the production and maintenance of plasticity (for plastic strains only), different degrees of environmentally-induced variation, and two alternative food sources (i.e., bacteria and nematodes' larvae), whose availability may vary over time at regular intervals. Using this model, I simulate several experimental scenarios with the aim of identifying relevant emerging patterns resulting from the interplay between the key variables that were introduced into the system.

Keywords

Phenotypic plasticity, Polysemy, Analogy, Agent-based model, Net-Logo, *Pristionchus pacificus*, Competition

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Introduction

The main aim of this work is not that of defending the critical role of *genes* in life and evolution, mostly because their importance has never seriously been doubted. Even a small *DNA* modification may generate stunning changes downstream, as we can see at any level of biological organisation, from alteration of cell structure to the determination of mating or eating behaviours, just to mention two of countless possibilities. This has always been a great source of wonder for me. The main biological motif of this thesis, *phenotypic plasticity*, is an admirable case of the capacity of genes, in concert with other genes and the environment, to shape the characteristics of any organism by channelling its development towards a multitude of possible paths which are not strictly established *a priori*. In a nutshell, the main objective of the following pages is to focus on the role of genetically inherited material as an activator of the ability of organisms to change themselves in response to the environment, rather than just as a constraint.

Almost all humans can learn skills, or change their own body through resistance workouts. When considering these common activities and their practical results, most people would likely support the possibility of a coexistence of both hereditary and environmental explanations. Nevertheless, it may appear somewhat reductive or counter-intuitive to think that these plastic mechanisms are *allowed*, rather than *limited*, by a long evolutionary history encoded in *DNA* strings, and ultimately embedded in genes inherited from parents. Indeed, what we really inherit, in a large number of cases, are wider or narrower ranges of variability, not characteristics sculpted in stone. This is also virtually true for any other taxonomic group, from bacteria to cetaceans. This study aims to defuse this apparent paradox, focusing on the ways through which environmental reactivity can evolve *due to* genes, not *in spite* of them.

Genes are not just the primary material of heredity. They are also the source of context-dependent plasticity, or biochemical tools through which life channels the huge amount of signals received from both the external and the internal environment. Environmental responsiveness may potentially emerge everywhere and for most heritable traits, as one can suspect by looking even at the most distant branches of the Tree of life: when some basic surrounding conditions are given, heritable plastic responses may proliferate locally, and this applies equally to protozoans, plants, or humans.

These environmentally-induced changes are the outcomes of what I call the *polysemy of genes* or, extending the notion so as to make it more suggestive (although also somewhat more vague), the *polysemy of life*. This kind of biological polysemy is carried by genes themselves, and it largely depends on their complex interactions with their *genomic* co-text and ecological context, just like words show their range of meanings when inserted in different sentences and used in alternative circumstances. Genes “decide” the width and the opening of the window of environmental influences, and they are uninterruptedly shaped by contingencies and evolutionary processes including *natural selection* and *mutation*. The analogy between polysemy and phenotypic plasticity is the concept around which all the other topics covered in this work gravitate, from the philosophical problem of genetic determinism to the evolutionary dynamics of biological *complex systems*.

For the breadth of perspectives adopted to study genetically-inherited environmental sensitivity, this investigation involves multiple, heterogeneous methods, in order to explore this very broad topic from different angles. In this study, I combine several approaches ranging from arguments to computer simulations, also including analogical reasoning and the implementation of biological knowledge into an interactive, dynamic model. This methodological heterogeneity is functional to ensure both a conceptual and an experimental treatment of the issue. More specifically, this thesis could be divided into two parts: the first part concerns the construction of a theoretical model based on the analogy above, whose function is to produce an effective heuristic tool to represent the salient characteristics of phenotypic plasticity in a simple manner; the second part regards the implementation of a computational model based on empirical data coming from an exemplary organism used to study

plasticity. This model is then used to produce large sets of simulations through which infer some statistical trends on the evolutionary interplay between a plastic and a non-plastic strain. These steps are encapsulated in five main chapters, whose contents are summarised below.

In Chapter 1, I introduce the philosophical background underlying the *nature versus nurture* debate. I argue that it is just a pseudo-problem which, however, hides a real misconception, that is, the idea that genes and their effects on organisms represent the biological embodiment of the concept of fate. In this chapter, I show that genetic reductionism and developmental and ecological holism may be successfully related to each other by focusing on the genetic inheritance of environmentally-responsive traits.

In Chapter 2, I discuss both the history of the concept of environmentally-induced responsiveness in organisms and the theoretical foundations underlying phenotypic plasticity and its evolution, supporting this conceptual overview with several case studies across many taxonomic groups.

In Chapter 3, I highlight both the value and the limits of analogical thinking, then I discuss in detail the contact points between phenotypic plasticity and polysemy, uncovering the suggestive similarities between biological and linguistic flexibility and, at the same time, warning about some relevant differences between these phenomena. I propose that this analogy could be used both to make plasticity more understandable for non-specialists and to suggest some new hypotheses in these areas of investigation.

In Chapter 4, I introduce complex systems and talk about the powerful possibilities opened by a conversion of the relevant aspects of such systems into computational models based on the simultaneous interaction between many digital entities. In particular, I focus on the features and use of the software for *agent-based modelling* NetLogo.

In Chapter 5, I use NetLogo as a simulation platform to implement an agent-based model aimed at replicating the behaviour of a real biological system. Then, I use the resulting model to carry out a computational research on the evolutionary interplay and competition between plastic and deterministic variants of the nematode worm *Pristionchus pacificus*, an emerging model organism used in recent studies on the evolution of phenotypic plasticity.

In addition to the main chapters, Chapter 6 (i.e., Appendix A) is the full code of the NetLogo model, while Chapter 7 and Chapter 8 (i.e., Appendix B and Appendix C) are interviews with two leading figures in the field of evolutionary developmental biology, Professor Alessandro Minelli and Professor Ralf J. Sommer, respectively.

Finally, the Glossary includes all definitions of the main technical terms which are used throughout the thesis. Except where indicated, these definitions are extracted from Rittner and McCabe (2004)¹.

¹Rittner, D. and McCabe, T. L. (2004). Encyclopedia of biology. Facts on File Science Library. Facts On File, New York, NY.

Chapter 1

Biological systems between evolution and development: flexibility powered by genes

Introduction

“To find that any trait, even one with formidable political implications, has a mainly genetic basis is not to find something set immutably in stone. It is merely to understand the nature upon which nurture is ever acting [...].”

(Watson, 2017)¹.

In the beginning, there was division. Although it was not clearly conceptualised until 19th century, the duality between *heredity* and *environment*, two factors acting generation after generation to determine the characteristics of organisms, is perhaps one of the most long-lasting in the history of mankind. As a matter of fact, different sensibilities and tendencies related to this issue may be found from the origins of Western thought, when some of the first, great philosophers were already discussing on crucial topics like those concerning life and cognition.

As often happens in the history of philosophy, the first symptoms of such an ancient dichotomy may be traced back to no less than the best-known duo of Greek thinkers, i.e., Plato and Aristotle. Nevertheless, differently from several other cases, their positions on

¹Watson, J. D. (2017). DNA, chapter 12. Knopf Doubleday Publishing Group, Westminster.

this theme were not incompatible at all, although they were not perfectly overlapping. They both believed in the simultaneous presence of two sources, one related to conservation and the other to variation, in the living world. Nowadays, we would call these sources *nature* and *nurture*, i.e., in the context of human development, the biological predisposition determined by the genetic makeup of the individual and all environmental aspects that can shape the individual throughout his life, respectively (Sravanti, 2017)².

On the one side, Plato seems to depict well the prodromes of hereditary thinking, emphasising the strong influx of the ancestry in determining the characteristics of the offspring: vertical transmission of gold, silver, or bronze natures between parents and children in the Republic (Stubbe, 1972, p.16)³ and innate presence of abstract ideas and knowledge at birth (Lewkowicz, 2011)⁴ are emblematic examples of that. On the other side, for instance, he argued that hand preference in humans was due to misguiding education towards young children, that is definitely not related to the ancestry in the genetic sense of the term. Conversely, Aristotle thought that asymmetry in handedness was universal and not distorted by environmental influences (Hopkins and Rönqvist, 2013)⁵ but, at the same time, he was also a proto-empiricist in thinking that knowledge is acquired through everyday experience. Finally, he had a deep interest in hereditary transmission of body characteristics too (Mayr, 1982, p.636)⁶. That said, who is the “nativist”, or perhaps the “naturist”? Who the “nurturist”? Indeed, it would be quite difficult to attribute them these labels, since both nature and nurture emerge from their partial disagreements and heterogeneous opinions. This was just the beginning of the debate, since Aristotle and Plato are just two of the first pioneers of a much longer and intricate conceptual history, that has traversed the meanders of philosophy and science until the present days.

One of the plausible questions one might think about is whether

²Sravanti, L. (2017). Nurture the nature. Indian Journal of Psychiatry, 59(3):385.

³Stubbe, H. (1972). History of genetics. MIT Press, Cambridge, Mass.

⁴Lewkowicz, D. J. (2011). The biological implausibility of the nature-nurture dichotomy and what it means for the study of infancy: Biological implausibility of the nature-nurture dichotomy. Infancy, 16(4):331–367.

⁵Hopkins, B. and Rönqvist, L. (2013). Human handedness: Developmental and evolutionary perspectives. In The Development of Sensory, Motor and Cognitive Capacities in Early Infancy, pages 191–236. Routledge.

⁶Mayr, E. (1982). The growth of biological thought. Belknap Press of Harvard Univ.Press, Cambridge, Mass.

this ancient debate may be represented in a binary way (i.e., something like the *yin/yang* or classical logic's *true/false*) or not. Although scientists have developed some rigorous methods to distinguish the hereditary contribution from the environmental one, it can well be said that one of the keys for all terrestrial life and biodiversity is nothing but the *interplay* between these two factors. However, that is almost universally accepted, both by specialists and common people. In other words, there is not a real debate on whether such an interplay exists or not. Nevertheless, the belief that biological ineluctability stands with the hereditary side, whereas the potential to induce changes is a prerogative of environmental influences alone, is still strong and alive, endowed with a plausible charm due to its simplicity, that makes it capable of spreading through human minds. In this chapter, I want to shape an alternative picture.

Hereditary material, also known as *DNA*, the main source of evolutionary change (nature), is nothing but the biochemical support that, with the help of a dense network of interactions and processes, regulates the responsiveness of any organism to its environment (nurture), allowing biological characteristics to show greater reactivity towards surrounding conditions or, conversely, more resistance to such influences. For this reason, I argue that investigating life from the genetic point of view (i.e., DNA's point of view) is far from being *necessarily* agnostic, or even distorting, towards the level of individual development. More specifically, in the following chapters, I explain in what sense DNA may be considered as the carrier of environmentally-induced developmental sensitivity, marginalising the outdated idea considering it and its famous protein-coding regions, *genes*, as mere constraints or even biological embodiments of the concept of fate, while keeping intact their centrality for evolution.

In Section 1.1, I show that the interplay between nature and nurture is already a well-acknowledged matter of fact in modern biology, as well as in common thought. Furthermore, I propose a perspective shift from considering DNA and genetic inheritance as the biochemical counterparts of the idea of fate, to considering them as the basic carriers of environmental responsiveness.

In Section 1.2, I focus on DNA and genes, emphasising their potential to allow environmentally-induced variation in organisms by means of basic evolutionary forces which include, among others,

mutation and natural selection.

In Section 1.3, I argue that environmentally-induced responsiveness is encoded in the most remote, simple, or even artificial minimal organisms, showing both the fundamental role of genetic flexibility for all life forms and the “ability” of DNA to respond to signals from outside the organism, even without complex developmental systems and coexistence of several levels of organisation.

In Section 1.4, I support a conceptual integration between one of the possible ways of looking at life and evolutionary processes, that is the so-called *gene’s eye view*, and important research areas claiming a more prominent role for development and environment, such as *evolutionary developmental biology*, keeping in mind the heuristic potential of such a composite perspective.

1.1 Genes, environment, and their deep interaction

Evolution and development are two highly complicated processes. Each single *trait*, that is, each particular characteristic isolated from all morphological, metabolic, or behavioural aspects of an organism, can be regarded as a small wonder of complex architecture, engineering (Dennett, 1996, pp.187-188)⁷, or tinkering (Jacob, 1977)⁸, depending on one’s perspective. Speaking generally and with a high degree of simplification, the evolution of a biological trait is the result of an interplay between: i) many random events, including those affecting the composition of the underlying hereditary material, like *mutations* and major chromosomal rearrangements, ii) a process related to the capacity of the organism to replicate within a given environment and that determines whether the hereditary material will be passed to the next generation (i.e., *natural selection*), and iii) several constraints of different types, e.g., developmental or simply depending on physical laws. On the developmental side, once the genetic basis involved in the formation of a specific trait is present, many other multi-level manipulations, processes, and paths are needed to connect the hereditary components to the expression of the final, visible outcome. Although evolution and de-

⁷Dennett, D. C. (1996). Darwin’s Dangerous Idea Evolution and the Meanings of Life. Penguin Books, Limited.

⁸Jacob, F. (1977). Evolution and tinkering. Science, 196(4295):1161– 1166.

velopment are, respectively, an inter-generational and an individual phenomenon, the understanding of both planes and their reciprocal interaction is the key to investigating life on Earth properly (Minelli, 2009b, p.212)⁹.

Among all the factors in play of the complex interaction between these two different planes, genes are those which had performed the most outstanding role for almost all of 20th century's biology (Keller, 2009, p.147)¹⁰. Although the definition of the concept of "gene" has continuously evolved, from Mendel's insight on the existence of some abstract hereditary units to the current era, marked by new sequencing techniques and countless databases, there is a general agreement on the fact that genes are DNA sequences capable of specifying some *RNAs/polypeptides/proteins*, which are molecules contributing, in a complex way, to the formation of traits (Portin and Wilkins, 2017)¹¹. In turn, DNA (deoxyribonucleic acid) is the fundamental chemical component of the *genome*, that can be thought as the informational entity representing the basis of the building of each organism, as well as the main (not always the exclusive) material of biological inheritance (Goldman and Landweber, 2016)¹². Although genes are usually a very small part of the whole genome, here I focus mainly on these regions and their pivotal role for living beings.

On the other side, *environment* is a very broad label, that may be used to indicate the summation of all variables external to the organism. Environmental conditions are usually far from being stable in space and time: temperature, availability of nutrients, presence of harmful substances, all these and many other factors are subject to a certain degree or rhythm of fluctuation, and such a fluctuation has been shown to be biologically critical in several different ways (Melbinger and Vergassola, 2015)¹³. Obviously, in this context, we are interested in environment insofar as it relates to living beings and affects their characteristics.

After this brief, yet necessary digression on the basic elements

⁹Minelli, A. (2009b). *Forms of becoming*. Princeton University Press, Princeton.

¹⁰Keller, E. F. (2009). *The Century of the Gene*. Harvard University Press, Cambridge.

¹¹Portin, P. and Wilkins, A. (2017). The evolving definition of the term "gene". *Genetics*, 205(4):1353–1364.

¹²Goldman, A. D. and Landweber, L. F. (2016). What is a genome? *PLOS Genetics*, 12(7):e1006181.

¹³Melbinger, A. and Vergassola, M. (2015). The impact of environmental fluctuations on evolutionary fitness functions. *Scientific Reports*, 5(1).

of the “game” of development and evolution, it is now possible to introduce gradually the core philosophical argument underlying most of this work. As a matter of fact, genes and environment *interact* deeply and frequently, and it is not surprising at all anymore. For instance, Gene-Environment interactions are so widely studied with respect to human health and behaviour that, in present days, debates over whether individual differences may be explained by nature or nurture (i.e., by genes or the environment) seem to be pretty outdated: they certainly contribute both (Boyce et al., 2020)¹⁴. One of the most important ways through which environmental factors affect living beings is found in the direct interaction of such factors with genes (and, more generally, with genomes). At the molecular level, the emblematic case of the *Escherichia coli* lactose operon (Jacob and Monod, 1961)¹⁵ is a basic representation of this capacity of converting environmental signals into changes in the metabolism of bacteria¹⁶. This is a genetically-based amplification of variation. I discuss in more detail the importance of the regulation of *gene expression*, referring to some organisms with minimal genomes, in Section 1.3.

As for the human species, genetic diseases are particularly suitable to observe the interplay between genes and the environment in action. Phenylketonuria (PKU) is a typical example of that: although it depends on the coexistence of two damaged copies of the gene phenylalanine hydroxylase (*PAH*) on the *chromosome* 12 (and on its homologous, since human chromosomes are organised in pairs), its severity is determined by the daily dietary intake of the *amino acid* phenylalanine. When it is untreated, it can generate developmental delay, intellectual disability, and other neurological

¹⁴Boyce, W. T., Sokolowski, M. B., and Robinson, G. E. (2020). Genes and environments, development and time. *Proceedings of the National Academy of Sciences*, 117(38):23235–23241.

¹⁵Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology*, 3(3):318–356.

¹⁶*E. coli* lactose operon is a DNA functional unit composed by both regulatory and structural regions. More specifically, its three structural genes are expressed, i.e., their sequences are first converted into the corresponding mRNA strand through the process of *transcription*, then the mRNA is converted into *enzymes* during *translation*, only in presence of an external component, that is lactose, and in the absence of a better energy source like glucose. This regulation depends on several interactions between allolactose (a spontaneous derivative of lactose), the transcription enzyme RNA polymerase, a protein that inhibits transcription when allolactose is not available (a repressor), and the non-coding regulatory elements of the operon.

dysfunctions (Hillert et al., 2020)¹⁷. Another example is asthma: this respiratory disease is polygenic, since it is associated with approximately ten genes, but it is also related to a variety of environmental factors like second hand smoke, air pollutants, deficiencies in maternal diet, and viruses (Turner, 2017)¹⁸.

Moreover, as might be expected, diseases are nothing but a small subset of all possibilities in Gene-Environment interaction. A genetic influence is detectable in *all* traits, but since genes always require an environmental cue to be expressed, *all* traits may show environmental influences as well (Schneider, 2007; Schlichting, 2021)^{19,20}. Just without moving from the realm of cognitive faculties, intelligence is a “positive”, yet perhaps controversial example of that, since high intelligence is the other side (or the other tail, if one wants to rely on a statistical terminology) of mental disorders. In general, intelligence may be seen as the aggregated cognitive ability to reason, solve problems, or learn quickly (Plomin and Deary, 2014)²¹. Although it strongly depends on a favourable environment experienced during child development, it is also rooted in genes: indeed, it is one of the most heritable human behavioural characteristics, as shown by the fact that some neurobiological correlates of intelligence are structurally similar among genetically-related individuals, especially considering frontal and lateral temporal regions (Goriounova and Mansvelder, 2019)²².

The debate on the role of genetic heredity and environment in determining human traits is a long-lasting one. Some authors high-

¹⁷Hillert, A., Anikster, Y., Belanger-Quintana, A., Burlina, A., Burton, B. K., Carducci, C., Chiesa, A. E., Christodoulou, J., Dordević, M., Desviat, L. R., Eliyahu, A., Evers, R. A., Fajkusova, L., Feillet, F., Bonfim-Freitas, P. E., Giżewska, M., Gundorova, P., Karall, D., Kneller, K., Kutsev, S. I., Leuzzi, V., Levy, H. L., Lichter-Konecki, U., Muntau, A. C., Namour, F., Oltarzewski, M., Paras, A., Perez, B., Polak, E., Polyakov, A. V., Porta, F., Rohrbach, M., Scholl-Bürgi, S., Spécola, N., Stojilković, M., Shen, N., Santana-da Silva, L. C., Skouma, A., van Spronsen, F., Stoppioni, V., Thöny, B., Trefz, F. K., Vockley, J., Yu, Y., Zschocke, J., Hoffmann, G. F., Garbade, S. F., and Blau, N. (2020). The genetic landscape and epidemiology of phenylketonuria. *The American Journal of Human Genetics*, 107(2):234–250.

¹⁸Turner, S. (2017). Gene-environment interactions—what can these tell us about the relationship between asthma and allergy? *Frontiers in Pediatrics*, 5.

¹⁹Schneider, S. M. (2007). On books: The tangled tale of genes and environment: Moore’s the dependent gene: The fallacy of “nature vs. nurture”. *The Behavior Analyst*, 30(1):91–105.

²⁰Schlichting, C. D. (2021). *Plasticity and Evolutionary Theory: Where We Are and Where We Should Be Going*, pages 367–394. CRC Press.

²¹Plomin, R. and Deary, I. J. (2014). Genetics and intelligence differences: five special findings. *Molecular Psychiatry*, 20(1):98–108.

²²Goriounova, N. A. and Mansvelder, H. D. (2019). Genes, cells and brain areas of intelligence. *Frontiers in Human Neuroscience*, 13.

lighted that this opposition has been central for Western societies even in remote times: for instance, Augustine of Hippo used to consider sin as a hereditary characteristic, capable of making children “naturally” inclined towards evil, so he believed that physical punishment was necessary to educate them (Wyman, 2005)²³. In the last four centuries, the debate has been greatly enriched. It has not only involved scientists like geneticist Richard Lewontin or entomologist Edward O. Wilson, but also thinkers of the caliber of John Locke, Thomas Hobbes (Pigliucci, 2001, p.253)²⁴, and Jean-Jacques Rousseau.

However, the locution *nature versus nurture* was made notorious by a man of the 19th century: Charles Darwin’s polymath cousin Francis Galton (Ridley, 2004; Tabery, 2023, p.71, p.2)²⁵²⁶ was the only personality of his time who focused on the distinction between the inherited material and the realisation of bodies based on those raw hereditary components (Mayr, 1982, p.781)²⁷. According to him, human twins were the perfect experimental subjects to evaluate and discern nature and nurture’s contributions to the individual development (Ridley, 2004, p.76)²⁸. As has been anticipated in the past few pages, nature may roughly be seen as a counterpart of genes, whereas nurture is into an analogous relation with the environment.

Galton’s idea of measuring the relative contributions of genes and environment paved the way for many further reflections and studies. Nowadays, finding a biologist who does not believe that traits are determined by both factors would be an impossible task, and there is no way it could be a serious matter of discussion. Massimo Pigliucci, an evolutionary biologist (and philosopher) whose interest towards Genes-Environment interactions was originally catalysed by the “nature versus nurture” debate, told it clearly in one of his most important books: advocates of both “factions” agree on the fact that the answer to this opposition falls somewhere in the

²³Wyman, R. J. (2005). Experimental analysis of nature-nurture interactions. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 303A(6):415–421.

²⁴Pigliucci, M. (2001). *Phenotypic plasticity. Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore, Md.

²⁵Ridley, M. (2004). *Nature Via Nurture*. HarperPerennial.

²⁶Tabery, J. (2023). *Beyond Versus The Struggle to Understand the Interaction of Nature and Nurture*. MIT Press.

²⁷Mayr, E. (1982). *The growth of biological thought*. Belknap Press of Harvard Univ.Press, Cambridge, Mass.

²⁸Ridley, M. (2004). *Nature Via Nurture*. HarperPerennial.

middle between the two extremes (although they strongly disagree on *where* it falls exactly) (Pigliucci, 2001, p.254)²⁹.

The idea of the actual existence of a duality is severely challenged by scientific knowledge accumulated until now. Indeed, since everyone seems to adhere to the “interactionist credo”, why does this conceptual division still persist over time? (Tabery, 2023, p.10)³⁰. Why has this simplistic dichotomy not yet vanished into the oblivion of “defused” paradoxes?

Even some apparently or partially solved issues, sometimes, could conceal certain nuances and hidden implications, so it may be beneficial to analyse them from different perspectives. For instance, as mentioned above, one possible way of dealing with this problem could be that of balancing between two ideal opposites, both of which are materially unfeasible in reality: on the one hand, genes, the molecular reification of fate, which would make the inherited characteristics fixed and unchangeable; on the other hand, the environment, seen as the only source of variation, malleability, or even “freedom” (from biological constraints). Investigating the interval between these two ends is probably way more reasonable than believing in the *exclusive* preponderance of genes or the environment in development. Nevertheless, I argue that even a continuum that is defined by those two purely hypothetical extremes would be as fallacious as the idea of the existence of the duality.

As discussed by philosopher and cognitive scientist Daniel Dennett, environmental constraints and individual experience can critically determine development and steer it in a specific direction, which are exactly the same effects that are attributed to the constraints related to genetic inheritance. As a matter of fact, major events in childhood can dramatically shape children, and can even affect heavily their adult life as well (Dennett, 2004, pp.157-158)³¹. Public education may also be effective in equalising, to some extent, large numbers of individuals showing alternative genetic makeups. This is also true outside humans: for instance, the reproductive cycle of the yellow perch (i.e., a fish) mainly relies on two variables linked to seasonal changes (i.e., temperature and *photoperiod*), similarly to

²⁹Pigliucci, M. (2001). Phenotypic plasticity. Syntheses in ecology and evolution. Johns Hopkins University Press, Baltimore, Md.

³⁰Tabery, J. (2023). Beyond Versus The Struggle to Understand the Interaction of Nature and Nurture. MIT Press.

³¹Dennett, D. C. (2004). Freedom evolves. Penguin books, London.

what happens in related temperate species. Proper artificial manipulations of these environmental factors may be used by breeders to induce out-of-season spermatogenesis (in males) or ovulation and hatching (in females), producing higher numbers of larvae throughout the year (Fontaine et al., 2015)³². The common aspect of the two examples above is the possibility of making different individuals (or even different populations) more homogeneous by changing their environment. Thus environmental influence, as well as genetic influence, may also be a source of constraints.

Just considering what has been said until now, it seems unquestionable that both genes and the environment may produce or repress variation. Furthermore, there are also cases where these two influences, other than being present simultaneously, cannot even be separated in any way (Pigliucci, 2001, p.60-61)³³. Altogether, these preliminary considerations are enough to make the overall picture much more inextricable than expected, as well as definitely not binary. In other terms, nature and nurture are more likely two blurred shades produced by the same figure, rather than well-defined, hypothetical ends of a range. For this reason, there is still much to be said about these two factors of biological variation and their complex relationship. This is the focus of the next sections of this chapter.

1.2 Genes as molecular activators of responsiveness

Quantitative geneticists have already been involved for a long time in a mathematical treatment of the issue of estimating the weights of the additive contributions of genes and the environment, as well as of Gene-Environment interaction. For instance, this could be done through the analysis of variance (ANOVA) (Sober, 2001)³⁴. Rather than trying to address that quite technical issue here, I want to explain in what sense nature, intended as genetic heredity, is the fundamental basis of nurture in terms of environmentally-induced

³²Fontaine, P., Abdulfatah, A., and Teletchea, F. (2015). Reproductive biology and environmental determinism of perch reproductive cycle. *Biology of perch*. CRC Press, Boca Raton, FL, USA, pages 167–192.

³³Pigliucci, M. (2001). Phenotypic plasticity. *Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore, Md.

³⁴Sober, E. (2001). *Separating Nature and Nurture*, pages 47–78. Cambridge University Press.

responses produced by organisms.

As a matter of fact, as the aforementioned case study of the operon of lactose clearly shows, genomes themselves can react to the environment. Although it may seem somewhat paradoxical, the possibility of producing environmentally-induced modifications is encoded in DNA and, more specifically, in genes. The existence of such basic molecular mechanisms subverts the idea that the genetic makeup is just a constraint or a limitation of the ability of organisms to develop and change themselves during their life cycles, repeatedly or irreversibly. Indeed, even the genome itself may be conceived as a subset of the environment, capable of interacting with both its microscopic neighbourhood and, through the organism to which it belongs, the external world. This high potential for interaction and feedback is the key to generate a plenty of shades of variation. However, I discuss more in depth this perspective later, in Chapter 3. For now, it would be enough to keep in mind that genes are the basic components through which organisms can be “flexible”. Using a fascinating expression, we could even consider genes as the “servants of experience” (Ridley, 2004, p.280)³⁵.

Considering genes under this slightly alternative light may recall Richard Dawkins’ argument in favour of the role of natural selection as a creative evolutionary force. People tend to consider natural selection as a purely “negative force”, that is, an elimination process of all unsuitable evolutionary outcomes. As explained by the British ethologist, this is false on the long distance, since reiterated survival and reproduction of some pre-existing forms to the detriment of others may build up complexity and diversity in many possible ways. A quite striking way through which this can happen is represented by arms races between predators and preys. An example of such dynamics is the evolution of sensory adaptations in sea stars to better sense glycoproteins released by barnacles’ shells which, in turn, had previously evolved to protect barnacles themselves from predators with a dietary predilection for exoskeleton-bearing animals (Dawkins, 1987; Zimmer et al., 2021, p. 169)^{36,37}. Genes, like natural selection, can be considered as “positive” or

³⁵Ridley, M. (2004). *Nature Via Nurture*. HarperPerennial.

³⁶Dawkins, R. (1987). *The blind watchmaker*. Longman Scientific & Techn., Harlow, Essex, reprint edition.

³⁷Zimmer, R. K., Ferrier, G. A., and Zimmer, C. A. (2021). Chemosensory exploitation and predator-prey arms races. *Frontiers in Ecology and Evolution*, 9.

“creative” evolutionary components as well. Thanks to regulatory mechanisms and networks, genes may be the ultimate enablers of biological variability, allowing the shaping of bodies and functions in accordance with ecological variables. Moreover, since environmental responsiveness also evolves by means of the combined effect of several processes affecting genes, including mutation and natural selection, it is clear that genetic inheritance is the foundation upon which environmentally-induced variation is built, rather than a source leading to a single, predetermined outcome.

Although genes can produce fixed traits which are independent of environmental stimuli, they can also enable a higher degree of reactivity, especially when it is beneficial for the organism in terms of reproductive success. Such reactivity, allowing its possessor a different interaction with the environment when compared with its more “deterministic” fellows, affects the probability of mutations promoting this ability to be selected and, therefore, to be transmitted to the progeny. In other words, basic Darwinian processes are sufficient to make organisms responsive to their surroundings.

1.3 The ubiquity of environmentally-induced responses: the case study of minimal cells

In the previous section, I proposed that genome may be considered as the basic toolkit regulating environmental reactivity across generations (i.e., its state can either reduce or enhance this property). In this case, the basic mechanisms of environmentally-induced responses would be found at the microscopic level of DNA sequences, rather than being necessarily related to a complex stratification of many organisation levels or to particular properties of developmental systems themselves (Charlesworth et al., 2017)³⁸. Assuming this perspective to be true, one would expect to observe environmentally-induced changes even in organisms lacking highly interactive developmental pathways.

Undoubtedly, developmental complexity is relevant in most multicellular organisms, like animals and plants. Indeed, it has been reported the existence of a positive correlation between complexity,

³⁸Charlesworth, D., Barton, N. H., and Charlesworth, B. (2017). The sources of adaptive variation. *Proceedings of the Royal Society B: Biological Sciences*, 284(1855):20162864.

intended as the number of distinct functions an organism can perform (i.e., the number of its specialised cells), and the size of the organism, due to the increased possibilities of division of labour (although some theoretical approaches have proven that it is not a universal rule) (Amado et al., 2017)³⁹. Since this kind of complexity would be a confounding factor to investigate the deep genetic roots of biological elasticity, multicellular species are not the best examples for achieving this goal. What we need is a species that cannot show such level of complexity, e.g., a unicellular one. In this context, unicellular organisms are ideal because the “distance” between genome and the external environment is very reduced, especially in terms of steps necessary to convert inherited information into visible characteristics.

The repertoire of unicellular organisms on Earth is huge. Given their simplicity, bacteria could be the most immediate candidates for this investigation. Furthermore, their modalities of environmental reactivity are well-known. In this domain of life, a relatively simple process of regulation of gene expression is used to allow organisms to respond to their environment. In this regard, I have already mentioned in Section 1.1 the classic operon model, that is the fundamental mechanism to accomplish regulatory tasks in *prokaryotic* species (Jacob and Monod, 1961; Ishihama, 2012)^{40,41}. Since the *Escherichia coli* operon model is extensively addressed in molecular genetics textbooks, it would be quite trivial using that to claim the importance of genetically-driven processes of conversion of environmental stimuli into visible variation. Nevertheless, many further related considerations may be produced to show at the same time the simplicity, antiquity, and ubiquity of gene-centred modalities of interaction with environmental inputs.

However, even bacteria’s level of complexity is not homogeneous at all. As a matter of fact, there is a large amount of diversity in this huge unicellular domain (Horner-Devine et al., 2004)⁴², and

³⁹Amado, A., Batista, C., and Campos, P. R. A. (2017). A theoretical approach to the size-complexity rule: Size-complexity rule. *Evolution*, 72(1):18–29.

⁴⁰Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology*, 3(3):318–356.

⁴¹Ishihama, A. (2012). Prokaryotic genome regulation: A revolutionary paradigm. *Proceedings of the Japan Academy, Series B*, 88(9):485–508.

⁴²Horner-Devine, M. C., Carney, K. M., and Bohannan, B. J. M. (2004). An ecological perspective on bacterial biodiversity. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1535):113–122.

E. coli is far from being its simplest representative. If one really wants to explore biological flexibility at the lowest level, thus considering organisms composed by their DNA strain and little else, he should take a look at the origins of life on Earth. Since these very remote times, two complementary tendencies in the relation between organisms and the environment have been recognisable: on the one hand, the necessity to develop structures capable of isolating early metabolisms from the outside; on the other hand, the need to communicate and interact with the external world. In other terms, living beings have always needed to be, at the same time, both resistant and responsive in relation to their surroundings.

LUCA (*Last Universal Common Ancestor*), the ultimate progenitor of the two most ancient life domains (i.e., Bacteria and Archea), links the abiotic phase of the history of Earth to the first microbial traces, dating back 3.8 - 3.5 billion years (Weiss et al., 2016)⁴³. Technically, LUCA is a theoretical entity corresponding to a primitive cell whose basic characteristics should reflect what is now shared by all organisms on Earth. Genomic analyses clarified that, for instance, this entity had *ribosome*, the current *genetic code*, *cell membrane proteins*, and DNA domains for a basic regulation of gene expression (Aravind et al., 2005; Forterre, 2005; Koonin and Novozhilov, 2008; Weiss et al., 2018)⁴⁴⁴⁵⁴⁶⁴⁷. The simultaneous presence of membranes and regulatory elements confirms the importance of both resistance to, and interaction with, the environment. Most importantly for our discussion, the existence of regulatory elements is a proof of both the rapid emergence and the “simplicity” of encoded environmental responses. Rudimentary molecular components stored in genes and used to regulate the expression of other genes are primitive characteristics of life. But there is more to be said about the relevance of this aspect in extremely “gene-centred”

⁴³Weiss, M. C., Sousa, F. L., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S., and Martin, W. F. (2016). The physiology and habitat of the last universal common ancestor. *Nature Microbiology*, 1(9).

⁴⁴Aravind, L., Anantharaman, V., Balaji, S., Babu, M., and Iyer, L. (2005). The many faces of the helix-turn-helix domain: Transcription regulation and beyond. *FEMS Microbiology Reviews*, 29(2):231–262.

⁴⁵Forterre, P. (2005). The two ages of the rna world, and the transition to the dna world: a story of viruses and cells. *Biochimie*, 87(9–10):793–803.

⁴⁶Koonin, E. V. and Novozhilov, A. S. (2008). Origin and evolution of the genetic code: The universal enigma. *IUBMB Life*, 61(2):99–111.

⁴⁷Weiss, M. C., Preiner, M., Xavier, J. C., Zimorski, V., and Martin, W. F. (2018). The last universal common ancestor between ancient earth chemistry and the onset of genetics. *PLOS Genetics*, 14(8):e1007518.

life forms.

Indeed, LUCA’s predicted genome is composed by nearly 600 genes (Mushegian, 2008)⁴⁸, which are still more than those estimated for the simplest organism conceivable. The *minimal cell* is, for present-days biologists, comparable to the hydrogen atom for 19th and 20th century physicists: while the latter, due to its simplicity, was used to gain intuitions on the structure and characteristics of more complex elements, the former may be used to understand life mechanisms (like environmentally-induced responses) at the most basic level (Glass et al., 2017)⁴⁹. Researchers are now able to build artificially whole synthetic organisms (more specifically, cells with a very basic genomic content) to address these questions. Of course, there are significant differences between the hydrogen atom and an artificial cell. First of all, while the former is found in nature, the latter is designed by humans. However, cells with minimal genomes are still crucial for the aim of investigating the relationship with the environment of radically simple organisms. Indeed, these designed organisms are expected to be as close as possible to the level of the genes encoding their own functions. Thus, assuming that genes are exclusively limiting factors, they would be the perfect demonstration of the unconditional expression of traits, something we could name “biological fate”.

Minimal genomes can be obtained with a bottom-up procedure, which involves design, synthesis, and installation of reduced genomes including only genes that are known, when disrupted, not to allow the performance of vital functions or a correct growth. In the artificial JCVI-Syn3.0, only essential and quasi-essential genes are kept in the final genome. Indeed, the inactivation of the former, that are indispensable to support cellular life, does not allow viability of cells and growth of the progeny, while the inactivation of the latter affects efficiency of functions and robust growth (Zhang,

⁴⁸Mushegian, A. (2008). Gene content of luca, the last universal common ancestor. *Frontiers in Bioscience*, Volume(13):4657.

⁴⁹Glass, J. I., Merryman, C., Wise, K. S., Hutchison, C. A., and Smith, H. O. (2017). Minimal cells—real and imagined. *Cold Spring Harbor Perspectives in Biology*, 9(12):a023861.

2004; Hutchison et al., 2016; Glass et al., 2017)⁵⁰⁵¹⁵². The resulting genome is composed by 473 genes, that is an impressive number. In particular, 9 of them are dedicated to regulation. That is quite surprising, since it means that encoded forms of interactivity with the environment are probably crucial for every conceivable living being, even for those with radically limited developmental processes, and whose life cycles are very poor of multilevel interactions. It is stunning to see that the basic elements of environmental responsiveness are already present in such conditions. This kind of responsiveness is probably one of the oldest features of genes, and it is fundamental for life even at a minimal level. For this reason, the idea of a one-to-one correspondence between genes and traits can be refuted at any level, and is anachronistic.

This short excursion to the lowest floors of life on Earth should leave us with a slightly more realistic idea of the true role of DNA. Preservation from, and interaction with, the environment, are both fundamental features of life, both encoded in *nucleotides* strings. The simultaneous execution of these roles makes genes far from being the exclusive guardians of biological fate. Instead, they are the fundamental enablers of nurture, thus the best allies of “nurturists”, who ironically are those who would like to diminish their importance (Ridley, 2004, p.6)⁵³. Even more surprisingly, the roots of this tendency of enabling environmentally-induced responses may be traced back to remote ancestors, and down to minimal “cousins”.

1.4 The evolution of development from the perspective of genes

A heated debate, whose origins are as old as the formulation of Darwinian theory itself, has recently grown around the core of evolutionary research, involving some ideas that are alternatively con-

⁵⁰Zhang, R. (2004). Deg: a database of essential genes. *Nucleic Acids Research*, 32(90001):271D–272.

⁵¹Hutchison, C. A., Chuang, R.-Y., Noskov, V. N., Assad-Garcia, N., Deerinck, T. J., Ellisman, M. H., Gill, J., Kannan, K., Karas, B. J., Ma, L., Pelletier, J. F., Qi, Z.-Q., Richter, R. A., Strychalski, E. A., Sun, L., Suzuki, Y., Tsvetanova, B., Wise, K. S., Smith, H. O., Glass, J. I., Merryman, C., Gibson, D. G., and Venter, J. C. (2016). Design and synthesis of a minimal bacterial genome. *Science*, 351(6280).

⁵²Glass, J. I., Merryman, C., Wise, K. S., Hutchison, C. A., and Smith, H. O. (2017). Minimal cells—real and imagined. *Cold Spring Harbor Perspectives in Biology*, 9(12):a023861.

⁵³M. (2004). *Nature Via Nurture*. HarperPerennial.

sidered either revolutionary and neglected by scholarship, or already acknowledged and under examination, depending on one’s perspective. Inheritance systems (e.g., genetic or non-genetic), role of development, feedback effects of habitat modifications, levels of natural selection, are currently hot spots for both philosophers and scientists (Laland et al., 2014; Welch, 2016)⁵⁴⁵⁵, especially those with some interests in the general, conceptual principles underlying biological change. Although it may be a fascinating way of approaching evolution, I have always looked at those “higher-level” disagreements as issues heavily relying on different emphasis, expectations, and confidence on possible extensions of several evolutionary processes. In a way, a matter of nuances and personal preferences. Nevertheless, broad and abstract perspectives may be heuristically useful to understand better some aspects of reality, or to catch new details from what is already documented. That is quite interesting, and it is also part of my present work.

In particular, I stress the fact that genes, rather than being *only* a source of developmental constraints, are the fundamental carriers of the environmental reactivity of biological systems. The degree of interaction with the environment is one of the functions of the genome that are regulated by natural selection and other related mechanisms. The more fine-tuned this degree is, the more the system will be adapted to its (sometimes highly changeable) context. That is far from being a new, radical perspective. Indeed, biologists already have massive empirical knowledge of these kinds of dynamics, some of which are also discussed in detail in Chapter 2. Nevertheless, here I want to build on this biological reality in an attempt to make of it a conceptual bridge between heterogeneous and, sometimes, even opposing visions. I shortly discuss them below.

One of the components of the perspective I propose here is the so-called *gene-centred view of evolution*, also known as *gene’s eye view*. It was subliminally present in biology from the age of the “reconciliation” between Darwinian and Mendelian theories operated by Ronald Fisher, Sewall Wright, and John Haldane’s pop-

⁵⁴Laland, K., Uller, T., Feldman, M., Sterelny, K., Müller, G. B., Moczek, A., Jablonka, E., Odling-Smee, J., Wray, G. A., Hoekstra, H. E., Futuyma, D. J., Lenski, R. E., Mackay, T. F. C., Schluter, D., and Strassmann, J. E. (2014). Does evolutionary theory need a rethink? *Nature*, 514(7521):161–164.

⁵⁵Welch, J. J. (2016). What’s wrong with evolutionary biology? *Biology & Philosophy*, 32(2):263–279.

ulation genetics (Ågren, 2021, p.26)⁵⁶, and it has been brilliantly communicated to a huge audience, even a non-scientific one, by the aforementioned ethologist Richard Dawkins since the publication of his most notable work, *The selfish gene* (Dawkins, 2009)⁵⁷. Relying on the ideas popularised by Dawkins, the gene’s eye view tenets can be summarised by saying that natural selection acts at the most basic level, that of genes, and that genes compete among them by forming alliances and building organic vehicles (i.e., organisms) in order to maximise the replication rate of their lineage, generation after generation (Sterelny, 2007, pp. 131-132)⁵⁸. For these and other reasons, this view has been accused of excessive genetic reductionism, paying the pledge for the lack of attention towards the crucial role of development in evolution, as it focuses only on genetic variants, rather than on organisms, to explain life (Ågren, 2021, p.2)⁵⁹.

A particularly interesting alternative point of view is that proposed by scholars operating within the framework of *evolutionary developmental biology*, or Evo-Devo, that is a well-established discipline, rather than a philosophical perspective. The main aim of Evo-Devo is to investigate the developmental “black box” represented by the generative processes responsible for the variation shown by individual organisms and to fit it into an evolutionary framework (Amato, 2020)⁶⁰. This is a remarkable frontier of biology, that is able to provide a much more complete picture of how evolution works. However, some other authors supporting the so-called *developmental systems theory*, mostly philosophers of biology, also place emphasis on developmental processes, while proposing some more drastic ideas. For instance, they support a decisive downgrading of the causal role of genes in evolution, claiming the centrality of highly interacting sets of multi-level entities (i.e., the developmental systems), and focusing on the action and inheritance of heterogeneous information sources across generations, ranging from molecular up to social and biogeographical factors. To sum up, it is all about

⁵⁶Ågren, J. A. (2021). *The gene’s-eye view of evolution*. Oxford University Press, Oxford, first edition edition.

⁵⁷Dawkins, R. (2009). *The selfish gene*. Oxford Univ. Press, Oxford, 2. ed., 30th anniversary ed. edition. Previous ed. : 1989.

⁵⁸Sterelny, K. (2007). *Dawkins vs. Gould*. Icon Books, Cambridge, [2nd, new ed.] edition.

⁵⁹Ågren, J. A. (2021). *The gene’s-eye view of evolution*. Oxford University Press, Oxford, first edition edition.

⁶⁰Amato, S. I. (2020). Evodevo: An ongoing revolution? *Philosophies*, 5(4):35.

complex interactions (Robert et al., 2001)⁶¹. I think it is a valuable conceptual contribution, with which I partially agree (as can be inferred from some of the following chapters).

Nevertheless, at the same time, I think it would be unjustified to abandon completely an evocative and powerful tool of thought like the gene-centred view surely is. Indeed, one of the potential risks of particularly radical positions in the opposite direction would be that of losing sight of the deep relevance of genes and natural selection, which might produce an undervaluation of *ultimate* explanations, i.e., population-level processes, in favour of *proximate* explanations, i.e., developmental dynamics. Since developmental changes themselves rely on genetically-based alterations, DNA transmission is still a fundamental part of the story. Explanations based on gene frequencies, on the one side, and explanations based on connecting genetic information and construction of bodies, on the other, are strictly related and complementary (Futuyma, 2015)⁶². That is specifically true when considering all mechanisms related to the amplification of individual variation induced by the environment: numerous interactions shape organisms during their life cycles, then natural selection has a spectrum or a set of alternative outcomes on which to act, generation after generation.

While partially agreeing with the criticisms moved to the gene-centred perspective and some of its excessive simplifications, here I want to reconsider it under a different light. This could be done by including experimental findings of biological research and theoretical components belonging to other, perhaps more complete, visions of life. Even some geneticists are calling for an integration between the gene-centred view and the current knowledge on gene networks, environmental effects, and development, which would need a combination of both a reductionist and a systemic approach. In addition, they also invoke a better public communication of all these relations (Hains, 2014)⁶³. However, that of saving the metaphor of the selfish gene and its conceptual ancestors at all costs is far from being my aim here. There is nothing like a “correct”, all-encompassing view

⁶¹Robert, J. S., Hall, B. K., and Olson, W. M. (2001). Bridging the gap between developmental systems theory and evolutionary developmental biology. *BioEssays*, 23(10):954–962.

⁶²Futuyma, D. J. (2015). *Can Modern Evolutionary Theory Explain Macroevolution?*, pages 29–85. Springer International Publishing.

⁶³Hains, B. (2014). *Dead or alive?* <https://aeon.co/essays/dead-or-alive-an-expert-roundtable-on-the-selfish-gene>. Accessed: 2024-07-01.

of evolution: there are just facts, hypotheses, and different focuses.

So, the main goal I would like to achieve is that of extending this once very fortunate perspective by incorporating the centrality of transmissible genetic variants across generations, typical of population genetics, with the critical importance of environmentally-induced changes in developmental pathways, including the feedback loops between these two components. By doing this, I would show that an evolutionary logic focused on “simple” DNA inheritance across multiple generations is not necessarily ignorant of all effects of the environment on developmental processes involved in each individual’s lifetime. In a nutshell, looking at evolution from genes’ point of view does not imply an underestimation of organisms and their complexity, at least if one keeps well in mind the profound relation between the genome, environmental cues, and development, even including whole life cycles (Fusco, 2019)⁶⁴.

⁶⁴Fusco, G. (2019). Evo-devo beyond development: The evolution of life cycles, pages 309–318.

Chapter 2

Phenotypic plasticity, or the ability to respond to the environment

Introduction

Although it is quite common in this discipline, many terms which are frequently used by biologists, like the fundamental concept of organism, still lack a well-defined, universally recognised meaning (Wilson, 2000)¹. Some other notions, such as dominant, that is, a widely used term in classical genetics, were reported to be even misleading in educational practice (Donovan, 1997)². Both the key concepts of this chapter, i.e., *genotype* and *phenotype*, are more than a century old and, although they are often used in technical contexts, they show a very high semantic stratification (Mahner and Kary, 1997)³. Some of the main influences on the diversification of their definitions came from the molecular approach flourishing around the mid-20th century, whose contribution reduced the original abstraction of classical genetics by focusing on the functional relationship between DNA, on the one side, and proteins and enzymes, on the other, leading to the successful integration between genetics and biochemistry that laid the foundations of a new research area (Morange, 2020, p.7)⁴.

¹Wilson, J. A. (2000). Ontological butchery: Organism concepts and biological generalizations. *Philosophy of Science*, 67(S3):S301– S311.

²Donovan, M. P. (1997). The vocabulary of biology and the problem of semantics: “dominant”, “recessive”, and the puzzling role of alleles. *Journal of College Science Teaching*, 6(26):381–382.

³Mahner, M. and Kary, M. (1997). What exactly are genomes, genotypes and phenotypes? and what about phenomes? *Journal of Theoretical Biology*, 186(1):55–63.

⁴Morange, M. (2020). *The black box of biology*. Harvard University Press, Cambridge.

Even the identification of a clear distinction between genotype and phenotype has been considered a serious, yet often neglected philosophical issue (Taylor and Lewontin, 2021)⁵, heavily relying on the boundaries of the above-mentioned definitions. However, the “conceptual armour” necessary to get through this and the following chapters does not include reaching this level of detail. What is needed to understand the next steps of this work are just some practical, even imperfect definitions, as long as they are widely used and understood by present-day researchers, so they may be used as a basic repertoire to build up an argument dealing with the deep relation between these two biological “types”. In particular, I decided to rely on some essential and well-recognised definitions borrowed directly from the fields of molecular and population genetics.

In a nutshell, the full set of genes of an individual is its genotype. Usually, as well as in the context of this thesis, this term is also used to refer to the genetic composition of an organism at a specific position on a chromosome (i.e., a locus) (Futuyma, 2017)⁶. The physical or biochemical expression of the genotype, that is usually influenced by subsets of genes and by environmental inputs, is called the phenotype. The phenotype can also be intended as the appearance of each individual (Elston et al., 2011; Hartl, 2020, p.2)⁷⁸. The complex layer of entities and processes lying between genotype and phenotype is one of the cornerstones of life. Consequently, it is a hot topic in all biological research as well. The same applies to the evolutionary side of this intricate interface.

Present-day biology, and more specifically *systems biology*, is deeply involved in the search for connections between the upper (and multiple) phenotypic levels and the lower level corresponding to the genotype. The main objective of important biological databases like KEGG (*Kyoto Encyclopedia of Genes and Genomes*) is to establish links between the genome and high-level functions and processes involving cells and organisms, from the molecular level up-

⁵Taylor, P. and Lewontin, R. (2021). The Genotype/Phenotype Distinction. In Zalta, E. N., editor, *The Stanford Encyclopedia of Philosophy*. Metaphysics Research Lab, Stanford University, Summer 2021 edition.

⁶Futuyma, D. J. (2017). *Evolution*. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts, fourth edition edition.

⁷Elston, R. C., Satagopan, J. M., and Sun, S. (2011). *Genetic Terminology*, pages 1–9. Humana Press.

⁸Hartl, D. L. (2020). *A primer of population genetics and genomics*. Oxford University Press, Oxford, fourth edition edition.

wards (Kanehisa et al., 2015)⁹. In this area of knowledge, the need for a combination between a meticulous investigation of single microscopic mechanisms and a multi-level approach looking at biological systems as a whole is clear, also including the succession of different physiological and developmental stages experienced by such systems over time (Markowetz and Boutros, 2020, p.139)¹⁰.

Let us consider for a moment the aforementioned database named KEGG. Even the simplest symbolic depiction of the interactions among genes and their products underlying the emerging behaviour of a cell (i.e., a biological *pathway*) strongly suggests that the relationship between genotype and phenotype is far from being one-to-one. The so-called *genotype-phenotype map* is a conceptual model that highlights this complexity. It was originally proposed as a mathematical function mapping genotype onto the phenotype, and it can be interpreted as the theoretically possible distribution of phenotypic variability generated by a set of genotypes (Alberch, 1991; Jaeger and Monk, 2014)¹¹¹².

Phenotypic plasticity is a property of the genotype-phenotype map. It is the capacity of a genotype to produce different phenotypes in response to environmental changes, both in a continuous or in a discrete way. A continuous variation is represented by the *reaction norm*, while discrete developmental changes are called *polyphenisms*. The reaction norm is the full spectrum of all possible phenotypic outputs (e.g., the height of a plant) resulting from a given genotype, whereas an example of polyphenism is the binary condition of presence or absence of a specific structure (e.g., wings

⁹Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. (2015). Kegg as a reference resource for gene and protein annotation. *Nucleic Acids Research*, 44(D1):D457–D462.

¹⁰Markowetz, F. and Boutros, M., editors (2020). *Systems genetics*. Cambridge series in systems genetics. Cambridge University Press, Cambridge.

¹¹Alberch, P. (1991). From genes to phenotype: dynamical systems and evolvability. *Genetica*, 84(1):5–11.

¹²Jaeger, J. and Monk, N. (2014). Bioattractors: dynamical systems theory and the evolution of regulatory processes. *The Journal of Physiology*, 592(11):2267–2281.

in an insect) (Pigliucci, 2001; Fusco and Minelli, 2010; Lachowiec et al., 2015; Nijhout et al., 2017)¹³¹⁴¹⁵¹⁶. For a visual example of a reaction norm, see Figure 2.1. Plasticity can affect any kind of characteristic, ranging from physiological to behavioural traits. It can be *adaptive*, *maladaptive*, or *neutral*, depending on whether it has positive, negative, or no consequences on *fitness*, that is, the success of an organism in terms of offspring produced. Furthermore, plastic responses induced by the environment during an organism’s lifetime can be either reversible or irreversible (Jebali et al., 2022)¹⁷.

¹³Pigliucci, M. (2001). Phenotypic plasticity. Syntheses in ecology and evolution. Johns Hopkins University Press, Baltimore.

¹⁴Fusco, G. and Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1540):547– 556.

¹⁵Lachowiec, J., Queitsch, C., and Kliebenstein, D. J. (2015). Molecular mechanisms governing differential robustness of development and environmental responses in plants. Annals of Botany, 117(5):795–809.

¹⁶Nijhout, H. F., Sadre-Marandi, F., Best, J., and Reed, M. C. (2017). Systems biology of phenotypic robustness and plasticity. Integrative and Comparative Biology, 57(2):171–184.

¹⁷Jebali, A., Sanchez, M. R., Hanschen, E. R., Starkenburg, S. R., and Corcoran, A. A. (2022). Trait drift in microalgae and applications for strain improvement. Biotechnology Advances, 60:108034.

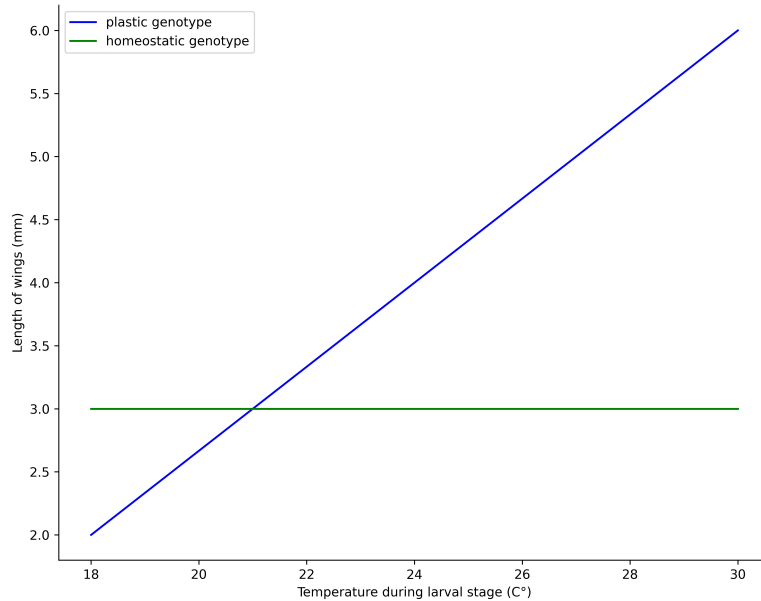


FIGURE 2.1: Two reaction norms referred to a hypothetical trait of an animal species, e.g., an *emimetabolous* insect with wing development following the larval stage, like dragonflies. In green colour, a flat reaction norm (i.e., the “deterministic” genotype). In blue colour, a plastic reaction norm (i.e., with slope $\neq 0$).

The mechanisms underlying plastic responses are multiple, not entirely clear, and still under investigation. Nevertheless, some of these mechanisms are already acknowledged in the literature. The most important one is regulation of gene expression via DNA transcription into mRNA (that has already been introduced in Chapter 1). Indeed, environmental factors can affect hormones or other signalling molecules inducing transcriptional changes. These changes modify the expression of genes (Futuyma, 2017, p.393)¹⁸. These basic dynamics were also shown by some recent studies, for instance, researchers found that alternative phenotypes within the same species, e.g., seasonal phenotypes in an Afrotropical butterfly, were related to remarkable differences in gene expression (Oostra et al., 2018)¹⁹. As a consequence of similar findings, that have been

¹⁸Futuyma, D. J. (2017). Evolution. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts, fourth edition edition.

¹⁹Oostra, V., Saastamoinen, M., Zwaan, B. J., and Wheat, C. W. (2018). Strong phenotypic

accumulating for a long time, some authors proposed the expression “*plasticity genes*” to refer to regulatory regions of the genome depending on environmental stimuli and controlling the transcription of structural genes (Schlichting and Pigliucci, 1993)²⁰. Since mutations can also occur in plasticity genes (like in any other gene), genetic variation in these regions is a highly plausible evolutionary explanation of plastic responses. Such dynamics are clear when looking at the effects of single-nucleotide mutations in *promoters* (i.e., regulatory elements dedicated to binding with RNA polymerase, an enzyme catalysing transcription) of *Saccharomyces cerevisiae* yeast. In this case study, mutations were shown to be critical in influencing the level of environmentally-induced plasticity of the structural genes linked to the promoter under analysis (Duveau et al., 2017)²¹. Among the other factors regulating plasticity, post-transcriptional events and some other *epigenetic* changes, that is, affecting gene expression rather than DNA sequences (e.g., *methylation*) may also play an important role (Peltier et al., 2018)²². Finally, some structural genes representing critical nodes within a gene regulatory network can also behave as developmental switches, i.e., they can induce irreversible decisions in development in response to environmental stimuli. Most importantly, some mutations in these genes were proven to modulate, or even repress, plasticity (Sieriebriennikov et al., 2018; Dardiry et al., 2023)^{23,24}. The dramatic importance of one of these key genes is investigated in detail in Chapter 5.

After this overview of the molecular causes of plasticity, let us focus on more general issues. Once again, it is important to keep in

plasticity limits potential for evolutionary responses to climate change. *Nature Communications*, 9(1).

²⁰Schlichting, C. D. and Pigliucci, M. (1993). Control of phenotypic plasticity via regulatory genes. *The American Naturalist*, 142(2):366–370.

²¹Duveau, F., Yuan, D. C., Metzger, B. P. H., Hodgins-Davis, A., and Wittkopp, P. J. (2017). Effects of mutation and selection on plasticity of a promoter activity in *saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*, 114(52).

²²Peltier, E., Sharma, V., Marti Raga, M., Roncoroni, M., Bernard, M., Jiranek, V., Gibon, Y., and Marullo, P. (2018). Dissection of the molecular bases of genotype x environment interactions: a study of phenotypic plasticity of *saccharomyces cerevisiae* in grape juices. *BMC Genomics*, 19(1).

²³Sieriebriennikov, B., Prabh, N., Dardiry, M., Witte, H., Roseler, W., Kieninger, M. R., Rodelsperger, C., and Sommer, R. J. (2018). A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. *Cell Reports*, 23(10):2835–2843.e4.

²⁴Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

mind the coexistence between environmental responsiveness and stable, “non-reactive” states. By definition, phenotypic plasticity can be regarded as a particular case of the former tendency, whereas the latter is largely corresponding to a condition known as *environmental homeostasis*. Environmental homeostasis is the production of fixed phenotypic responses, regardless of stimuli which are independent of the genetic structure of the organism. Since this kind of homeostasis makes the developmental system resilient to the environmental changes, it may be thought as the inverse of plasticity (Pigliucci, 2010b, pp.358-359)²⁵. Both capacities may be experienced by the same organism, when looking at different phenotypic characteristics.

As mentioned in the previous chapter (for instance, when talking about cell membranes and gene expression regulation), the interpenetration between resilience and flexibility seems to be common to all biological systems. Indeed, the mechanisms underlying fixed and plastic responses to the environment may be studied by focusing on the *current* conditions of the organisms showing such responses. However, this kind of investigation could only provide a partial perspective on the “dialectic” between plasticity and homeostasis.

If paleontologists of the past had conducted their research based solely on the current configuration of the biosphere, that is, ignoring the necessity of providing a narrative of changes to phenotypes and their functions over time and among taxonomic groups (Jablonski and Shubin, 2015)²⁶, the fixist perspective prevailing until the 19th century would never have been challenged. That approach is still highly valuable today. The complex relationship between genotype and phenotype can be better understood only if one looks at its variation over time, as well as at the evolutionary processes inducing that variation. Given a proper interval of time and some contextual conditions, homeostasis can emerge from plasticity and, conversely, plasticity can emerge from homeostasis.

For this reason, the main aim of this chapter is to emphasise strongly the evolutionary side of the interplay between genotype and phenotype, highlighting how the layer between them can be rewired in many alternative, surprising ways. All the following pages are

²⁵Pigliucci, M. (2010a). *Evolution, the Extended Synthesis*. The MIT Press Ser. MIT Press, Cambridge.

²⁶Jablonski, D. and Shubin, N. H. (2015). The future of the fossil record: Paleontology in the 21st century. *Proceedings of the National Academy of Sciences*, 112(16):4852–4858.

preliminary to understanding how deep is the connection between a gene-focused view and a higher-level, developmental one, and why it is crucial to think about a new theoretical perspective aimed at combining the existent ones.

Section 2.1 is a historical overview including information from the first intuitions about a possible distinction between genotype and phenotype to the current status of the debate on the emergence, role, and relevance of phenotypic plasticity in the evolution of life on Earth.

Section 2.2 is a general overview of the numerous case studies involving phenotypic plasticity in nature. All information is extracted from the recent literature and referred to the most diverse taxonomic groups, for instance, animals (both vertebrates and invertebrates) and single-cell *eukaryotes*. Note that no specific statistical criteria were used to select the studies reported in this section.

In Section 2.3, I talk about the evolution of plastic responses, focusing on case studies involving the passage from plastic to homeostatic states, and on the reverse process. Both these dynamics are of particular interest for what is presented in the following chapters.

2.1 Genotype, phenotype, and their interplay: a very concise historical overview

The history of genotype, phenotype, and the complex layer connecting them, as it is often the case with scientific notions, is a very long and articulated one. For this reason, it would be virtually impossible to provide the reader with a comprehensive perspective of this history, or even with some good approximations of that. Nevertheless, knowing some of the most relevant parts of it is critical to place correctly the central topic of this work within its broader theoretical context.

The idea of the existence of a distinction between an inherited source of biological information and the “construction” of an organism based on that source precedes by far the origin of the words which would be later used to distinguish these two levels. The fact that this idea dates back to antiquity can be easily explained considering the foundational role it has played in life sciences from their very origins. Indeed, it could be reasonably claimed that the his-

tory of such idea spans most of the history of genetics itself, although they are not completely overlapping. In fact, the theory of biological inheritance was originally included, together with concepts more related with current reproductive biology, in a larger field that the ancient Greeks used to call *generation* (Stubbe, 1972, p.12)²⁷.

The first, notable debate emerging from this heterogeneous area of speculation arose from the opposition between Hippocrates (460-377 p.e.v.) and Aristotle (384-322 p.e.v.) (Mayr, 1982, p.635)²⁸, who were both formulating hypotheses on the transmission of traits between parents and their offspring. Hippocrates promoted a hypothesis which would be later known as *pangenesi*s: a seminal material coming *directly* from each anatomical district would converge, after fertilisation, from parents to the fetus, endowing him with the parental characteristics. The Aristotelian criticism to this view opened a breach in the previous “unitary” vision of biological generation. The counterexample proposed by the philosopher was both simple and meaningful at the same time: how is a direct and continuous transmission of characteristics from parental to filial organs possible, given the evident cases where the offspring shows characteristics that parents had not yet developed at the moment of fertilisation, such as grey hair or baldness? Or, on the other hand, how is it possible that progeny does not show some characteristics that parents already had at the moment of fertilisation, such as mutilations (Sturtevant, 2001, p.1)²⁹? The core of the theoretical separation of inheritance into two different levels was already traceable in these arguments.

In the Middle Ages, the Dominican friar and prominent naturalist Albert the Great noticed the importance of environmental variables in influencing the growth path of plants from seed to adult phase, accounting for the observable variation between wild and cultivated strains (Stubbe, 1972, p.64)³⁰. Although he generally underestimated the hereditary side of the issue, he was also one of those who highlighted the relevance of the ecological context in affecting widely the individual development. However, as for the issue of the

²⁷Stubbe, H. (1972). History of genetics. MIT Press, Cambridge, Mass.

²⁸Mayr, E. (1982). The growth of biological thought. Belknap Press of Harvard Univ.Press, Cambridge, Mass.

²⁹Sturtevant, A. H. (2001). A history of genetics. Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY. Previously published: New York : Harper & Row, 1965.

³⁰Stubbe, H. (1972). History of genetics. MIT Press, Cambridge, Mass.

separation between the informational level and the level of formation of bodies, it would have taken several other centuries before becoming a topic of scientific debate again.

Charles Darwin, lacking information on the mechanisms generating inherited variation (that is the “raw material” of the process of natural selection), and a view on inheritance based on discrete entities, supported the old theory of pangenesis and the Lamarckian hypothesis of inheritance of acquired characteristics (Charlesworth and Charlesworth, 2009)³¹. Conversely, the first scientist who grasped the duality between the aforementioned biological levels was probably Gregor Mendel (1822-1884) (Pigliucci, 2001, p.2)³². With his research, he suggested the separation between hereditary “elements” and visible characteristics, to the point that such distinction would soon become the implicit prerequisite of the whole, newly arisen field of genetics.

That conceptual split was made even clearer by August Weismann’s (1834-1914) reflections and experiments. The most influential one was the sequential cut of mice’s tails for 22 inbred generations, which did not cause any reduction of newborns’ tail size (Sturtevant, 2001, p.18)³³, as it would be expected within the pangenesis (or Lamarckian) framework. This experiment fueled the belief in the separation between biological inter-generational material (the *germ line cells*) and the intra-generational one (the *somatic line*). More drastically, in some of his statements, Weismann was close to asserting that development was regulated by a genetic program (Mayr, 1982, p.702)³⁴. Apart from all the details, this handful of elements together suggests very clearly the key role of Weismann in establishing the dichotomy between inherited information and physical formation of the organism. Moreover, his work strongly influenced the following theoretical speculations, probably including those of Wilhelm Johannsen (1857-1927) (Pigliucci, 2001, p.48)³⁵.

From a scientific point of view, Johannsen truly represents a

³¹Charlesworth, B. and Charlesworth, D. (2009). Darwin and genetics. *Genetics*, 183(3):757–766.

³²Pigliucci, M. (2001). Phenotypic plasticity. *Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore.

³³Sturtevant, A. H. (2001). A history of genetics. Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY. Previously published: New York : Harper & Row, 1965.

³⁴Mayr, E. (1982). The growth of biological thought. Belknap Press of Harvard Univ.Press, Cambridge, Mass.

³⁵Pigliucci, M. (2001). Phenotypic plasticity. *Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore, Md.

pioneering personality. He was a botanist endowed with both a scientific and a humanistic culture, and he coined some words that would have become an integral part of 20th century biology: not just the strikingly famous “gene”, but also “genotype”, and “phenotype”. The appreciation he expressed towards Aristotle in his paper *On the soul of plants*, published in 1889 (Roll-Hansen, 2008)³⁶, is of particular interest from the historical point of view, since it evokes the ancient Greek “roots” of the inheritance issue. Furthermore, as was also the case for Albert the Great, plants were again central in the discussion on the relative contributions of heredity and the environment in the formation of traits. However, if the influence of proto-genetic Aristotelian ideas on the Danish botanist remains nothing but conjectural, the contribution of the rediscovered Mendelism from the early 20th century, instead, is clearly ascertainable, as Mendelian laws and experimental results were important reasons to believe in the existence of a stable genotype beneath all the visible variations shown by organisms. At that point, the challenge for scientists like Johannsen and his contemporaries was precisely to distinguish between hereditary and non-hereditary variation (Roll-Hansen, 2008)³⁷.

From then on, in particular after the formulation of the *central dogma of molecular biology* by the DNA discoverer Francis Crick (Crick, 1958)³⁸ (i.e., the flux of biological information is one-way, from genes up to proteins), the existence of some kind of conceptual separation between genotype and phenotype, at least for purely operational purposes, has never been seriously questioned. However, the debate on the complexity of the “black box” between genotype and phenotype and its evolutionary change was already emerging at the end of the 19th century, when psychologist James Baldwin (2017)³⁹ started talking about the interaction between behavioural phenotypes and genotypes from a Darwinian perspective, proposing the evolution of fixed modules from an ancestral plastic state (I discuss more in detail his hypothesis in Section 2.3). The awareness of the existence of such complexity was inevitably amplified in

³⁶Roll-Hansen, N. (2008). Sources of Wilhelm Johannsen’s genotype theory. *Journal of the History of Biology*, 42(3):457–493.

³⁷Roll-Hansen, N. (2008). Sources of wilhelm johannsen’s genotype theory. *Journal of the History of Biology*, 42(3):457–493.

³⁸Crick, F. H. (1958). On protein synthesis. *Symposia of the Society for Experimental Biology*, (12):138–163.

³⁹Baldwin, J. M. (2017). A new factor in evolution. *Diacronia*, (7).

a remarkable way during the first decades of the 20th century, as more and more extensions to classical Mendelian inheritance were discovered. Most importantly, the discovery of *epistasis*, that is the interaction of several (if not many) products of different genes in the construction of a single phenotypic characteristics, contributed to the recognition of the non-biunivocal relationship between genes and traits (Bateson, 2010)⁴⁰.

Another cornerstone of this debate was established in 1909, when Richard Woltereck introduced the previously discussed concept of reaction norm. It was such an important step forward that one of the main founders of the Evolutionary Modern Synthesis, Theodosius Dobzhansky, claimed that heredity was all about the transmission of reaction norms, i.e., spectra of developmental possibilities encoded in the genome, rather than about the transmission of well-defined and fixed characteristics. In the following decades Conrad Waddington, following the Ukrainian zoologist Ivan Schmalhausen, introduced new concepts like that of *genetic assimilation*, i.e., a Baldwin-like process based on natural selection and minimising the effects of environmental noise on a trait (Waddington, 1942; Futuyma, 2021)^{41,42}.

Two decades later, in 1965, Anthony Bradshaw highlighted that the stability of traits can be downregulated or upregulated through a selection process. Consequently to this conclusion, which came to his mind by reviewing a significant amount of experiments on the *artificial* selection of plants and *Drosophila* fruitflies, Bradshaw also began to wonder how *natural* selection could shape this degree of stability in natural populations. In his valuable scientific works, he emphasised many general and fundamental issues: the *genetic control* of plasticity, the conditions favouring its evolution, and the diversity of plastic responses (Pigliucci, 2001; Futuyma, 2021, pp.53-58)^{43,44}. For all these reasons, he can be easily regarded as one of the most crucial authors of this historical digression.

⁴⁰Bateson, W. (2010). Mendel's principles of heredity. Dover Publications.

⁴¹Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature*, 150(3811):563–565.

⁴²Futuyma, D. J. (2021). How Does Phenotypic Plasticity Fit into Evolutionary Theory?, pages 349–366. CRC Press.

⁴³Pigliucci, M. (2001). Phenotypic plasticity. *Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore, Md.

⁴⁴Futuyma, D. J. (2021). How Does Phenotypic Plasticity Fit into Evolutionary Theory?, pages 349–366. CRC Press.

Since 1980s, it has been well-acknowledged that many plastic responses are the result of natural selection, and some highly influential quantitative genetics models have been designed to explain these evolutionary phenomena, e.g., by Via and Lande (1985)⁴⁵. Finally, more recent studies showed that *adaptations* to new environments can happen both by increasing or decreasing plasticity, depending on plasticity-related costs on the individual’s survival and reproduction (Scheiner et al., 2017; Futuyma, 2021)^{46,47}. This is another critical achievement. Thus, in line with Dobzhansky’s expectations, the view of a biological world dominated by a diffused interaction, inheritance, and change over time of a multitude of reaction norms seems to be supported by empirical data, combining genotype and phenotype in a much more complex way than it was thought in the past.

This non-exhaustive section had the only aim to depict an overall picture of the extremely articulated history of genotype, phenotype, and phenotypic plasticity. But little has been said yet on more specific case studies across different kingdoms and domains of life. Actually, this is the aim of the next section of the chapter.

2.2 The striking diffusion of plastic responses across taxonomic groups

A conspicuous number of characteristics and processes is frequently mentioned in discussions concerning the definition of the word “life”: the presence of cells and metabolism, DNA replication, growth, and several others. Considering their extensive diffusion in so many different groups of organisms, environmentally-induced responses fully deserve to be in a prominent place of the same list. In particular, this flexibility is made possible by another general feature of biological systems. As a matter of fact, differently from basic physical interactions between bodies, where the distance between action and reaction is null (for Newton’s third law of motion), living entities are

⁴⁵Via, S. and Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39(3):505.

⁴⁶Scheiner, S. M., Barfield, M., and Holt, R. D. (2017). The genetics of phenotypic plasticity. xv. genetic assimilation, the baldwin effect, and evolutionary rescue. *Ecology and Evolution*, 7(21):8788–8803.

⁴⁷Futuyma, D. J. (2021). *How Does Phenotypic Plasticity Fit into Evolutionary Theory?*, pages 349–366. CRC Press.

characterised by the increased distance between the biological counterparts of action and reaction, i.e., *stimulus* and *response*. Such distance is crucial to enable the manipulation of genetic information through multiple layers and steps resulting in the final, visible outcomes. Furthermore, it allows the environment to have a significant impact on this complex process of conversion. This impact is frequently observed in a wide variety of species.

Indeed, environmentally-induced responses are easily found by looking at the countless cases of plastic traits exhibited by the most diverse organisms: from algae to birds, from crustaceans to bacteria, and so on. For this reason, the regulation of environmental reactivity is a long-lasting evolutionary challenge for most, if not all, living beings. As shown by the following paragraphs, it affects a wide range of functions, from molecular to behavioural ones, deeply influencing sex, predation, nesting, survival, communication, and many other facets of life.

Nutrient intake and quality are very common environmental stimuli, capable of changing organisms dramatically, as shown by human obesity and muscle hypertrophy. This also applies to many insect species. The Asian brown planthopper shows facultative wing growth in relation to the concentration of glucose in rice plants. Since glucose is involved in the so-called *insulin pathway*, this phenomenon can be explained by assuming the presence of a discrete plastic condition (i.e., a polyphenism) depending on hormone regulation. Indeed, glucose concentration of the host plant, which was artificially regulated by researchers to test this hypothesis, was shown to influence the development of planthoppers, inducing either the short-winged (reproductive) morph or the long-winged (migratory) one in relation to plants growth. In nature, such plants move from lower to higher sugar concentration as approaching to senescence, which is the best time for insects to escape and colonise new areas. This is consistent with the finding that higher glucose concentration induces long-winged individuals, whereas lower concentration promotes the development of short-winged planthoppers. Thus, in this case, plasticity is easily identified as a surprisingly adaptive feature (Lin et al., 2018)⁴⁸.

However, one of the best-known case studies involving insects is

⁴⁸Lin, X., Xu, Y., Jiang, J., Lavine, M., and Lavine, L. C. (2018). Host quality induces phenotypic plasticity in a wing polyphenic insect. *Proceedings of the National Academy of Sciences*, 115(29):7563– 7568.

undoubtedly that of honey bees (*Apis mellifera*) caste differentiation based on a single genotype. Food concentration is recognised as one of the variables influencing the development of a female larva either into a sterile worker or into a long-lived fertile queen. The two types are morphologically divergent for several characteristics (in the first place, body size). As in the case of planthoppers, a dietary input is crucial to induce this switch. More specifically, some researchers claimed a primary role for the protein Royalactine, one of the components of royal jelly, in order to explain the whole process of differentiation. Nevertheless, this is a much more debated topic than planthoppers wing polyphenism. The Royalactine primacy has been criticised in favour of more comprehensive explanations of a very complex phenomenon (Maleszka, 2018)⁴⁹, which shows that identifying the causes of phenotypic plasticity is often far from being a simple task.

Sometimes, such explanations must be carefully sought in several processes occurring at the molecular level. Another insect species, which is closely related to bees and whose societies are also divided in castes, the bumble bee *Bombus terrestris*, shows increased phenotypic diversity due to a process known as *alternative splicing*, that is a mechanism producing different proteins based on the same DNA sequence whose *non-coding* regions are removed in such a way as to remove some interposed *coding* sequences as well (Price et al., 2018)⁵⁰.

Arthropods (i.e., insects, spiders, centipedes, millipedes, scorpions, and crustaceans) are an inexhaustible source of examples of plasticity. In the geophilomorph centipede *Strigamia maritima*, all body segments are formed during embryonic development. Thus, environmental factors influencing segments formation must act at this stage. Temperature was found to be pivotal in determining the total number of segments of the centipede (Vedel et al., 2008)⁵¹. This study is of particular interest, not only because it regards a complex developmental characteristic, but also because its conclu-

⁴⁹Maleszka, R. (2018). Beyond royalactin and a master inducer explanation of phenotypic plasticity in honey bees. *Communications Biology*, 1(1).

⁵⁰Price, J., Harrison, M. C., Hammond, R. L., Adams, S., Gutierrez- Marcos, J. F., and Mallon, E. B. (2018). Alternative splicing associated with phenotypic plasticity in the bumble bee *bombus terrestris*. *Molecular Ecology*, 27(4):1036–1043.

⁵¹Vedel, V., Chipman, A. D., Akam, M., and Arthur, W. (2008). Temperature-dependent plasticity of segment number in an arthropod species: the centipede *strigamia maritima*. *Evolution & Development*, 10(4):487–492.

sions could be perhaps extended to other related groups, since this is probably the first demonstration of temperature-dependent segment number plasticity in any arthropod. More specifically, temperature and segment numbers were found to be positively related.

Plasticity can be also considered a key component of *sexual selection*, more specifically, when considering mate choice by females. For instance, body size, that is often a critical feature of both intra and inter-sexual competition, is frequently context-dependent. Here again, temperature is one of the main variables influencing this morphological trait, with males typically exhibiting a greater sensitiveness in body size variation than females, as shown by *Callosobruchus maculatus* beetles (García-Roa et al., 2020)⁵².

All the cases mentioned so far should not lead the reader to think that plasticity is limited to insects and their related groups alone. Molluscs, along with arthropods, are another great invertebrate phylum showing cases of phenotypic plasticity. Just to mention one such case, temperature was shown to be an important factor in the regulation of physiological and biochemical processes of *Helix lucorum* land snails. Researchers carried out a mutual population exchange between two different populations living at different altitudes, then checked changes induced by temperature (and altitude) in the expression of some protein groups indicating cellular stress response. The results of the analysis supported a phenotypic plasticity of most of these biochemical factors as a response to environmental stress induced by each habitat (Staikou et al., 2021)⁵³.

Moving towards aquatic environments, another important case study in this research area is represented by the small crustacean *Daphnia pulex*, which has been noted for experiencing age-related phenotypic plasticity. Juvenile individuals are normally subject to predation by insects' larvae, and the chemical signals from predators induce the development of defense devices called "neckteeth". A temperature increase was used by researchers to slow down the development, which allowed them to find significant correlations between neckteeth induction and time spent in the vulnerable stage

⁵²García-Roa, R., García-Gonzalez, F., Noble, D. W., and Carazo, P. (2020). Temperature as a modulator of sexual selection. *Biological Reviews*, 95(6):1607–1629.

⁵³Staikou, A., Feidantsis, K., Gkanatsiou, O., Bibos, M. N., Hatzioannou, M., Storey, K. B., and Michaelidis, B. (2021). Seasonal cellular stress phenomena and phenotypic plasticity in land snail *Helix lucorum* populations from different altitudes. *Journal of Experimental Biology*, 224(24).

(Klintworth and von Elert, 2021)⁵⁴. This discovery suggests that life cycles and developmental rhythms should also be considered carefully when searching for plastic responses, which is one more step towards a better understanding of the big picture underlying plasticity.

As for vertebrates, cartilaginous fishes like sharks constitute another pool of meaningful examples, because they are a very old class showing well-preserved traits over many millions of years. The Port Jackson shark (*Heterodontus portusjacksoni*), a widely diffused species in Australian temperate waters, is an ideal case study to investigate intra-specific variation in *thermal tolerance*, i.e., the capacity to survive a normally lethal heat-stress (Norris and Hightower, 2000)⁵⁵. Shark eggs from two geographically distinct populations were reared in several temperature conditions. More specifically, present-day mean temperatures, predicted end-of-century temperatures, and reciprocal-cross conditions for each location. Researchers observed that this environmental inversion induced significant variations in the resting metabolic rates of juvenile individuals, suggesting the plasticity of this physiological characteristic (Gervais et al., 2020)⁵⁶.

Shifting again the focus from water to land, one of the most remarkable examples of phenotypic plasticity regards temperature-dependent sex determination in crocodiles, lizards, and turtles. This feature is critical in influencing the general behaviour of all these species. For instance, East Pacific green turtles (*Chelonia mydas*) nest-site selection was found to be relevant in influencing sex ratios, which could be a successful ability in order to face climate change through perhaps genetically-determined behavioural corrections. Indeed, since in this species higher temperature induces the production of female offspring, while lower temperature induces male offspring, the tendency to nest in shaded and cooler areas is probably favoured to keep balanced this fundamental aspect of phenotypic diversity (i.e., sexual diversification), preventing from extremely biased offspring (e.g., 100% females) (Heredero Saura et al.,

⁵⁴Klintworth, S. and von Elert, E. (2021). Effect of developmental time on chaoborus-induced phenotypic plasticity. *International Review of Hydrobiology*, 106(3–4):164–172.

⁵⁵Norris, C. E. and Hightower, L. E. (2000). The Heat Shock Response of Tropical and Desert Fish (genus *Poeciliopsis*), pages 231–243. Elsevier.

⁵⁶Gervais, C. R., Huvneers, C., Rummer, J. L., and Brown, C. (2020). Population variation in the thermal response to climate change reveals differing sensitivity in a benthic shark. *Global Change Biology*, 27(1):108–120.

2022)⁵⁷.

Some astonishing cases of phenotypic plasticity are also found in species whose life cycles involve multiple discrete developmental steps, such as the amphibians belonging to the Urodela order. In salamanders like *Triturus alpestris*, the potential of sexually mature individuals to keep juvenile morphs over time in response to environmental inputs is genetically inherited. This characteristic has relevant ecological implications, since it leads the same population towards a separation into subgroups based on the partitioning of food resources between “normal” individuals (which spend most of their lives out of the water) and *paedomorphic* individuals, which maintain the juvenile fish-like morph in adult age and spend their whole life underwater (Denoël et al., 2005)⁵⁸.

However, from a non-biologist point of view, bird songs are definitely more familiar than most of the previous cases. The plastic nature of these songs is a higher level manifestation of environmental sensitiveness. In fact, in this case, it involves important behavioural characteristics of very intelligent animals, allowing the diversification of their sounds into the so-called “dialects”. Learning process itself (on which dialects are based) can be considered a form of neural plasticity. Through learning, bird species can adjust their songs to the appropriate acoustic conditions for their area, which is a feature of huge importance for females, since they can choose males that are more fit for a particular habitat, leading to reproductive divergence and even to *speciation*, the emergence of new species (Slabbekoorn and Smith, 2002)⁵⁹.

Primates are not exempt from plasticity. Indeed, plasticity is a plausible explanation of their skull diversity. For instance, captive individuals of Japanese macaques are square-jawed with longer tooth rows than wild animals, showing a pattern resembling *sexual dimorphism* (i.e., anatomic differences between males and females). The former morph is masculinised, and researchers hypothesise that this may be due to the perturbations in the social environments (social plasticity), which are ultimately disrupting the balance of

⁵⁷Hereadero Saura, L., Janez-Escalada, L., L’opez Navas, J., Cordero, K., and Santidri’an Tomillo, P. (2022). Nest-site selection influences offspring sex ratio in green turtles, a species with temperature-dependent sex determination. *Climatic Change*, 170(3–4).

⁵⁸Denoël, M., Joly, P., and Whiteman, H. H. (2005). Evolutionary ecology of facultative paedomorphosis in newts and salamanders. *Biological Reviews*, 80(4):663–671.

⁵⁹Slabbekoorn, H. and Smith, T. B. (2002). Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 357(1420):493–503.

androgen hormones (Kamaluddin et al., 2019)⁶⁰.

Not only animals, but also plants show phenotypic plasticity. Plant height is a character influenced by both genetic factors and environmental variables. For instance, the diurnal temperature range (DTR) was proven to be important in regulating stem elongation. In a recent study, a population of *Sorghum bicolor*, a grass species cultivated to produce grain, was evaluated for its height in seven natural field conditions. Authors found that DTR was effective to change the effects of three genetic *loci* (Dw1, Dw3 and qHT7.1), focusing on the relation between environmental gradient and developmental stage (Mu et al., 2021)⁶¹.

Analogous mechanisms are also found in mushrooms. The Asian mushrooms of the species *Ganoderma sinense* show forms of plasticity related to both biotic and abiotic variables. In (Hapuarachchi, 2019)⁶², a set of specimens characterised by identical nucleotide sequences showed plastic responses induced by a wide variety of extrinsic factors, such as air pressure, elevation, nutrient concentrations, pH, vegetation types around, and several others.

Even simple, microscopic organisms show phenotypic plasticity for some of their traits. (Solari et al., 2011)⁶³ reported the evidence of phenotypic plasticity in four aquatic species of the order *Volvocales*, which are photosynthetic organisms endowed with a motor structure (i.e., *flagellum*). Populations of very different sizes were bred in environments which differed from the standard ones, then their characteristics were analysed by microscopy. When the considered species were cultivated in a low-nutrient medium, the unicellular ones developed smaller cells, while those characterised by a colonial condition (i.e., those belonging to the *Volvox* genus) increased collective flagellar fluid stirring, in order to enhance nutrient uptake. Both those cases support the presence of environmentally-induced responses.

⁶⁰Kamaluddin, S. N., Tanaka, M., Wakamori, H., Nishimura, T., and Ito, T. (2019). Phenotypic plasticity in the mandibular morphology of Japanese macaques: captive-wild comparison. *Royal Society Open Science*, 6(7):181382.

⁶¹Mu, Q., Guo, T., Li, X., and Yu, J. (2021). Phenotypic plasticity in plant height shaped by interaction between genetic loci and diurnal temperature range. *New Phytologist*, 233(4):1768–1779.

⁶²Hapuarachchi, K. (2019). High phenotypic plasticity of *Ganoderma sinense* (Ganodermataceae, Polyporales) in China. *Asian Journal of Mycology*, 2(1):1–47.

⁶³Solari, C. A., Drescher, K., Ganguly, S., Kessler, J. O., Michod, R. E., and Goldstein, R. E. (2011). Flagellar phenotypic plasticity in volvoclean algae correlates with phylogenetic number. *Journal of The Royal Society Interface*, 8(63):1409–1417.

Analogous cases were also found in asexual species: the rodent malaria parasite *Plasmodium chabaudi* changes its phenotype both in response to changes *of* host and changes *in* the host internal conditions. In (Birget et al., 2019)⁶⁴, authors altered red blood cells (where the parasite replicates) resource availability within the same host, then transferred parasites to new, unperturbed hosts to access the ability of the parasite to properly adjust its phenotype to the new conditions. By doing this, they found high responsiveness to frequent within-host changes.

As discussed in Chapter 1, even extremely basic, unicellular, and prokaryotic organisms like bacteria can experience phenotypic plasticity. *Pseudomonas aeruginosa* can regulate the expression of a cooperative trait, i.e., the production of pyoverdine, an iron-scavenging molecule, in response to the surrounding cell density. It was observed that, when put into an iron-rich environment, pyoverdine production per cell at higher cell densities decreased significantly, because of the substantial contribution of cooperative conspecifics (Kummerli et al., 2009)⁶⁵.

Finally, I mention another case study linking genotype and phenotype in a controlled way within a lab context, this time using a physical stressor (Matuła et al., 2019)⁶⁶. Researchers exposed *E. coli* bacteria to numerous collisions of *ZnO nanorods* (i.e., nanoscale objects used in nanotechnology), observing the phenotypic changes induced by the impact of nanorods on cell walls: in a nutshell, they found enhanced spherical shape, and a 30% increase in the average cell wall thickness. The immediateness of both the impact and the phenotypic responses induced by the stressor were, by themselves, remarkable proofs of environmentally-induced plasticity, since there was no time for gradual genetic adaptations.

The main aim of all the above-mentioned case studies was just to provide a small overview of the extraordinary diffusion of different

⁶⁴Birget, P. L. G., Prior, K. F., Savill, N. J., Steer, L., and Reece, S. E. (2019). Plasticity and genetic variation in traits underpinning asexual replication of the rodent malaria parasite, *Plasmodium chabaudi*. *Malaria Journal*, 18(1).

⁶⁵Kummerli, R., Jiricny, N., Clarke, L. S., West, S. A., and Griffin, A. S. (2009). Phenotypic plasticity of a cooperative behaviour in bacteria. *Journal of Evolutionary Biology*, 22(3):589–598.

⁶⁶Matuła, K., Richter, L., Janczuk-Richter, M., Nogala, W., Grzeszkowiak, M., Peplińska, B., Jurga, S., Wyroba, E., Suski, S., Bilski, H., Silesian, A., Bluysen, H. A. R., Derebecka, N., Wesoły, J., Łoś, J. M., Łoś, M., Decewicz, P., Dziewit, L., Paczesny, J., and Hołyst, R. (2019). Phenotypic plasticity of *Escherichia coli* upon exposure to physical stress induced by *ZnO* nanorods. *Scientific Reports*, 9(1).

forms of phenotypic plasticity in all domains of life. However, genotypes and traits showing a certain degree of plasticity, as any other product of evolution, should not be intended as “static” features of organisms. Indeed, a dynamic and inter-generational approach must be adopted to represent correctly the change over time of phenotypic variation induced by plastic responses. Therefore, the core question is: how do evolutionary forces shape plastic responses over time?

2.3 The evolutionary perspective: transitions between homeostasis and responsiveness

One of the most basic discoveries in evolutionary biology is that the current biodiversity is nothing but a very small fraction of the global biodiversity that has spread throughout the history of life, which would stand out if it were possible to trace that history backwards. Plastic responses of phenotypic traits change over time as well, since environmental reactivity may be either decreased or increased by evolutionary forces and dynamics. Indeed, a particular trait can approach a more constitutive (or “deterministic”) condition from a more flexible one, and vice-versa. Both these tendencies evolve through the same mechanisms regulating variation over time in any other aspect of an organism. In the first place, the elimination of phenotypes with reduced reproductive success, namely natural selection.

As it might be guessed, the intrinsic chronological nature of evolution complicates biological investigation considerably. As a matter of fact, for much of the discipline’s history, evolutionary patterns and processes have been studied adopting a descriptive approach only, due to the impossibility of replicating such processes and their outcomes under controlled conditions. Nevertheless, experimental evolutionary studies are an emerging area of great interest, even in the field (i.e., outside of laboratories) (Irschick and Reznick, 2009)⁶⁷. As for lab approaches, some successful and well-known research can be easily mentioned: for instance, the bottom-up approach used by Nobel Prize winning Frances Arnold for the synthesis of new en-

⁶⁷Irschick, D. J. and Reznick, D. (2009). Field Experiments, Introductions, and Experimental Evolution A Review and Practical Guide, pages 172–193. University of California Press.

zymes by applying artificial selection on bacteria, and the long-term evolution experiment carried out by Richard Lenski (Lenski, 2017)⁶⁸, which is based on the interplay between mutation and selection in *E. coli* colonies and is still ongoing, involving tens of thousands of generations following one another for three decades. The evolution of inherited environmental sensitiveness is based on the same fundamental mechanisms, and can be now studied over the long term as well, considering changes occurring over multiple generations of organisms.

Now, as mentioned earlier, there are two possible directions in the evolution of plasticity. One is from a plastic condition to a homeostatic one, the other one is quite the opposite, that is, from a homeostatic condition to a plastic one. In other words, evolutionary processes change the reactivity of a genotype, so it can be converted either in a variety of different phenotypic responses or in a single, fixed way. The dramatic importance of this topic in biology can also be guessed by looking at the age of the first scientific hypotheses which were closely related to this kind of dynamics. The most notable example dates back to the late 19th century, when philosopher and psychologist James Baldwin (Baldwin, 2017)⁶⁹ formulated a theoretical argument which was later called the *Baldwin effect* after him. Indeed, the Baldwin effect is an emblematic case of reciprocal causation between individual development and genetic inheritance, with a focus on behavioural characteristics and learning (that, as it was said before, is itself a form of phenotypic plasticity):

“Suppose a population encounters a new environmental condition, in which its old behavioral strategies are inappropriate. If some members of the population are plastic with respect to their behavioral program, and can acquire in the course of their lifetime new behavioral skills that fit their new surroundings, these plastic individuals will survive and reproduce at the expense of less flexible individuals. The population will then have the chance to produce mutations that cause organisms to exhibit the new optimal behavioral profile without the need for learning. Selection will favor these mutants, and in time the behaviors which

⁶⁸Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, 11(10):2181–2194.

⁶⁹Baldwin, J. M. (2017). A new factor in evolution. *Diacronia*, (7).

once had to be learned will be innate.” (Godfrey-Smith, 2003)⁷⁰.

In short, a Baldwinian process is basically the effect of natural selection acting on an ancestral, environmentally-induced phenotype and leading to the fixation of more advantageous “hard-wired” genotypes in the considered population. The Baldwin effect is very similar to another process proposed by Conrad Waddington (Waddington, 1953)⁷¹, the so-called *genetic assimilation*. Although several authors have criticised the actual correspondence between these two processes (Longa, 2006; Crispo, 2007)⁷²⁷³, the Baldwin effect and genetic assimilation may be considered largely corresponding for practical reasons (Futuyma, 2021)⁷⁴, so here I am talking about the Baldwin effect in a broad sense, including assimilation and other closely related concepts.

It is important to notice that, despite the appearances, processes of fixation of constitutive genotypes are far from being a new form of Lamarckism, that is, the pre-Darwinian doctrine stating the existence of an inter-generational transmission of traits acquired during an individual life cycle. Indeed, in the Baldwin effect, the overcoming of the plastic phase happens when the development of the considered trait is no longer dependent on an environmental input, that is a direct consequence of the Darwinian process of natural selection of a fixed genetic variant underlying that trait.

Since the Baldwin effect is a very complex, multi-step mechanism acting over time and generations, and since its outcomes can be obtained computationally in alternative ways, its frequency and biological significance have been seriously questioned by some scholars (Santos et al., 2015)⁷⁵. Nevertheless, some important case studies confirm that the Baldwin effect can explain some of the current

⁷⁰Godfrey-Smith, P. (2003). Between Baldwin Skepticism and Baldwin Boosterism, pages 53–68. The MIT Press.

⁷¹Waddington, C. H. (1953). Genetic assimilation of an acquired character. *Evolution*, 7(2):118–126.

⁷²Longa, V. M. (2006). A misconception about the baldwin effect: Implications for language evolution. *Folia Linguistica*, 40(3–4).

⁷³Crispo, E. (2007). The baldwin effect and genetic assimilation: Revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution*, 61(11):2469–2479.

⁷⁴Futuyma, D. J. (2021). How Does Phenotypic Plasticity Fit into Evolutionary Theory?, pages 349–366. CRC Press.

⁷⁵Santos, M., Szathmari, E., and Fontanari, J. F. (2015). Phenotypic plasticity, the baldwin effect, and the speeding up of evolution: The computational roots of an illusion. *Journal of Theoretical Biology*, 371:127–136.

evolutionary patterns observed in real animal populations: for instance, a systematic review on North-American finch maternal cares (Badyaev, 2009)⁷⁶ and, above all, a recent research investigating a famous model organism, the nematode worm *Caenorhabditis elegans* (Vigne et al., 2021)⁷⁷. The latter work is of particular interest, since its authors identify with precision the genetic mutation producing the constitutive (i.e., non-plastic) genotype. This mutation affects the gene *KCNL-1*, inducing a single amino acid change that provokes retention of eggs in the uterus and the consequent matricidal hatching, since it determines a drastic decrease in the environmental sensitiveness promoting egg-laying stimuli. In a nutrient-scarce environment, plasticity in hatching behaviour is adaptive, because worms can retain eggs inside their uterus facultatively, allowing their larvae to survive at the expense of their own survival. In other cases where the absence of food is stable over time, the originally plastic genotype may be replaced by the “deterministic” one as a form of genetic reinforcement.

This evolutionary trend is observed as well in some unicellular organisms, for instance, *Cyanobacteria*. Cyanobacteria are bacteria gaining their energy from photosynthesis, like plants do. The case study involving the filamentous cyanobacterium *Trichodesmium* is a recent research work of some relevance (Walworth et al., 2016)⁷⁸. This study investigates the genetic fixation of previously plastic biochemical responses in the context of natural habitats which are deeply influenced by human activity. In particular, the integration into the genome of ancestrally plastic responses was investigated considering increasing carbon dioxide levels (CO₂) in oceans. Researchers found genetic expression changes in genes and pathways involved in photosystems, transcriptional regulation, and other cell functions, which supported the hypothesis of a replacement of short-term plastic reactions to high CO₂ levels with more stable and inherited responses.

⁷⁶Badyaev, A. V. (2009). Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the baldwin effect. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1520):1125–1141.

⁷⁷Vigne, P., Gimond, C., Ferrari, C., Vielle, A., Hallin, J., Pino- Querido, A., El Mouridi, S., Mignerot, L., Frøkjær-Jensen, C., Boulin, T., Teotonio, H., and Braendle, C. (2021). A singlenucleotide change underlies the genetic assimilation of a plastic trait. *Science Advances*, 7(6).

⁷⁸Walworth, N. G., Lee, M. D., Fu, F.-X., Hutchins, D. A., and Webb, E. A. (2016). Molecular and physiological evidence of genetic assimilation to high CO₂ in the marine nitrogen fixer *Trichodesmium*. *Proceedings of the National Academy of Sciences*, 113(47).

However, genetic fixation of previously plastic traits is not limited to egg laying behaviour and adaptation of microorganisms to new biochemical conditions. In fact, similar processes have also been proposed as explanations of some crucial cognitive abilities, like those underlying human language. More specifically, several researchers hypothesised that an originally learned syntax may have become fixed due to genetic integration of genotypes reproducing the same outcomes without learning (Szabolcs et al., 2009)⁷⁹. At this regard, some scientists claim a key role for the Baldwin effect in the origin of language in *Homo sapiens* (Pinker and Bloom, 1990)⁸⁰, which would be one of the most significant examples of evolution of an ancestrally plastic trait into a fixed one.

The reverse process involving an ancestrally fixed trait that evolves towards increased phenotypic plasticity is the other side of the coin. This inverse polarity is commonly associated with the ability of organisms to keep surviving and reproducing into a new environment showing unprecedented challenges or, more specifically, with the necessity to deal with a changeable ecological context, characterised by rapid environmental fluctuations (Fierst, 2011)⁸¹ which do not favour the selection of a “specialised” genetic variant inducing the expression of a single, fixed phenotype. Phenotypic plasticity is *adaptive* when a plastic genotype increases the probability of producing a higher number of offspring compared to the corresponding “deterministic” genotype. In other words, plasticity is beneficial and may prevent extinction over a range of environments if the phenotypes produced by the plastic genotype have a high fitness across such environments, making the organism a “generalist” for that characteristic (Chevin et al., 2013)⁸².

It is interesting to notice that, while recent experimental studies have shown that plastic responses tend to reduce due to subsequent genetic mutations (as it happens with processes like the Baldwin ef-

⁷⁹Szabolcs, S., James R., H., Dorothy V. M., B., Terrence W., D., Francesco, d., Julia, F., Kazuo, O., Eors, S., and Stephanie A., W. (2009). What Are the Possible Biological and Genetic Foundations for Syntactic Phenomena?, pages 207–236. The MIT Press.

⁸⁰Pinker, S. and Bloom, P. (1990). Natural language and natural selection. Behavioral and Brain Sciences, 13(4):707–727.

⁸¹Fierst, J. L. (2011). A history of phenotypic plasticity accelerates adaptation to a new environment: Phenotypic plasticity accelerates adaptation. Journal of Evolutionary Biology, 24(9):1992–2001.

⁸²Chevin, L.-M., Gallet, R., Gomulkiewicz, R., Holt, R. D., and Fellous, S. (2013). Phenotypic plasticity in evolutionary rescue experiments. Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1610):20120089.

fect), it seems that an increased level of plasticity might be favoured as a mechanism of readaptation to ancestral environments, rather than as a basis for the adaptation to novel environments. This was tested with a reciprocal transplant experiment carried out using lowland and Tibetan chickens. The experiment informed about the prominent role of plastic responses (to the detriment of genetic adaptations) in the case of return of the organisms to their ancestral environments (Ho et al., 2020)⁸³.

Nevertheless, other experiments showed that colonisation (or invasion) of new environments is still one the main reasons why plasticity of some characters may evolve and be favoured by natural selection. Sessile (i.e., non-moving) organisms like plants are perfect candidates to observe the evolution of adaptive plasticity, since they cannot escape from adverse environments (i.e., they cannot circumvent the problem of survival by simply seeking a more favourable area). This was tested in the herbaceous plant *Centaurea stoebe*, where experiments simulating different climatic conditions showed the success, in terms of fitness, of plastic responses in traits associated, for example, with rapid growth (Hahn et al., 2012)⁸⁴.

Analogous research is found, among the other groups, in insects. Indeed, many of these studies were carried out on fruit flies, like those of the species *Drosophila simulans*, which were used as experimental subjects by (Mallard et al., 2020)⁸⁵. The authors examined 64 generations of flies. When a hot environment was used, plasticity in gene expression increased in 75% of all genes whose level of plasticity changed after the experiment, indicating that just a minority of them, instead, reduced their plasticity. Genes that increased their plasticity were key genes associated with the energetic metabolism (e.g., involved in *glycolysis*). In this case, it is important to point out that these traits were already plastic in the ancestral populations, but they evolved, at least partially due to selection, a greater environmental sensitiveness over time.

Although it is effective to show the *fact* of positive selection in favour of increased environmental reactivity, this very narrow

⁸³Ho, W.-C., Li, D., Zhu, Q., and Zhang, J. (2020). Phenotypic plasticity as a long-term memory easing readaptations to ancestral environments. *Science Advances*, 6(21).

⁸⁴Hahn, M. A., van Kleunen, M., and Muller-Scharer, H. (2012). Increased phenotypic plasticity to climate may have boosted the invasion success of polyploid *centaurea stoebe*. *PLoS ONE*, 7(11):e50284.

⁸⁵Mallard, F., Nolte, V., and Schlotterer, C. (2020). The evolution of phenotypic plasticity in response to temperature stress. *Genome Biology and Evolution*, 12(12):2429–2440.

specimen of studies is certainly not representative of the whole, as it may be easily inferred by looking at the number and variety of case studies summarised in Section 2.2. Indeed, the very existence (and persistence) of so many cases of plastic traits in nature is *itself* an indication of their plausible adaptive potential. Furthermore, as it will be shown in a more detailed way later, the evolution of phenotypic plasticity is currently the focus of a lot of mathematical and computational models, searching for the relevant conditions which have to be satisfied in order to select some forms of plasticity within a population (Lalejini and Ofria, 2016; Edelaar et al., 2017; King and Hadfield, 2019)⁸⁶⁸⁷⁸⁸. The flourishing of all these heterogeneous works is a sign of the scientific relevance of plasticity research, that was sometimes aimed at highlighting the theoretical and philosophical aspects underlying this biological property (Pigliucci, 2001, pp.253-261)⁸⁹.

The inherent richness of the topic, the constantly increasing amount of data from empirical research, and the need to broaden the theoretical scope of this important concept within the framework of the modern debate on evolutionary theory require an equally multifaceted treatment, like that proposed in the following chapters. What is really important to keep in mind for the next steps we are going to take is that phenotypic plasticity is a critical aspect of life evolving over time, and its evolution is more and more experimentally testable and computer-simulated. Mutation and natural selection are among the key factors influencing its change, which can happen in two opposite directions: either allowing environmental influences to affect the final phenotypic output, or strengthening constraints which keep phenotypes independent from contextual variations. These few notions are enough to introduce the following steps, where plasticity is considered as a more comprehensive, ubiquitous, even generalisable characteristic of life, operating on multiple levels, and readable from several and, to some extent, unexpected

⁸⁶Lalejini, A. and Ofria, C. (2016). The evolutionary origins of phenotypic plasticity. In Proceedings of the Artificial Life Conference 2016, ALIFE 2016. MIT Press.

⁸⁷Edelaar, P., Jovani, R., and Gomez-Mestre, I. (2017). Should i change or should i go? phenotypic plasticity and matching habitat choice in the adaptation to environmental heterogeneity. *The American Naturalist*, 190(4):506–520.

⁸⁸King, J. G. and Hadfield, J. D. (2019). The evolution of phenotypic plasticity when environments fluctuate in time and space. *Evolution Letters*, 3(1):15–27.

⁸⁹Pigliucci, M. (2001). Phenotypic plasticity. *Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore, Md.

perspectives, like those of philosophy of language (Chapter 3) and agent-based modelling (Chapters 4 and 5).

All the marvellous case studies mentioned so far are shades projected from a single, central “body” representing the evolution of flexibility in living systems. After this very concise examination of its biological basis, it is now time to explore this kind of phenomena on a higher, conceptual level, to the point that one could perhaps replace non-technical expressions such as “flexibility of life” with another non-technical, yet much more evocative expression such as “*polysemy* of life”.

Chapter 3

The polysemy of genes

Introduction

Phenotypic plasticity, that is, the capacity to produce different phenotypes based on the same genetic information in response to different environmental stimuli, is a widespread peculiarity of life. In addition to its ubiquity, this capacity is of dramatic importance when it comes to defining what a living being is. Indeed, environmental responsiveness is one of the reasons why organisms are *special* with respect to inanimate bodies, since this property is the result of the evolution of an intricate system of mediation between action and reaction, or stimulus and response. The previous chapter should be considered primarily as a short, introductory journey across some of the many manifestations of phenotypic plasticity in organisms belonging to the most diverse taxonomic groups. More specifically, some of the studies cited throughout the text were focusing on the evolutionary paths leading to the spread or repression of plastic responses in natural populations. If I have successfully put together at least some fragments of this colourful portrait, the crucial role of plasticity as a central characteristic shared across all domains of life should now be clear enough to the reader.

Other than being numerous, studies involving phenotypic plasticity and its evolution currently constitute a well-recognised area of biological research. Nevertheless, all data extracted from the literature and discussed in the previous pages still lack a common theoretical framework capable of placing them within a broader conceptual context. This is an important gap in biological thinking, which needs to be filled in order to provide a philosophical perspective that can

combine and clarify the salient aspects of this multiplicity of highly heterogeneous studies. I argue that such perspective may be based on a linguistic analogy.

A “linguistic way” of intending phenotypic plasticity can be easily grasped just by recalling its definition and the most basic characteristics of life: a code composed by a quaternary *alphabet* (i.e., the four letters identifying nucleotides, A, T, C, and G), is used to generate, according to certain biochemical rules, *words* and *expressions*, e.g., single or multiple genes, whose “*meanings*” are represented by proteins, enzymes, or higher-level traits such as eye colour, wing length, and countless others. The critical point is that the determination of these products depends on the way and the timing with which the code is read during development, which are influenced to some extent by contextual elements like the biotic or abiotic factors from the local environment. Indeed, diet, temperature, and many other variables affect, often in a remarkable way, molecular mechanisms of pivotal importance like gene transcription, changing the final phenotypic output. This multiplicity of related, yet alternative outcomes depending on a single genomic source is what I would call the *polysemy* of genes. Once again, all changes affecting these biological “meanings” over time are regulated by relatively simple evolutionary processes, like mutation and natural selection. This is a possible linguistic way of looking at biology (which, as I explain in one of the following sections, is not new) but, above all and more specifically, it is a linguistic way of looking at the evolution of environmental responsiveness, that is the core of this central chapter of the thesis.

I argue that this fundamental biological issue would highly benefit from the conceptual operation I propose here, at least for two different reasons: in the first place, the linguistic analogy may improve the understanding of this multifaceted biological phenomenon, making its functioning more intelligible for the readership without a sound biological background; no less importantly, a new bridge between biology and theory of language might promote the reciprocal exchange of contents and viewpoints, allowing a possible deepening of issues belonging to these areas of investigation by using the knowledge already available from both fields. Furthermore, the two theories could perhaps reveal to be related in a more profound way and maybe, in the future, a common substrate shared by plastic-

ity and polysemy could be found by interested scholars from both “factions”, who could then analyse the mechanisms underlying the flexibility of all types of languages of life as a unitary philosophical and scientific issue. However, regardless of this desirable outcome, I am convinced that the analogy may bring more clarity and scientific suggestions than possible confusion, that is sufficient motivation to decide to persist in this direction.

The coexistence of multiple meanings is the cornerstone of all the topics discussed in this chapter. First of all, this coexistence is clear in the concept of analogy, whose function is that of transferring meanings from a specific area to another one, e.g., from polysemy to phenotypic plasticity, and vice versa. But it is even clearer when considering polysemy itself, since it is the property of words or expressions owning different related meanings. Keeping in mind both elements, namely the characteristics of analogy (that is the cognitive tool I use for the following investigation) and the linguistic notion of context-dependent determination of meanings, my main aim is to talk about the composition of alternative biological traits in a different way, showing “familiar” phenomena under a new light. Moreover, although this chapter does not contain new experimental results and does not add new research data to the current knowledge on plasticity and polysemy, my aspiration is to go beyond the mere stylistic choice of representing established facts with an unusual terminology. Indeed, the following argument is an attempt to provide a philosophical framework to the way of looking at life I introduced in Chapter 1, combining the centrality of genes and natural selection with the effects of the environment on developmental pathways within the concept of *biological polysemy*.

In Section 3.1, I discuss in detail the role of analogy in human cognition and scientific research, highlighting both its heuristic potential and intrinsic limitations.

In Section 3.2, I talk about some of the most relevant theoretical and terminological overlaps between biology and linguistics, investigating both the past and the present of the relationship between these two disciplines, ranging from Aristotelian biolinguistics to present-days code biology.

In Section 3.3, I define polysemy from the linguistic point of view, calling the attention to some of the most interesting philosophical issues involving this peculiarity of language. This in-depth

examination is a necessary preliminary step in order to transfer useful information from the linguistic *source* to the biological *target* of the proposed analogy.

In Section 3.4, I develop step by step the analogy between phenotypic plasticity and polysemy, looking for significant corresponding aspects between these two properties and pointing out how an information transferring is fruitful to improve understanding and to expand the conceptual foundation underlying the theory of phenotypic plasticity.

3.1 The analogical way to knowledge

Why should *analogies* be relevant in science? Before trying to answer this very broad question, it would be important to consider a specific definition of the word “analogy”. An interesting and simple one is that deriving from Dedre Gentner’s *structure mapping theory*. Gentner’s model defines an analogy as a mapping of knowledge from a “base” domain onto a “target” domain. The key characteristic of the map is that objects belonging to the base are linked to each other by a system of relations that is also found in the target (Brown and Salter, 2010)¹. The concept of analogy can also be related to the more technical ones of *homomorphism* and *isomorphism*, which are both notions drawn from mathematics, more specifically, from abstract algebra. A homomorphism is mapping one mathematical set to another set in such a way that the result obtained from an operation on the elements of one group is mapped to the result obtained from a corresponding operation on the related images in the other set. In other words, the two sets share a common, basic structure. Therefore, *some* characteristics of a group are also found in its homomorphic group. An isomorphism is a particular case of homomorphism characterised by a one-to-one relationship between each element of one set and its related image in the other set (i.e., each element of one set is mapped to *exactly* one element of the isomorphic set) (Cheng, 2023)². I anticipate that the analogy presented in this chapter is closer to the broader concept of homomorphism

¹Brown, S. and Salter, S. (2010). Analogies in science and science teaching. *Advances in Physiology Education*, 34(4):167–169.

²Cheng, B. (2023). Homomorphism, isomorphism, and their applications in group theory. *Highlights in Science, Engineering and Technology*, 47:71–74.

than the more restrictive one of isomorphism, since not all elements from one of the considered domains (i.e., phenotypic plasticity) have their corresponding element in the other domain (i.e., polysemy), although such domains share a very similar basic structure. Before we get there, however, let us start with a general discussion on the role of analogy.

The ability to grasp or, at least, to search common systems of relations between different domains seems to be deeply ingrained in the human mind, which is quite intriguing for its implications on cognition and reasoning. Analogical processes might be the heart of human thought. As argued by the brilliant polymath Douglas Hofstadter, analogy is such an essential mechanism that it could be reasonable to consider it as the basis of most of our cognitive life. Indeed, in his perspective, analogy is far from being a particular case of reasoning or problem-solving approach, in spite of the way it is often presented. Furthermore, Hofstadter's position is so radical that he considers all our concepts as nothing but bundles of analogies, whose reciprocal interrelationships and connections are analogical processes themselves (Hofstadter, 2001)³. As specified by the author, this is not a widely recognised view, and it is plausible that, at some point, it may turn out to be inaccurate (or even wrong). Nevertheless, it is still a highly evocative view, which is clearly emphasising the spontaneous and continuous, basic process of production of analogies, regardless of their complexity or validity. If the generation of new analogies is really such a basic tendency, it is likely to be affected by several biases and other problems.

In fact, due to this spontaneous propensity in identifying analogical pathways linking different ideas, it follows that it is very easy to get distracted by superficial and fallacious juxtapositions, false similarities, and unclear correspondences. If this is true for people in general, it should also be true, to some extent, for that specific subset of human beings known as scientists. Although scientists are largely guided by their accumulated knowledge, empirical data, and hypotheses (at least, in their work), they certainly share with common people the impulse to produce new analogical associations of ideas. This is the reason why the aforementioned Richard Dawkins once said that an important ability of a true scientist is that of dis-

³Hofstadter, D. R. (2001). Epilogue: Analogy as the Core of Cognition, pages 499–538. The MIT Press.

carding bad analogies, rather than discovering new ones (Dawkins, 1987, pp.195-196)⁴, an elimination procedure of unfit links which is also curiously similar (or, perhaps, *analogous*) to the functioning of *artificial selection*, that is normally put into practice by breeders for domestication aims. As a matter of fact, the ease with which inappropriate analogies can be invented is a critical issue for both research and public communication of science: are analogies more like enhancers of scientific imagination, or, instead, should they be regarded as psychological obstacles to real scientific advance? The answer to this question is directly associated with a long-lasting issue in philosophy: can figures of speech like analogies be relevant in increasing knowledge and, more generally, is a creative use of language applicable in argumentation?

The latter topic is largely overlapping with the traditional (and possibly fallacious) dichotomy between *persuasion* and *truth*, or, in other terms, between a pure operation of conviction of the audience and “neutral” reasoning, a duality based on the split between the stylistic and the emotional aspects of language from the argumentative one. A similar approach is even found in several present-day theories dealing with rhetoric (Piazza, 2004, pp.9-13)⁵, but the very existence of a sound partitioning between these different aspects of speech, paradoxically, is still challenged by the ideas of an Ancient Greek philosopher. Aristotle, despite being rightly regarded as one of the fathers of logic and deductive inference, used analogies and metaphors frequently in his treatises, attributing to them a cognitive value which goes far beyond the mere ornamental aspect. In this context, *metaphors* are as relevant as analogies (other than being very close concepts), because they are words or expressions used in place of other words or expressions to indicate a likeness between the two linked objects. Aristotle talked explicitly about their importance in speech. For him, a good metaphor could drive the intuitive perception of the similarity in dissimilars (Driscoll, 2012)⁶, insofar as it serves as a pleasurable cognitive enhancer due to its ability to involve the listener (or the readership) in a more active and direct way in the assimilation of a new concept (Piazza, 2004,

⁴Dawkins, R. (1987). *The blind watchmaker*. Longman Scientific & Techn., Harlow, Essex, reprint edition.

⁵Piazza, F. (2004). *Linguaggio, persuasione e verità*. Number 477 in *Studi superiori*. Carocci, Roma, 1. ed edition.

⁶Driscoll, S. (2012). Aristotle’s a priori metaphor. *Aporia*, 1(22):20– 31.

pp.148-149)⁷. Although this ancient insight is not widely recognised by present-day philosophical debate, the idea that analogical thinking is valuable for aspects other than style is sometimes highlighted by some modern scientists. For instance, biologist Arvid Agren (Ågren, 2021, p.186)⁸ argued in favour of the importance of such linguistic constructions, pointing out their role of enablers of unexplored paths among concepts and their potential in highlighting connections which would be otherwise invisible.

Of course, the formulation of a conceptual bridge between different domains should be regarded as a preliminary step to testing the hypotheses suggested by this process. Otherwise, the risk is to fall into a situation similar to that of Wittgenstein’s beetle in a box: in this thought experiment, everyone has a box and everyone claims that there is a “beetle” inside his box. In fact, these boxes might contain anything (or nothing). Nevertheless, each person refuses to show others the content of his box, or even to describe its characteristics. Therefore, “beetle” is just a word indicating the content of each box, but knowledge of this content is totally subjective and remains precluded to others (Wittgenstein, 1994, p.100)⁹. In order not to be irrelevant for scientific aims, analogical thinking needs to be dropped into reality.

In other words, analogies and metaphors can play the role of excellent cognitive supports only during the *discovery* process, that logical empiricists used to distinguish from *justification*. Indeed, while discovery is a subjective event concerning the invention of scientific hypotheses, justification is all about testing and evaluating the latter in an objective way (Kordig, 1978)¹⁰. Analogical thinking may catalyse the emergence of new ideas in the minds of individual scientists. Although the specific path leading to the genesis of each new idea is unknown to anyone other than the person who formulated it, this idea may turn out to be valuable from a scientific point of view after undergoing a rational and objective scrutiny in a subsequent step.

Just to keep focused on biology, an emblematic example of a

⁷Piazza, F. (2004). Linguaggio, persuasione e verità. Number 477 in Studi superiori. Carocci, Roma, 1. ed edition.

⁸Agren, J. A. (2021). The gene’s-eye view of evolution. Oxford University Press, Oxford, first edition edition.

⁹Wittgenstein, L. (1994). Philosophical investigations. Blackwell, Oxford [u.a.], 3. ed., repr. edition.

¹⁰Kordig, C. R. (1978). Discovery and justification. Philosophy of Science, 45(1):110–117.

good use of analogical thinking by a famous scientist is the conceptualisation of the process of natural selection by Charles Darwin. In his great masterpiece *On the origin of species*, Darwin grasped the existence of an automatic, natural mechanism driving the differentiation of species by referring explicitly to the techniques employed by humans in the domestication of animals and plants, namely artificial selection (that is also mentioned above). In other terms, he believed that the principles of this natural mechanism were *analogous* to those underlying artificial selection, and he was convinced that this comparison would have improved the understanding of the events leading to the origin of new species (Sterrett, 2002)¹¹, which actually happened. Beyond the boundaries of life sciences, another notable case of use of analogy in a scientific theory is represented by some observations of Johannes Kepler, who can undoubtedly be regarded as an able and spontaneous creator of illuminating analogies. The most important among them was probably that linking the light from the sun and a hypothetical force, emanating from the sun itself, which would regulate the movement of planets by travelling invisibly from its source to all the planets. In particular, such interaction would be based on an inverse proportionality relationship between the intensity of the force and each planet's distance from the sun, which would explain why the speed of a planet decreases with distance from the sun (that is analogous to the diminishing of brightness with distance from a light source). The relevant thing here is that the force theorised by the German astronomer may be considered a precursor of gravity (Gentner, 2002)¹². Thus, several great discoveries have originated from analogies.

As could easily be guessed, these are just two exemplary cases, among the many possible, of analogies used by well-renowned scientists of the past. However, as mentioned earlier, some present-day scientists are well aware of the importance of analogies in their own activity, and many of those who do not reflect explicitly on this point make use of analogies in their work as well. Indeed, researchers have to deal frequently with this type of thinking, for instance, when it comes to communicating efficiently their findings to the readership,

¹¹Sterrett, S. G. (2002). Darwin's analogy between artificial and natural selection: how does it go? *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, 33(1):151–168.

¹²Gentner, D. (2002). *Analogy in Scientific Discovery: The Case of Johannes Kepler*, pages 21–39. Springer US.

developing their hypotheses, or even interpreting their results (Taylor and Dewsbury, 2018)¹³. In the light of all this, the idea that analogy is a useful cognitive tool in empirical science seems to be supported by facts.

However, although the issue we are dealing with here clearly fits into the natural sciences, some important arguments on the value of analogy are found in mathematics, as brilliantly explained by French mathematician Andr  Weil in some passages of a memorable letter addressed to his philosopher sister Simone. In this interesting letter, whose aim was to explain properly the work of a mathematician to a person without that background, Andr  Weil highlighted the final, desirable goal each analogy can aspire to achieve while talking about the way of doing theory in mathematics:

“Let us examine this analogy more closely. Once it is possible to translate any particular proof from one theory to another, then the analogy has ceased to be productive for this purpose; it would cease to be at all productive if at one point we had a meaningful and natural way of deriving both theories from a single one. In this sense, around 1820, mathematicians (Gauss, Abel, Galois, Jacobi) permitted themselves, with distress [angoisse] and delight, to be guided by the analogy between the division of the circle (Gauss’s problem) and the division of elliptic functions. Today, we can easily show that both problems have a place in the theory of abelian equations; we have the theory (I am speaking of a purely algebraic theory, so it is not a matter of number theory in this case) of abelian extensions. Gone is the analogy: gone are the two theories, their conflicts and their delicious reciprocal reflections, their furtive caresses, their inexplicable quarrels; alas, all is just one theory, whose majestic beauty can no longer excite us.” (Weil, 1979, pp.244-255)¹⁴.

In Weil’s perspective, analogy is the first step to unify two theories which are initially not clearly linked with one another. When it is possible to translate a theory into another one, the analogi-

¹³Taylor, C. and Dewsbury, B. M. (2018). On the problem and promise of metaphor use in science and science communication. *Journal of Microbiology & Biology Education*, 19(1).

¹⁴Weil, A. (1979). *Oeuvres scientifiques*. Springer, New York.

cal architecture loses its original function. More specifically, this happens when it is possible to derive both theories from a third, all-encompassing one. This is probably the maximum scientific aspiration for a newly coined analogy, a goal preceded by a spectrum of degrees of “commensurability”, in the sense that the interconnections between the two compared domains can have different strength and reliability.

Keeping in mind this spectrum, a more basic scientific role an analogy can aspire is that of being “just” a heuristic process, a catalyst of thought capable of combining available information from different areas to create unprecedented visions. In other words, although the correspondence between the two domains can still be somewhat superficial (i.e., the compared phenomena are not completely superimposable), the comparison process itself may promote the emergence of an alternative perspective on already known concepts. In this work, my intention is to start shaping a new analogy capable of fuelling new insights and strengthening understanding of a biological process through a comparison with a particular property of human language. More specifically, the structure of the following analogy is based on the similarities between environmentally-induced responses in biological traits and a linguistic phenomenon called *polysemy*. In the next sections, I explain in detail why, apart from some notable differences that deserve to be discussed as well, polysemy can be regarded as an overall well-functioning analogical counterpart of plasticity, paving the way for a better understanding of both phenomena and for a theoretical exchange of information in both directions.

At the same time, a further aim of this operation is to sketch a new philosophical perspective encompassing both what I have previously called the *gene’s eye view* and the developmental approach typical of evolutionary developmental biology (see Chapter 1). Indeed, I argue that this new theoretical framework could be efficient in representing, at least partially, the complex interplay between evolution via genes and the role of development, reinforcing an integrative vision and, hopefully, suggesting new ideas on the highly intertwined relationship between heredity and changes in life cycles induced by the environment. Whether or not the analogy between plasticity and polysemy may become, in the future, a more corroborated and punctual research program is beyond my current

intentions and aims. At the present moment, the crucial step is to provide a single, coherent big picture taking into account all main interacting factors involved.

However, before introducing and discussing in detail this polysemic view of life, it is indispensable to place this analogy in the correct context, giving at least an insight into the historical background behind the linguistic treatment of biological dynamics and phenomena, from the earliest speculation about these topics to the molecular revolution of 20th century genetics and back.

3.2 Biology and language: two deeply communicating domains

The first, extensive description of the biochemical structure of DNA by James Watson and Francis Crick dates back to 1953. The discovery of a large number of details on the fundamental molecule of hereditary transmission was the major event promoting the so-called Molecular Revolution in genetics. Other than being a real milestone in biology, that scientific breakthrough changed the way people looked at life dramatically. As Watson himself wrote in one of his books, all these advancements of astonishing importance made humans aware that, one day, they might have a power that had always been thought to belong only to gods. In his opinion, the discovery of DNA and the understanding of its crucial role inside every cell and organism made clear that the secret of life was all about biochemical reactions and sets of instructions stored in chromosomes and passed across generations (Watson, 2017, Coda)¹⁵.

Nevertheless, at the time of the establishment of this huge cornerstone, functional details about heredity at the molecular level were still largely unknown. Indeed, genome was still considered as a book written in a mysterious language, and it took a few more years before its basic mechanisms began to be understood with some accuracy (Hayes, 1998)¹⁶. New findings, like those of many processes and microscopic entities, which were obviously far away from common sense and intuition, led molecular geneticists to search a new way to talk about biological dynamics at this level, relying on words which

¹⁵Watson, J. D. (2017). DNA, chapter 12. Knopf Doubleday Publishing Group, Westminster.

¹⁶Hayes, B. (1998). The invention of the genetic code. *American Scientist*, 86(1):8.

resembled those typically used only by linguists and other scholars also dealing with language, such as *transcription*, *translation*, *code*, and so on. As could be guessed by simply looking at all these terms, the informational nature of DNA suggested those scientists to make their area more and more permeable to terminological influences from the linguistic domain. Thus, at least to some extent, molecular biology was searching for some analogies and metaphors drawn from the study of language in order to explain the chemical basis of life in a more understandable way. However, all these notions are well-known to anyone who has studied biology, and not necessarily at a university level.

It is less known that, in the same century, the reverse need of explaining language using words originally coined to talk about biological concepts is also found in the literature. In fact, just three decades after the description of the double helix, in a completely different research area, a suggestive book by the Armenian-American theoretician of linguistics Sebastian Shaumyan, *A semiotic theory of language*, introduced two new terms in the theory of language, namely *genotype* and *phenotype*. At that time, these two words were not used in his field. Nevertheless, they should be familiar to the reader, since they were originally introduced by one of the most important scientists mentioned so far, Wilhelm Johannsen (see Chapter 2), other than being the fundamental concepts underlying phenotypic plasticity. In a nutshell, in their original domain, they were used to distinguish Mendel's hereditary "factors" and the observable characteristics of organisms. Similarly, Shaumyan used them to indicate two different levels of grammar, a stable one and one subject to variation (respectively, the genotypic and the phenotypic level) (Šaumjan, 2010, p.97)¹⁷.

Although the above-mentioned cases are just two specific manifestations of interaction in both directions (i.e., from the linguistic domain to the biological one, and vice versa), if one looks further into the history of this interaction, it seems reasonable to hypothesise they could be just the tip of the iceberg. Indeed, it is quite fascinating to reflect about the very nature of these terminological intersections, and whether they could be regarded as accidental examples of superficial contact or, on the contrary, as symptomatic

¹⁷Saumjan, S. K., editor (2010). *A semiotic theory of language*. Advances in semiotics. Indiana University Press, Bloomington.

cases of a deeper connection between biology and theory of language. As a matter of fact, even present-day biology is involved in the search for profound common features between its main topics and linguistics, as shown by some attempts to apply formal language theory in molecular genetics. For instance, grammars were proved to be suitable to represent DNA and RNA folding in an abstract way, and modelling the syntactic structure of genes seems to be a worthwhile effort as well (Searls, 2010)¹⁸. These are further indications that there might be a real, strong relatedness between these two domains.

If one wants to pursue this idea, evolutionary biology is, once again, a treasure trove of meaningful concepts and suggestions. Indeed, this field has been interested in language at least since publication of Darwin’s *Descent of man* in 1871 (Pagel, 2016)¹⁹. Actually, the British naturalist had already expressed his curiosity about the topic in his previous masterpiece, *On the origin of species*, where he suggested that species are like languages, because they are both subject to geographical constraints and experience some evolutionary dynamics which are based on a low level of continuous change:

“Thus, on the view which I hold, the natural system is genealogical in its arrangement, like a pedigree [...] It may be worthwhile to illustrate this view of classification, by taking the case of languages. If we possessed a perfect pedigree of mankind, a genealogical arrangement of the races of man would afford the best classification of the various languages now spoken throughout the world; and if all extinct languages, and all intermediate and slowly changing dialects, had to be included, such an arrangement would, I think, be the only possible one. Yet it might be that some very ancient language had altered little, and had given rise to few new languages, whilst others (owing to the spreading and subsequent isolation and states of civilisation of the several races, descended from a common race) had altered much, and had given rise to many new languages and dialects. The various degrees of difference in the lan-

¹⁸Searls, D. B. (2010). *Molecules, Languages and Automata*, pages 5–10. Springer Berlin Heidelberg.

¹⁹Pagel, M. (2016). Darwinian perspectives on the evolution of human languages. *Psychonomic Bulletin & Review*, 24(1):151–157.

guages from the same stock, would have to be expressed by groups subordinate to groups; but the proper or even only possible arrangement would still be genealogical; and this would be strictly natural, as it would connect together all languages, extinct and modern, by the closest affinities, and would give the filiation and origin of each tongue.” (Darwin, 1859, pp.867-868)²⁰.

Darwin also compared the unpronounced letters of words to vestigial organs, which are parts of the body that have lost most of their function over (evolutionary) time (Bralley, 1996)²¹. Apart from this prominent example, a much more recent case study is contained in a notable work by Szathmáry and Maynard Smith (1995)²², where the concept of *evolutionary transitions* was introduced for the first time. An evolutionary transition is a very rare event of huge relevance, capable of channelling the evolution of life on Earth deeply. In evolutionary transitions, a unit which was formerly able to replicate independently is typically integrated into a new, higher-level unit, as shown by the transition between prokaryotic and eukaryotic cells, or between unicellular and multicellular organisms. Other than that, another common trend is the emergence of new hereditary systems, e.g., in the transition from the initial RNA world to the new DNA centrality, or in the rising of cultural transmission in some species (Szathmáry, 2015)²³. In line with the latter observation, the two authors juxtaposed the origin of the genetic code and that of human language as emblematic cases of evolutionary transitions, so their theory provides an explicit connection between biology and language.

Although the latter perspective emphasises in a clear-cut manner the continuity between biology and language, it is still placed within a context where the overlapping between heterogeneous areas of investigation is the exception, rather than the rule. In modern times, it is generally acknowledged that each area is independent from all others for both methods and contents, and this is particu-

²⁰Darwin, C. R. (1859). On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London, UK.

²¹Bralley, P. (1996). An introduction to molecular linguistics. *Bio- Science*, 46(2):146–153.

²²Szathmáry, E. and Maynard Smith, J. (1995). The major evolutionary transitions. *Nature*, 374(6519):227–232.

²³Szathmáry, E. (2015). Toward major evolutionary transitions theory 2.0. *Proceedings of the National Academy of Sciences*, 112(33):10104–10111.

larly true when looking at biology, on the one hand, and theory of language, on the other. Nevertheless, the assumption of the existence of a rigorous disciplinary partitioning is not a historical constant. Indeed, for instance, it was not accepted by ancient Greeks. The fact that some important philosophers were more like universal thinkers than specialised scholars is clear when looking at their tendency to combine reflections on human language and the study of living beings. Keeping in mind this approach, it could be said that the separation between biology and linguistics is not an *ancestral* condition, but rather a *derived* one. In other words, the merging of different disciplines was the first step, then separation came later.

In particular, Aristotle was a leading advocate of this holistic way of seeing science and philosophy, since his linguistic thought was heavily influenced by biological knowledge and suggestions. This was not limited to the use of terms drawn from biology, which, however, was a widespread trend among Greek thinkers. Aristotle's peculiarity was his highly evocative perspective on the nature of language, that was also based on the viewpoints of some of his cultural ancestors, foremost Plato. He discussed explicitly this position in his treatise *De anima*, where he talked about human thought and its linguistic embodiment as a superior form of living being, actually, an *organism* in all respects. Furthermore, the integration of language and life sciences in Aristotelian writings is supported even more strongly by a comparison between the 20th chapter of the *Poetics* and the second book of the *De partibus animalium*, from which the isomorphism between the steps involved in the construction of the linguistic organism and those involved in the development of the biological organism unfold (Laspia, 1997, 2018, pp.21-22, p.1, 40)²⁴²⁵. This was undoubtedly one of the first, remarkable analogical operations in the history of philosophy, connecting knowledge about biological entities and processes (e.g., development) and linguistic parts and rules so deeply as to create a unitary field of speculation. Aristotle's work on this topic is a milestone of speculations on the relationship between life and language, stemming from the holistic approach that characterised all ancient Greek philosophers. In Aristotle's time, differently from the recent revival of expressions drawn

²⁴Laspia, P. (1997). L' articolazione linguistica. Number 16 in Ricerche. Nuova Italia Scientifica, Roma.

²⁵Laspia, P. (2018). From biology to linguistics. UNIPA Springer series. Springer, Cham, Switzerland.

from linguistics and used in biology, it was linguistics that *needed* biology to express its concepts and mechanisms of composition of sentences.

However, the ancient approach is not unique in history. A prominent, long-lasting and very articulate example of systematic thought binding together biology and linguistics in a deep way is found in the 19th century, that was the time of *pragmatist semiotics*. An interesting detail on pragmatist semiotics is that this philosophical movement originally arose from the theoretical disputes between Charles Peirce and the Darwinian thinker Chauncey Wright (Cimatti and Fadda, 2016)²⁶, which recalls the biological roots of this tradition of thought, that would later manifest themselves fully with the emergence of *biosemiotics*. While semiotics is the qualitative science investigating systems capable of producing meanings, biosemiotics is its biological counterpart, i.e., the science based on the assumption that meaning production exists in biological systems in general, to the point that this feature could be regarded as the foundation of life sciences, as proposed by the late 19th century biologist Jakob Von Uexküll (Barbieri, 2009; Kull, 2015)²⁷²⁸.

Although Uexküll's position may seem somewhat radical, it does not significantly differ from those supported by some present-day biologists, who are still looking for detailed correspondences between several aspects of human language and peculiarities of DNA and its expression inside cells. For instance, letters of the alphabet may correspond to nucleotides, words constituting the lexicon may be counterparts of structural genes, and so on (Ji, 1999; Minelli, 2014b)²⁹³⁰. This approach is also probably influenced by the most recent developments of biosemiotics, that has meanwhile gone far beyond Uexküll and his ideas. Indeed, biosemiotic thinking has bifurcated into two strands in the 1960s, a molecular and a zoological one, when biologists like Howard Pattee began to talk about cell as a physical system controlled by symbols, and Thomas Sebeok

²⁶Cimatti, F. and Fadda, E. (2016). *Biologia e pragmatismo*, pages 201–219. Carocci editore.

²⁷Barbieri, M. (2009). A short history of biosemiotics. *Biosemiotics*, 2(2):221–245.

²⁸Kull, K. (2015). *Introduction to Biosemiotics*, pages 521–533. Springer Netherlands.

²⁹Ji, S. (1999). The linguistics of dna: Words, sentences, grammar, phonetics, and semantics. *Annals of the New York Academy of Sciences*, 870(1):411–417.

³⁰Minelli, A. (2014b). *Geni come parole - uno, nessuno e centomila*, pages 107–117. Roma-Messina: Corisco Edizioni.

questioned the fact that the use of signs is restricted to humans only. Further branches were added later, as is the case with *code biology* (Barbieri, 2009)³¹.

In code biology, the main focus is the distinction between *information* and *meaning*, where copying is thought to be about information, whereas coding would involve meaning (Barbieri, 2012)³², since codes themselves, in the perspective of code biology’s supporters, are processes generating meaning. According to them, the discovery that hereditary material is carried by genetic sequences implies that information exists in cells, and the following discovery that protein synthesis follows the rules of the genetic code should imply that meaning exists as well in cellular contexts. The reluctance in introducing the concept of meaning in biology can be traced back to several issues, first of all, the fact that the genetic code is not a *real* code, because it does not deal with arbitrary aspects and its rules are strictly determined by chemistry (Barbieri, 2020)³³.

This criticism is also shared by the two aforementioned advocates of the theory of evolutionary transitions, Smith and Szathmáry, who claimed that, while in human language meanings of phrases depend on formal rules and syntax, at the molecular level “meanings” are not derived from a logical reasoning, but rather by the laws of physics and chemistry. For this reason, the analogy between linguistic and biological meanings would be fallacious and not useful (Maynard Smith and Szathmáry, 2010, p.169)³⁴. Nevertheless, supporters of code biology refer to some experimental evidence showing that, in the translation process, bonds between nucleotides (i.e., genes building blocks) and amino acids (i.e., proteins building blocks) are not deterministic, but rather arbitrary (Barbieri, 2020)³⁵. However, apart from the theoretical debate about the actual existence of biological meanings, some biologists keep relying on this part of the analogy by talking about the fact that genetic information is made of digital data (i.e., mRNA sequences) plus meaning (i.e., the translation of mRNA into a functional protein) (Chatterjee and Yadav, 2019)³⁶. Since the concept of information and, more im-

³¹Barbieri, M. (2009). A short history of biosemiotics. *Biosemiotics*, 2(2):221–245.

³²Barbieri, M. (2012). Code biology – a new science of life. *Biosemiotics*, 5(3):411–437.

³³Barbieri, M. (2020). The semantic theory of language. *Biosystems*, 190:104100.

³⁴Maynard Smith, J. and Szathmáry, E. (2010). *The origins of life*. Oxford University Press, Oxford.

³⁵Barbieri, M. (2020). The semantic theory of language. *Biosystems*, 190:104100.

³⁶Chatterjee, S. and Yadav, S. (2019). The origin of prebiotic information system in the

portantly, the concept of meaning are very complex, there is a high risk of using them in a wrong way. Thus, both notions are defined and explored in detail in Section 3.4, when I describe my linguistic analogy step by step.

The renewed interest towards what we could perhaps call “the linguistics of life” is promoting both a return to the old Aristotelian linguistics and, for other aspects, to its reverse, i.e., the tendency to describe the functioning of biological mechanisms and dynamics by using theories and notions drawn from linguistics and related fields, which is the inverse of the original intention of explaining language using prior biological knowledge. This historical path shows a sort of “palindromic” nature: while the oldest and the most recent segments are inverted in their order, they still share most of their key components.

Now, the next step is to introduce a specific, point-like analogy involving phenotypic plasticity, on the biological side, and polysemy, on the linguistic one. It should be noted that this analogy is far from being an attempt to demonstrate the validity of the whole theoretical scaffolding underlying perspectives that promote the intrinsically linguistic or semiotic nature of life (e.g., code biology). Indeed, even if none of these views had come to our attention, and even if it turns out that an all-encompassing analogy between linguistics and biology is inaccurate or inexact as a whole, I argue that the small fragment of analogy discussed in the following sections would still be a valuable catalyst of understanding, intuition, and hopefully discovery.

3.3 Some basic facts about polysemy and the debate around it

“mouse -n. (pl. mice) 1 small rodent, esp. of a kind infesting houses. 2 timid or feeble person. 3 (pl. -s) Computing small hand-held device controlling the cursor on a VDU screen.”

(Thompson, 1993)³⁷

peptide/rna world: A simulation model of the evolution of translation and the genetic code. *Life*, 9(1):25.

³⁷Thompson, D., editor (1993). *The Oxford dictionary of current English*. Oxford pa-

This section is a very concise introduction to some of the main tenets of the theory underlying *polysemy*, which, as shown in the next few lines, can be rightly considered a critical aspect of human language, regardless of its local and contingent manifestations determined by historical and geographical variables.

Before defining this property of language in detail and discussing briefly some highly relevant conceptual issues involving it, it may be useful to start with a simple example of polysemy. The opening quote of this section is an excerpt from an English dictionary entry, and is a well-known case of polysemy, since it is a single word with several alternative meanings. As any average English speaker knows, the term *mouse* can be used to refer to a small mammal, a computer hardware designed to control the cursor on the screen, or other entities. But this is just one of countless possible examples, in English as well as in most, if not *all* languages used around the world. Even Mandarin Chinese, that is based on logograms rather than words, is not exempt from this phenomenon (Ursini et al., 2021)³⁸, although in this chapter I mainly focus on alphabetic languages. For instance, let us examine the case of the word *line*: we can draw a *line*; read a *line*; we can also have a *line* around eyes, or we can even make a *line* of bad decisions (Falkum and Vicente, 2015)³⁹. In Russian, the term *glava* can refer to such things as a head, a chief, a cupola, a chapter, and others (Shimorina and Grachkova, 2011)⁴⁰. In Italian, *arco* can mean either a bow or an arch, and so on.

In this regard, a premise is necessary. As has been said both in this section and in some of the previous ones, polysemy is based on the controversial concept of *meaning*. When it comes to talking about meaning, it should be noticed we are dealing with a multifaceted issue, studied from different perspectives by heterogeneous research areas, such as linguistics, philosophy, psychology, and even

perbacks. Oxford University Press, Oxford [u.a.], 2. ed., oxford university press paperback edition. Previous ed. published as: 8th ed. of The pocket Oxford dictionary of current English, 1992.

³⁸Ursini, F.-A., Rao, Q., and Zhang, Y. S. (2021). The polysemy and hyponymy of mandarin spatial prepositions and localisers: Building semantic maps from the ground up. *Frontiers in Communication*, 6.

³⁹Falkum, I. L. and Vicente, A. (2015). Polysemy: Current perspectives and approaches. *Lingua*, 157:1–16.

⁴⁰Shimorina, A. and Grachkova, M. (2011). Identification of context markers for russian nouns. In *Proceedings of the 18th Nordic Conference of Computational Linguistics (NODAL-IDA 2011)*, pages 344–347.

computer science. As a consequence of that, this interdisciplinarity is also largely shared with the study of polysemy (Falkum and Vicente, 2015)⁴¹. Defining meaning in an unequivocal way is a very difficult task, both at the level of single words and at the level of full sentences, as proven by the existence of many alternative definitions which have been formulated over the centuries (Marsen, 2009; La Mantia, 2012, pp.14-15)^{42,43}. In particular, it is important to notice that some prominent philosophers of language have been highly critical towards the classical notions of meaning. For instance, Wittgenstein wrote that, in most cases, the meaning of a word is its *use* in the language (Wittgenstein, 1994, p.20e)⁴⁴. Other authors, mainly belonging to the analytical tradition, have shown scepticism on the very existence of facts about meanings of linguistic expressions. If one assumes as true a perspective similar to those of Quine (2002)⁴⁵ or Kripke (1982)⁴⁶, then the foundations of the theory of polysemy discussed here are inevitably weakened. In this regard, it is important to keep in mind that all the following arguments are mainly based on a “suspension of judgement” about this scepticism, so they do not pretend to be compatible to the same extent with all the most prominent theories of meaning available.

Given such a long-lasting debate, it is easy to imagine that developing a further, broader definition of “meaning” including, for instance, the proteins corresponding to DNA nucleotide sequences, would be a thorny and controversial operation that would require a very careful approach. Nevertheless, since the goal of this chapter is to build an analogy between polysemy and phenotypic plasticity, this issue needs to be addressed. So, like some of the aforementioned authors interested in the relationship between the linguistic and biological domains did, we too should ask ourselves whether the idea of assuming the existence of some “biological meaning” is reasonable or not. However, since this important point is specifically

⁴¹Falkum, I. L. and Vicente, A. (2015). Polysemy: Current perspectives and approaches. *Lingua*, 157:1–16.

⁴²Marsen, S. (2009). Is meaning information? some thoughts on linguistic ambiguity, embodied emotion, and the making of meaning. *Semiotica*, 2009(174).

⁴³La Mantia, F. (2012). Che senso ha? Number 2 in *Semiotica e filosofia del linguaggio*. Mimesis, Milano, Udine.

⁴⁴Wittgenstein, L. (1994). *Philosophical investigations*. Blackwell, Oxford, 3. ed., repr. edition.

⁴⁵Quine, W. V. (2002). *Word and object*. MIT Press, Cambridge, Mass.

⁴⁶Kripke, S. A. (1982). *Wittgenstein on rules and private language*. Harvard Univ. Pr., Cambridge, Mass., 2. ed. edition.

investigated in the following section, I suggest to put it aside for a moment and to focus on scratching the surface of the concept of polysemy first, that is an essential preparatory step for what comes next.

Now, other than showing some practical examples, one of the possible ways to introduce a new concept is to start with its etymology. Actually, in many if not most cases, this step is unnecessary, and sometimes it is even misleading. Nevertheless, the etymology of the word “polysemy” is of some interest for this argumentation: indeed, it shows that the issues concerning the relation between words and meanings date back at least to the ancient Greeks, like many other things we still discuss in our times. The term is derived from the Greek *polys-* (meaning “many”) and *sēma-* (which means “sign”). The roots of this concept reveal an early theoretical interest towards the remarkable potential of words to be linked to more than one meaning. However, such interest usually took the form of criticism, both among ancient Greeks and later thinkers. Indeed, rather than looking at polysemy as a manifestation of the productive side of language (that is common opinion among present-day scholars), they tended to blame it due to its intrinsic misleading connotation (Kovacs, 2011)⁴⁷.

However, although polysemy is not considered a mere degeneration of human language anymore, an important question may still arise: why should it be regarded as a relevant aspect of our linguistic and cognitive equipment? In the first place, such relevance can be justified from a quantitative point of view. As was said before, polysemy is a ubiquitous property of any human language (for sure, of all alphabetic languages), since it is found in speakers from all parts of the world, that is itself a fact of huge interest. In addition to their wide diffusion across different languages, one of the most remarkable things about polysemous words is their frequency *within* each language. It has been estimated that 40-80% of all English words exhibit some degree of polysemy (Floyd et al., 2020)⁴⁸, and

⁴⁷Kovacs, E. (2011). Polysemy in traditional vs. cognitive linguistics. *Eger Journal of English Studies*, (11.):3–19.

⁴⁸Floyd, S., Goldberg, A. E., and Lew-Williams, C. (2020). Toddlers recognize multiple meanings of polysemous words. pages 2799– 2804. Publisher Copyright: © 2020 The Author(s). This work is licensed under a Creative Commons Attribution 4.0 International License (CC BY); 42nd Annual Meeting of the Cognitive Science Society: Developing a Mind: Learning in Humans, Animals, and Machines, CogSci 2020 ; Conference date: 29-07-2020 Through 01-08-2020.

that approximately 93% of most used words in English texts are polysemous (Lee, 1990)⁴⁹.

Other than being very frequent, and probably also *because* it is so frequent, the use of polysemy by speakers and hearers can be considered a critical linguistic skill, as clearly shown by the precocity with which it emerges in individuals of our species. In this regard, a case study involving 40 English native speakers with ages ranging from 24 up to 36 months informed that even toddlers are able to associate a term to multiple meanings, and to add further meanings to their repertoire based on the similarity between the new ones and those which had already been learned in the past. Researchers obtained these results by showing each toddler a sequence composed by couples of images projected on a screen, that corresponded to two conventional meanings of polysemous words in the English language. Other pictures corresponding to additional meanings of each word (which are reported in the Spanish language, but *not* in English) were projected on the screen as well. Toddlers' choices were induced by a phrase (e.g., "look at the *cap!*") and the direction of their gaze was monitored with proper tools. The authors of the study found that the frequency of toddlers which were able to guess the right meaning was, in all cases, above random expectancy, especially (but not only) when considering the choice between the two alternative English meanings. These outcomes support the hypothesis that even very young individuals own a network of meanings for each word (that is due to their first months of experience), as well as the idea that even children can link a term to meanings they have never met before, based on the similarity with those which are already stored in their memory (Floyd et al., 2020)⁵⁰.

This research is both meaningful and useful for the purpose of introducing an accurate definition of polysemy and some of the theoretical issues related to this concept. As for the definition, a classic one was provided in 1897 by French linguist Michel Bréal, who was also the scholar who introduced the term "polysemy" in linguistics.

⁴⁹Lee, C. J. (1990). Some hypotheses concerning the evolution of polysemous words. *Journal of Psycholinguistic Research*, 19(4):211– 219.

⁵⁰Floyd, S., Goldberg, A. E., and Lew-Williams, C. (2020). Toddlers recognize multiple meanings of polysemous words. pages 2799– 2804. Publisher Copyright: © 2020 The Author(s). This work is licensed under a Creative Commons Attribution 4.0 International License (CC BY); 42nd Annual Meeting of the Cognitive Science Society: Developing a Mind: Learning in Humans, Animals, and Machines, CogSci 2020 ; Conference date: 29-07-2020 Through 01-08-2020.

In his *Essai de Sémantique* (Bréal, 1983)⁵¹, he argued that polysemy occurs when a newly arisen meaning of a word is added to the pre-existing ones *without* replacing any of them. Although Bréal's observation is crucial and has been retained in modern definitions, some other valuable details must be added to this basic description, so it can be synthesised as follows: polysemy occurs when multiple, distinct, but *related* meanings coexist within the same word or expression. The latter characteristic (in italics) is of fundamental importance within the theoretical framework I am outlining in this chapter.

Indeed, a *line* of research (here is a further meaning of the word *line*) of particular relevance in polysemy studies, which is also suggested by the paper involving toddlers, is that focusing on the ease with which a speaker free of cognitive deficits can grasp the most appropriate meaning of a polysemous term, quickly and effortlessly, in any possible situation of everyday life. As it usually happens when it comes to investigate an apparently simple and spontaneous cognitive skill, the difficulty in its execution by speakers is negatively related to that faced by theoreticians when they try to explain it exhaustively. However, one of the plausible explanations for this phenomenon is the following: the correct recognition of totally new meanings in young children suggests that a close *semantic relatedness* among meanings of a polysemous word might be a crucial factor in determining their choices, which also resembles the general functioning of basic analogical reasoning. This is one of the reasons why the assumption of relatedness seems to be essential in the definition of polysemy, in addition to the fact that it also distinguishes polysemy from another linguistic phenomenon called *homonymy*, i.e., when words with different and unrelated meanings share the same pronunciation and/or spelling (Kovacs, 2011; La Mantia, 2012, p.13)⁵²⁵³.

The continuity between different meanings of a polysemous word paves the way for another critical issue in polysemy studies, namely that concerning the so-called *meaning potential*. To intro-

⁵¹Bréal, M. (1983). *Essai de sémantique*. @Imago mundi. G. Monfort, Brionne, reprint [nachdr. d. ausg. paris 1913] edition.

⁵²Kovacs, E. (2011). Polysemy in traditional vs. cognitive linguistics. *Eger Journal of English Studies*, (11.):3–19.

⁵³La Mantia, F. (2012). Che senso ha? Number 2 in *Semiotica e filosofia del linguaggio*. Mimesis, Milano, Udine.

duce this concept, it is necessary to say a few words on the problem of *lexical meaning placement*, that is, identifying where the meaning of words is “positioned”, for example, when considering a full sentence. Several alternative views on this problem are found in the literature. According to the so-called *atomists*, each word is directly linked to a specific meaning. In this case, the context is irrelevant, and the global meaning of an expression is due to a simple composition of partial meanings. In its purest sense, this is to some extent a naive perspective, as shown by the existence of polysemy itself, that does not allow a one-to-one association between words and their meanings. A prominent alternative view is proposed by *contextualists*, who believe that words are like empty shells, lacking any potential to generate meaning on their own. This view has also some problems, because it would imply that words are not useful at all to compose meanings.

A further position that has been added more recently to this list is a sort of a *cellular theory* of meaning: in this perspective, a semantic unit is initially undifferentiated (*totipotent*, using a term drawn from developmental biology), then it gradually differentiates in relation to its linguistic *co-text*, that is, the other words within the sentence, and *context*, that is, all the aspects which are “external” to the sentence, a process leading to a temporary fixation of the most relevant meanings for that specific situation. In this case, meaning is determined dynamically at each new linguistic activity, through provisional steps characterised by stability (La Mantia, 2012, pp.16-20, 130-133)⁵⁴. All this is consistent with the existence of a spectrum of possible, virtual meanings for each lexical unit (i.e., the meaning potential), whose concrete implementation is deeply influenced by some variables which are located at all levels of the enunciation, including the linguistic “environment” within which the act is performed (Hanks, 2013, pp.65-83)⁵⁵. The cellular perspective is particularly enlightening because it combines the main positive points of the two mentioned before, overcoming some of their limitations. Furthermore, it gives a lot of value to the concept of meaning potential, that is discussed from an alternative point of view in the next section.

Other than the issues related to the composition of meanings,

⁵⁴La Mantia, F. (2012). Che senso ha? Number 2 in *Semiotica e filosofia del linguaggio*. Mimesis, Milano, Udine.

⁵⁵Hanks, P. (2013). *Lexical analysis*. The MIT Press, Cambridge, Mass.

i.e., the *synchronic* side of the matter, another topic of particular interest in the context of this work, due to its clear evolutionary connotation, is the *diachronic* aspect of polysemy. Although the focus of linguistic studies, during the 20th century, switched from the latter to the former approach (Kovacs, 2011)⁵⁶, some notable case studies dealing with the paths of change of meaning potentials over time are also found in the literature. As for the engine influencing these paths, MacCormac (MacCormac, 1990)⁵⁷ proposed that words can assume new meanings via *metaphorical extensions*. More specifically, a newly arisen metaphor can be the fuel of the process of change by introducing an additional meaning showing similarities between a previously acknowledged domain and an unusual one. Then, the new application of the concept can be fixed in the dictionary, as long as it is successful when introduced into the community of speakers. For instance, in the 14th century, the term *birth* was exclusively used in the strict biological sense, whereas the first figurative uses of the word (e.g., the *birth* of an idea) date back to the 16th century. In present-day English language, the new meaning is no longer considered figurative, then it can be regarded as completely fixed in the English dictionary.

In this respect, a study carried out three decades ago (Lee, 1990)⁵⁸ checked whether some categories of terms were richer of alternative meanings than others or not, with the intention of investigating patterns underlying any differences in the number of meanings among groups. In particular, a multiple regression analysis based on a sample of 200 random nouns was conducted to determine whether the date of entry and frequency of use of the selected nouns were predictive with respect to polysemy (intended as the number of meanings of a single word). In fact, the study informed that polysemy is a function of both the date of entry and frequency of use of each word or, in other terms, that these variables are good predictors of polysemy in nouns. This kind of studies, although perhaps out of the focus of most current research, is of fundamental importance because of its potential to open the way to new hori-

⁵⁶Kovacs, E. (2011). Polysemy in traditional vs. cognitive linguistics. *Eger Journal of English Studies*, (11.):3–19.

⁵⁷MacCormac, R. (1990). *A cognitive theory of metaphor*. A Bradford book. MIT Press, Cambridge, Mass. [u.a.], 3rd printing edition. A Bradford book.

⁵⁸Lee, C. J. (1990). Some hypotheses concerning the evolution of polysemous words. *Journal of Psycholinguistic Research*, 19(4):211– 219.

zons for an all-encompassing understanding of polysemy, combining findings on development and others on the evolution of polysemous words.

Before discussing in detail the analogy between polysemy and phenotypic plasticity in the next section, I synthesise here some of the main conclusions of this introductory section on polysemy as a linguistic phenomenon, at least for what concerns the arguments I am most interested to for building this conceptual bridge:

- A polysemous word is a word with multiple, coexisting, and related meanings;
- Each word has a meaning potential, namely its capacity to be “converted” in a variety of different meanings according to the local conditions of each enunciation;
- According to some theories of language, meanings in polysemous words are temporary, stable states induced by each word’s co-text and context;
- Polysemous terms evolve over time. More specifically, they can increase their degree of polysemy through subsequent implementations of newer meanings selected by the community of speakers among metaphorical extensions;
- Older and more frequently used words tend to have a higher number of meanings compared to the others.

3.4 A bridge between genotype and phenotype: the “polysemy” of genes

In this section, I introduce and discuss the analogy that has already been suggested and mentioned several times from the beginning of the thesis up to this point. To my knowledge, the conceptual relationship between polysemy and phenotypic plasticity has never been explicitly considered in the literature. The closest allusion to this topic is found in a suggestive paper by French linguist Bernard (Victorri, 2006)⁵⁹. In his article, Victorri engages in the discussion about the analogy between biology and language by focusing on the

⁵⁹Victorri, B. (2006). Analogy between language and biology: a functional approach. *Cognitive Processing*, 8(1):11–19.

deep similarity between the utterance of a sentence and the release of a protein in the intra or extracellular environment, based on the fact that both events lead to some effects on the local situation, e.g., some changes in the behaviour of listeners or in metabolic reactions within the cells of a tissue. More specifically, Victorri talks about polysemy when considering the alternative effects of certain proteins, such as insulin, depending on the specific anatomical district where these proteins are released. In other terms, he recalls the *context-dependent* behaviour of insulin. However, since Victorri does not go into much detail on the subject in his paper, I suppose it would not be wrong to present the analogy between polysemy and plasticity as new, which implies a higher probability to generate some ambiguities.

This is the reason why, in order to make the analysis conducted in this core section of the thesis deeper and reach an acceptable level of accuracy, the following arguments need to be combined with some preliminary conceptual warnings of high theoretical relevance. In this respect, I recall the definition of analogy given in Section 3.1: when considering an analogical relationship between different domains, elements in the source and the target domain are linked to each other because they share a similar role in their respective structures. It may also be said that the preserved component of any analogy is the structure *itself*. My attempt to investigate the sharing of a common structure between polysemy and phenotypic plasticity is certainly not free of aspects for which the analogical scaffolding does not seem to hold very well. Nevertheless, it should be pointed out that a newborn and “informal” analogy like this is inevitably more vulnerable to possible limitations and misaligned elements than a well-established theoretical framework. At the same time, saying that an analogy is still rudimentary does not imply it is compromised or useless. Indeed, I argue that this conceptual bridge has potential as a tool for the understanding of some scientific ideas and for promoting the exchange of knowledge from one domain to another, or even as a source of inspiration to design some empirically testable hypotheses.

Moreover, some weaknesses can even be found in the most famous and inspiring analogies involving biology and evolution, like the one underlying the whole argument presented in *The selfish gene*

(Dawkins, 2009)⁶⁰. As a matter of fact, this successful book is based on the assumption that it is appropriate, or at least *useful*, to consider genes (intended as discrete hereditary units) as selfish agents. In other words, genes are presented as entities whose “aim” is the maximisation of their individual advantage in terms of replication (technically speaking, their *fitness*). Although the selfish gene analogy has been hugely widespread over the last half-century, it has also been criticised by many scholars since its publication, sometimes using reasonable arguments, other times less so. In the first place, say Dawkins’ opponents, genes do not have intentions, and the existence of any design in evolution is obviously nothing more than a mere appearance (so, suggesting that genes may have some “aims” can even be misleading for some readers). But there is more. The concept of gene itself is vague and still debated. Furthermore, genes do not always behave “selfishly” and, as discussed in the previous pages, phenotypes are usually the outcome of a complex interaction between multiple genes and the environment, which makes things much more complicated than they seem when reading Dawkins’ book.

Nevertheless, other than being a valuable conceptual tool, capable of making evolution understandable even by people with no biological background, and a catalyst for research on transposable DNA elements (Orgel and Crick, 1980)⁶¹, the analogy that considers genes as fitness-maximising agents was even used as a basis to develop a precise mathematical model combining optimisation theory and population genetics, that was also able to generate some reliable results (Gardner and Welch, 2011)⁶². This is just one possible example of an analogy with several controversial points and flaws reported in the literature, but still useful for science. Clearly, the possible future implications of the analogy proposed here cannot be predicted. However, achieving a goal similar to that achieved by Dawkins (with the necessary proportions), that is, to produce an imperfect and objectionable analogy of some use for scientific purposes, would be a valuable result. At worst, it may become one more piece in the mosaic of connections between life and language

⁶⁰Dawkins, R. (2009). *The selfish gene*. Oxford Univ. Press, Oxford, 2. ed., 30th anniversary ed. edition. Previous ed. : 1989.

⁶¹Orgel, L. E. and Crick, F. H. C. (1980). Selfish dna: the ultimate parasite. *Nature*, 284(5757):604–607

⁶²Gardner, A. and Welch, J. J. (2011). A formal theory of the selfish gene: A formal theory of the selfish gene. *Journal of Evolutionary Biology*, 24(8):1801–1813.

which have been suggested from Darwin to the present day.

Now, it is very likely that several objections might be addressed towards this analogy, but I focus on the one I regard as the most relevant, that needs to be discussed before any further step is taken to clear the field of some possible conceptual troubles. This is a challenging issue, since it involves the basic principles of the whole operation I am building up in these pages, namely the applicability of the concept of *meaning* in biology. As shown in the previous section, that of meaning is a controversial idea itself, since there is not universal agreement even on its actual existence and definition, that is clear when looking at the scepticism in the works of some authors, such as Quine (2002)⁶³. However, even if one decides to overlook this scepticism to make things simpler, it is still not trivial at all whether an extension of the concept of meaning beyond its original field of application may be considered valid. Furthermore, the notions of polysemy and phenotypic plasticity belong to two very distant areas of investigation whose contents are apparently incommensurable: while the former deals with communication and understanding between intelligent beings, the latter is all about biochemistry, heredity, and developmental pathways. So, the biggest question is: can we identify some kind of meaning in biological systems, or at least a reasonable counterpart of what meaning represents for human language?

As discussed in Section 3.2, several authors working in different branches of biosemiotics and other related fields have argued extensively in favour of the actual existence of meaning within biological systems, especially at the molecular level. Nevertheless, as shown by the criticism expressed by Maynard Smith and Szathmáry, this controversy is far from being resolved. Now, it is possible to enter the debate and give some other reasons to believe in a more general concept of meaning which could also be applied outside human language. However, I think this would weaken the analogy, tying it too closely to a particular notion of meaning among many possible ones, or inevitably emphasising the differences between linguistic meaning and the type of meaning which might be found in organisms. Instead, I argue that the most appropriate approach is a *downgrade* of the concept of polysemy, to make it more compatible with biological entities and processes without compromising the pivotal aspects of

⁶³Quine, W. V. (2002). *Word and object*. MIT Press, Cambridge, Mass.

the analogy.

The concept of *information* is, at the same time, very close to that of meaning and much less controversial. I believe this term could be a good starting point because, first of all, biologists have been very familiar with it since the developing of the *central dogma of molecular genetics* by Francis Crick in 1958 (see Section 2.1). Moreover, and this is even more important, it has also proven to have a very wide scope, well beyond the boundaries of its original area of application. Engineering, physics, linguistics, physiology, genetics, and several other fields have all been influenced by this idea, which was born in the context of *communication technology*. Indeed, the minimal sense of information was defined by Claude Shannon, whose aim was to design a model of communication. In Shannon's view, everything that has a number of alternative states that might be realised on a particular occasion is a *source of information*. More specifically, a *signal* carries information about a source if the signal itself is a predictor of the state of the source, and a variable contains information about the source if its state is correlated with the state of the source. The more the state of a signal is a good predictor of the source, the more information it carries about the source. So, Shannon information is a causal or correlational concept, that is particularly useful because it can also be quantified (Godfrey-Smith, 2007)⁶⁴.

Considering this minimal sense, it can be said without particular problems that genes carry information about the proteins they make, as well as about the higher-level phenotypes they concur to determine, without the need to postulate the existence of any language. A stronger sense would be that of *semantic information*, which is the type of information we would expect to observe in a system where meaning is present. In the biological domain (excluding what concerns cognition), there seems to be an important lack of some fundamental features of semantic information. For instance, a key feature of messages carrying semantic information is their capacity to *misrepresent* what they are about. This capacity of error is not included in Shannon's definition of information. As a matter of fact, there are no such things like a false information

⁶⁴Godfrey-Smith, P. (2007). Information in biology. In Hull, D. L. and Ruse, M., editors, *The Cambridge Companion to the Philosophy of Biology*, pages 103–119. Cambridge University Press.

(Godfrey-Smith, 2007)⁶⁵. In addition, the hypothesis that genes or other biological entities carry semantic information should also suggest that their messages should be composed and read by intelligent systems, which is obviously not the case, then this hypothesis would still require some further sophistication and a different definition of semantic information (Godfrey-Smith and Sterelny, 2016)⁶⁶.

In spite of these obstacles, some philosophers and biologists have argued in favour of the use of this strong sense of information when talking about genes. While some authors go as far as to claim that semantic properties are own by everything having a causal role in the developmental processes (including environmental factors), many others agree that a true semantic connotation is observed only by looking at the function of genes to encode amino acids for proteins or, in other words, that semantics can only be found in protein synthesis (Barbieri, 2003; Godfrey-Smith, 2007)⁶⁷⁶⁸. The latter perspective is perhaps less problematic, and could admit the use of the stronger concept of meaning instead of relying on that of information (in the minimal sense). However, such perspective does not involve higher-level phenotypes, so it would still be unsuitable to cover some of the implications of the present analogy, that is intended to deal with all kinds of biological traits which can show some degree of plasticity, from proteins to behavioural aspects.

This is the reason why I am insisting on the idea of information, and I designed the analogy in the light of what has just been said about information sources and transmission. Indeed, I believe it is a good strategy for dealing with the high density of issues related to meaning and semantics without losing important parts of my theory along the way. Shannon’s idea of information is good enough to keep the discussion at a broader and less theoretically problematic level. In addition, it applies to the causal role of both genes and the environment in the production of a whole-organism

⁶⁵Godfrey-Smith, P. (2007). Information in biology. In Hull, D. L. and Ruse, M., editors, *The Cambridge Companion to the Philosophy of Biology*, pages 103–119. Cambridge University Press.

⁶⁶Godfrey-Smith, P. and Sterelny, K. (2016). Biological Information. In Zalta, E. N., editor, *The Stanford Encyclopedia of Philosophy*. Metaphysics Research Lab, Stanford University, Summer 2016 edition.

⁶⁷Barbieri, M. (2003). Biology with information and meaning. *History & Philosophy of the Life Sciences*, 25(2):243–254.

⁶⁸Godfrey-Smith, P. (2007). Information in biology. In Hull, D. L. and Ruse, M., editors, *The Cambridge Companion to the Philosophy of Biology*, pages 103–119. Cambridge University Press.

phenotype (Godfrey-Smith, 2007)⁶⁹. In other words, this solution is not invalidating the general structure of my analogy, in the sense that it does not limit its heuristic potential, nor does it require too many corrections to the salient features of polysemy I pointed out in the previous section.

These remarks on meaning and information allow us to reconsider some assumptions of wide-ranging theoretical enterprises like biosemiotics, and their intrinsic limits. In the case of biosemiotics, its entire theoretical scaffolding invests too much on the idea that meaning actually exists in biological systems (in contexts other than human language and cognition), which is perhaps a too problematic postulate, inducing a huge quantity of conceptual implications of difficult treatment. Furthermore, biosemiotics is a too broad research program, to such an extent that it resembles an all-encompassing philosophical and scientific framework. This is why I am not proposing a biosemiotic theory, nor am I arguing in favour of its assumptions, although I also share its primary aim of improving the dialogue between life sciences and linguistics. Differently from biosemiotics, I consider some notions drawn from information theory as the ideal middle ground between these two fields.

Keeping this in mind, it is now time to introduce the analogy by presenting two extended, slightly modified definitions of polysemy and phenotypic plasticity. While the following definitions are mostly relying on those I have already mentioned, they are adjusted on the basis of the “conceptual downgrade” from meaning to information. As should be clear by looking at these propositions, although it may sound somewhat paradoxical, much of what has been said about polysemy is preserved, even if it is not dealing anymore with the concept of meaning. Indeed, although polysemy is obviously related to the concept of meaning ever since its original definition and etymology, the downgrade to the information level requires only minimal changes, so I keep using the original word at the expense of perhaps more precise, yet much less elegant neologisms (which could also be misleading for readers of the previous chapters). In other terms, I do not use words such as “polyinformation” instead of polysemy. Here are the updated definitions of *polysemy* (**A**) and *phenotypic plasticity* (**B**):

⁶⁹Godfrey-Smith, P. (2007). Information in biology. In Hull, D. L. and Ruse, M., editors, *The Cambridge Companion to the Philosophy of Biology*, pages 103–119. Cambridge University Press.

Definition A: *the property of a word to recall multiple, related information by combining the state of the word itself, the state of other words which are parts of its co-text, and the state of extra-linguistic contexts. The global information resulting from the interaction of linguistic and extra-linguistic partial information is then received and used in some ways by other speakers;*

Definition B: *the property of a genotype to produce multiple, alternative phenotypes by combining the state of its own DNA sequence, the state of other related sequences of the genome, and the state of environmental variables. The resulting phenotypes are a kind of information which is then sifted by natural selection, that determines the increase or decrease of the frequency of genes involved in that phenotype.*

Many reflections spontaneously arise from these modified definitions. In the first place, let us start with a detail. More specifically, an apparent difference: the relatedness among alternative resulting information linked to a single word is clear in the definition of polysemy, but it is not made explicit in that of plasticity. Nevertheless, it should be recalled that all phenotypes that are based on a single genotype are in fact related to each other, either in a continuous way (e.g., see the case of dragonflies wing length in the plot of Figure 2.1) or in a discrete way (e.g., the presence or absence of the “cap” in small crustaceans of the *Daphnia* genre).

As for more crucial theoretical aspects, it should be noticed that I adjusted these propositions so that the information coming from two different sources (i.e., genetic and environmental in the case of plasticity, linguistic and contextual in the case of polysemy) merged into a single, more complex signal, namely the resulting phenotype or a “global” linguistic information. In other terms, given certain inputs from different sources, the global information resulting from the combination of such inputs is what is ultimately transmitted through the signal. This choice makes the updated definitions more akin to the context of classical information theory. Indeed, similarly to some notable authors like John Maynard Smith (2002)⁷⁰ and Eva

⁷⁰Maynard Smith, J. (2002). The Concept of Information in Biology, pages 689–699. Springer Netherlands.

Jablonka (2002)⁷¹, I take my inspiration directly from the famous model of linear communication originally developed by the already mentioned Claude Shannon, together with Warren Weaver (Shannon and Weaver, 1998)⁷². Again, it is important to repeat that there is no claim to assert that there is a perfect match between this fortunate model of communication technology and the structure of my analogy. As specified at the beginning of this section, misalignments are just around the corner. Nevertheless, I think that the similarities/differences ratio plays in favour of this comparison.

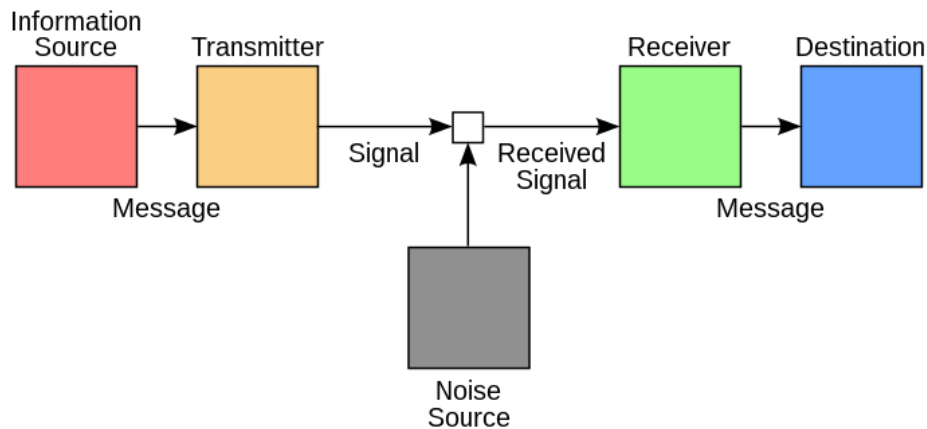


FIGURE 3.1: The original Shannon-Weaver model of communication.

The original Shannon-Weaver model (see Figure 3.1) is a simple representation of the process of transmission of information, and it consists of several steps. The starting point is an *information source*, that is what produces the message to be transmitted. The *transmitter* operates on the message in order to make it suitable to be transmitted through a *channel*, that is the medium used to transmit the signal. When the signal is transmitted through the channel, it may be perturbed by a noise source before reaching a *receiver*, whose role is to reconstruct the message from the signal. Finally, the *destination* is the person or thing to whom the message

⁷¹Jablonka, E. (2002). Information: Its interpretation, its inheritance, and its sharing. *Philosophy of Science*, 69(4):578–605.

⁷²Shannon, C. E. and Weaver, W. (1998). *The mathematical theory of communication*. Univ. of Illinois Press, Urbana.

was sent (Shannon and Weaver, 1998, pp.33-34)⁷³. I rely on some of the key characteristics of this basic description to sketch a couple of schemes which represent the transmission of information in the context of human language and biological systems, respectively. In doing so, I emphasise the common mechanism underlying polysemy and plasticity, based on the two definitions given in this section. I first describe both these models individually, pointing out some of their general aspects, then I move on to a comparison between them.

The diagram below (Figure 3.2) recalls the steps of the Shannon-Weaver model, adapted to the transmission of alternative information coming from a single word and addressed to one or more *hearers* (if one considers the evolution of this alternative information over time, the entire community of speakers). One of the major departures from the standard structure of the model is the parity attributed to both information sources (i.e., words and contexts). Indeed, in the case of human communication, *words* used and their *co-textual surroundings* are considered one source of information, while the extra-linguistic, *contextual variables* are equally important to complete the information directed to the destination. Then, the message transmitted through the signal, namely the result of the confluence of information from the two complementary sources, is some kind of global *linguistic information*, that is, what we previously called meaning, which is capable of influencing the behaviour of hearers/speakers, both in the short and in the long term. In other words, there is an equal contribution from each source, so none of them can be regarded as noise, but rather as an integral part of the model. Finally, as could be easily noticed, this simple diagram and the idea on which it relies neglect for simplicity the encoding and decoding steps, which are normally performed, respectively, by the transmitter and the receiver.

⁷³Shannon, C. E. and Weaver, W. (1998). The mathematical theory of communication. Univ. of Illinois Press, Urbana.

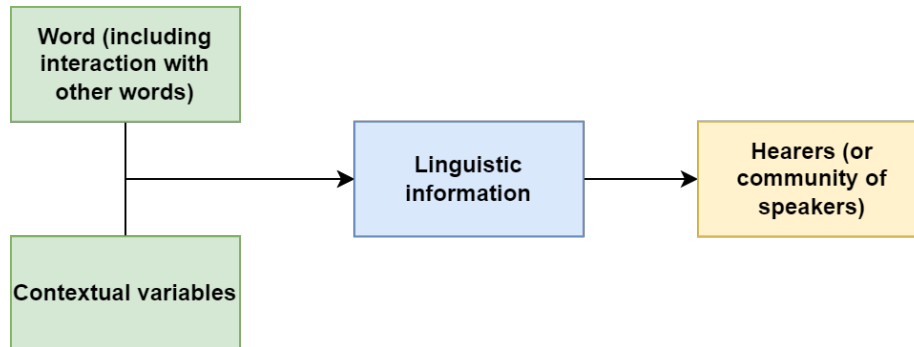


FIGURE 3.2: A simplified representation of the Shannon-Weaver linear communication model adapted to polysemy.

Similar considerations can be done for the biological side of the analogy (see Figure 3.3). As just shown for polysemy, this is another case where the two information sources make an equal contribution to the determination of the outcome: on the one hand, a particular *genotype* and the *network of other genetic loci* interacting with the former; on the other hand, all possible *environmental variables* (e.g., temperature, diet, and so on). An interesting thing when talking about plasticity is that it has long been regarded as the result of background noise generated by the environment, based on the assumption that all non-genetic factors are nothing but simple sources of perturbation of the “true” message encoded by the genome. Since some scholars have already criticised explicitly this misleading idea (Pigliucci, 2001, p.4)⁷⁴, the attribution of a causal parity to both genes and the environment in this diagram is just a visual representation of their criticism. So, in this model, both these complementary sources transmit their information, which is then combined into aggregate information, *phenotype*. The last step is perhaps more subtle, and is related to evolutionary dynamics: since phenotype is “readable” by a totally unconscious and blind mechanism, namely *natural selection*, that is responsible for the differential reproductive rates of genetic variants (based on the phenotype they build in concert with the environment), natural selection itself may be interpreted as the destination of this biological “message”.

⁷⁴Pigliucci, M. (2001). Phenotypic plasticity. Syntheses in ecology and evolution. Johns Hopkins University Press, Baltimore, Md.

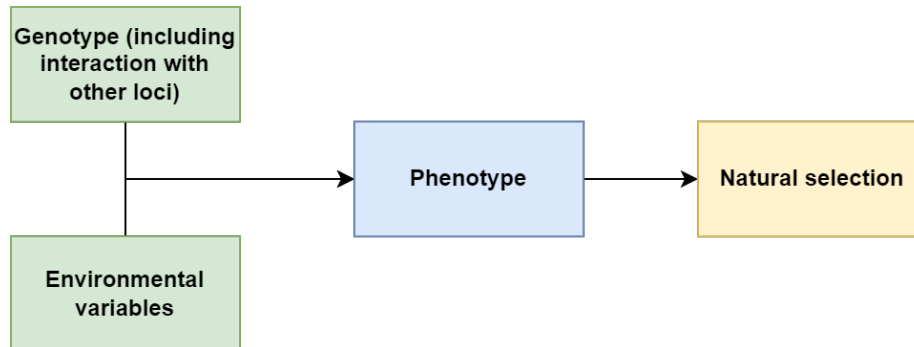


FIGURE 3.3: A simplified representation of the Shannon-Weaver linear communication model adapted to plasticity.

As for the unusual destination reported in the diagram (and in Definition B), it is in line with the idea of natural selection proposed by Maynard Smith (2002)⁷⁵, that is, a mindless process which “produces” genomes that are more able than others to survive and replicate within a given environment in a totally mechanical manner. Differently from the case of human language, there is no way this mechanism can *understand* any message, but it is not important at all for our aims: it is sufficient that it can make mechanical decisions, no more than a light switch. Thus, natural selection is similar to an abstract “judge” of phenotypic information, that is the only type of information it is able to “read”.

After this short overview, it is necessary to talk about these two models adopting a comparative approach. In the perspective suggested by the analogy, all different genomes in nature are considered sequences containing some sets of elements endowed with an informational value. These sets of elements are reciprocally interacting genes, both regulatory and structural. Each gene has an evolutionary origin, usually far back in time, and several variants, which are also products of evolution. The fact that genes are subject to change over time is of critical importance, because it implies that each different gene is specialised in transmitting different information when compared with other genes, while some genetic variants of the *same* gene (i.e., some alleles) can widen or narrow the range of alternative, yet related information transmitted. The latter phenomenon corre-

⁷⁵Maynard Smith, J. (2002). The Concept of Information in Biology, pages 689–699. Springer Netherlands.

sponds to a change in the reaction norm (see Section 2.1), that is the spectrum of all potential information on the phenotype that the considered genotype can transmit in response to the environmental gradient, and whose width is regulated by natural selection and the other evolutionary forces. Put another way, genotypic information, that is the genotype including all interactions with other regions of the genome concurring in the determination of a specific phenotypic trait, is combined with environmental information transmitted by a wide variety of environmental variables to produce a global information, phenotype. Then, phenotype is ready to be “read”, that is, for instance, decreased or increased in frequency by the blind mechanism of natural selection. This, in short, is the biological side of the analogy. Let us compare what has just been said, point by point, with concepts drawn from the theory underlying polysemy and from my reinterpretation of that theory presented in this section.

Human alphabetic languages contain as well some elements which can be regarded as information sources, i.e., words. Different words have different historical origins, and each word usually contains different information when compared to the others. Furthermore, the *same* word can undergo some changes, so the width of the range of potential alternative information it can transmit may either increase or *decrease*, as discussed next. This process is strictly related to the concept of meaning potential introduced in Section 3.3, and these ranges of possible information are *analogous* to reaction norms. So, just like it happens with genes, the range of potential informational contribution is the outcome of some kind of evolutionary process, where the community of speakers selects unconsciously the width of such range on the basis of the current communicative necessities. Independently by that coming from the considered word, the information transmitted by linguistic factors also includes the contribution of all other words which constitute the surroundings of that word once it is placed within a sentence (its co-text). This linguistic information is then combined with that transmitted by the utterance context, that may be extremely changeable from time to time. Finally, the resulting combined information (before the clarifications contained in this section, we would have simply called it “meaning”) is ready to be received by other speakers, whose collective linguistic behaviour provides the pressures affecting the information potential of the considered word in the long term.

To put the analogy into a real biological scenario, I propose a very specific example, which is also experimentally investigated in the last chapter. Indeed, a case study that is particularly suitable for showing that even some single genetic loci may behave *exactly* as polysemous words is represented by a widely studied locus that has been reported to be sensitive to several environmental cues (e.g., diet). The name of the corresponding gene is *eud-1*, that encodes the enzyme sulfatase in the nematode species *Pristionchus pacificus*, an exemplary organism known for showing a morphological and behavioural plastic response related to its mouth form. The wild-type allele of *eud-1* enables the developmental decision inducing one of two alternative mouth forms in response to the ecological context experienced by each worm (Ragsdale et al., 2013; Dardiry et al., 2023)⁷⁶⁷⁷. In particular, such binary decision depends on the interaction between this critical locus and its genomic co-text, as it is part of a regulatory network. So, the functioning of *eud-1* is comparable to that of a polysemous word with two available “meanings”. For more details on *eud-1* and this remarkable nematode species, see Chapter 5.

In a nutshell, this is the theoretical framework within which I am placing my investigations on phenotypic plasticity, proposing a different manner of thinking about it. This description of the basic elements of the theory paves the way for some possible issues and criticism, as well as for some heterogeneous, yet complementary insights. One possible way to discuss these issues and suggest these insights is to spend a little more time refining the analogy with some further conceptual clarifications. More specifically, I want to focus on two important points.

As stated several times throughout these pages, there is a substantial difference between *genes* and *alleles*. Just as a reminder, genes are segments of a chromosome producing a particular polypeptide, regardless of the variations which can be found by comparing the same segment from different organisms of the same species. So, different genes have a different position in the genome, i.e., they are

⁷⁶Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

⁷⁷Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

different segments. Alleles are just alternative forms of the same gene, and are the result of some changes in its sequence (e.g., induced by mutations). Now, different words are exactly like different genes, so as each word has its information (i.e., “meaning”) potential, each gene has its reaction norm. Since the match between alleles and their linguistic counterparts is not as perfect as that, it requires some further details. In this perspective, a linguistic allele of a word is the same word, but with some possible variation in its information potential. The misalignment with biology is the following: the sequence of letters composing a word (in an alphabetic language) is usually kept unchanged even when some changes in its information potential occur, which is not the case with DNA. Indeed, one of the basic facts about genetics is that each particular coding sequence is strictly related with its protein products, as well as with the range of possible phenotypes induced by those products. So, different reaction norms imply different sequences (of the same genes).

While this issue represents a clear, yet still minor difference between the two domains (because it does not affect any relevant feature of the analogy), there is at least another aspect deserving some further deepening. The discussion on the positive correlation between the date of origin and degree of polysemy in each word (Section 3.3) highlights an important characteristic of polysemous words: they tend to evolve by cumulative addition of new information which overlaps with the preexisting one. This consideration allows to grasp the similarity with one of the *two* possible outcomes observable when considering the evolution of plasticity, that is, from a more constitutive condition to an increased degree of plasticity, meaning that genes may increase the quantity of alternative potential information inducing different phenotypic traits. This supports the analogy, but also raises a question: are there in linguistics some cases showing the reverse dynamics? Or, to put it another way, is it possible for a word to *lose* some previously acquired meanings, just like plasticity may be replaced by deterministic responses (as observed in the Baldwin effect), rather than accumulate newer ones over time?

I think the answer is affirmative, as we can see from a couple of the many possible examples of what linguists call *semantic restriction*. Just consider the English word “art”: in the 13th century,

it was used to denote “skill” in a very broad sense, including as a result of knowledge and experience, as well as to refer to *trivium* and *quadrivium* sciences. From the 16th century onwards, it has started to be used to refer to the application of skills to painting, sculpture, or architecture. So, its information potential has been *reduced* over time (Oklah, 2014)⁷⁸. The same reasoning applies to the Italian word “spezie”: in the present days it is used to refer to spices, whereas in the Middle Ages it was also used to refer to the human species. So, it seems that this aspect is also respected by the analogy, which could also suggest further comparisons between this process of restriction and biological mechanisms like the Baldwin effect. Interestingly, returning to the example of the *eud-1* gene, also in that case researchers have shown that several mutations affecting that locus can make the developmental response independent from environmental cues (Ragsdale et al., 2013; Casasa et al., 2023)^{79,80}, narrowing the “meaning potential” of that particular mutated genotype. As I do in Chapter 5, it could be hypothesised that mutations reducing the polysemy of this gene may also occur in nature, and could then be selected in response to some specific pressures.

Of course, both details and controversial elements might be many more than those reported here. I argue that some of the theoretical strengths of this way of looking at things, apart from the necessary attention to be paid to the problematic concept of meaning, may already be seen from this general introduction to the “theory” of the *polysemy of genes*. Talking about genes, words, and their respective functioning simultaneously is not just an alternative way to present very technical biological issues to those who are not biologists. I think there are several communicative, philosophical, and scientific reasons why this idea may be regarded as truly useful.

Indeed, the idea of the polysemy of genes is more consistent with current biological theory than other existing perspectives, since it combines the centrality of genes and natural selection (that is already a cornerstone of the gene’s eye view) with processes underlying the shaping of phenotype via complex interaction among genes

⁷⁸Oklah, S. (2014). Semantic change.

⁷⁹Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

⁸⁰Casasa, S., Katsougia, E., and Ragsdale, E. J. (2023). A mediator subunit imparts robustness to a polyphenism decision. *Proceedings of the National Academy of Sciences*, 120(32).

and between genes and the local environment, highlighting the fact that these two aspects are *not* in opposition, as it could seem at a first sight, but are rather *deeply interrelated*. Evolution and development are really two faces of the same coin. The fundamental units of heredity contain information for a range of alternative developmental outcomes, which are determined together with the information coming from the environment (Sultan, 2017)⁸¹, and that range can be regulated by natural selection. From a philosophical point of view, the general idea underlying the polysemy of genes is a way of emphasising the fact that genetic heredity, although it has some constraints, cannot be reduced to the biochemical support of an inescapable fate. Genes and genomes are more complex than it was thought decades ago, so our view on what they represent needs to be updated. Furthermore, this perspective holds even without considering polysemy and plasticity as two properties obeying to a common set of rules, i.e., without assuming the necessity (or even the possibility) of a unique, higher-level theory of context-dependence shared by biology and human societies.

I think that designing analogical models aimed at showing in a different light biological phenomena like phenotypic plasticity could be a fascinating way in which philosophy and science can communicate and prove their possible continuity. Showing this continuity is the main objective that guided both the realisation of this thesis and the construction of the model I have just presented in these chapters, with the hope that it could provide a good basis for future, valuable interactions between these two fields. In particular, a promising starting point could be the above-mentioned causal parity between information coming from the genome and information coming from the environment. In fact, other than being one of the most important hints this analogy can uncover, it also suggests a further theoretical background underlying the processes of composition of both linguistic and phenotypic information, that is, *complexity science*. The theory of language dealing with polysemy has already shown that it is possible to consider words, co-texts, and contexts as equally contributing components affecting the global behaviour and outcomes of a linguistic system through the spontaneous establishment of temporary states of stability (i.e., *attractors*) (La Mantia,

⁸¹Sultan, S. E. (2017). Developmental plasticity: re-conceiving the genotype. *Interface Focus*, 7(5):20170009.

2012, pp.139-140)⁸². All these concepts represent a crucial connection with biology, at least since Conrad Waddington introduced *epigenetics* in 1942, that is a term he used to indicate all multiple interactions between the environment and genes leading to the development of phenotype (Tronick and Hunter, 2016)⁸³.

Multiple entities, spontaneous order, and patterns emerging from interaction, are all defining characteristics of *complex systems*, so the next step to be taken to further strengthen the link between linguistic and biological context-dependence is the use of principles and methods belonging to complexity science to investigate phenotypic plasticity and its evolution from a different perspective. Indeed, I argue that complexity is the core of the association between polysemy and plasticity. This is the reason why in the second, more technical section of the thesis, I introduce one of the approaches which are typically used for the study of complexity, that is, *agent-based modelling*. In Chapter 5, this technique is used to simulate the evolution of a digital population including two alternative strains whose ability to respond to the environment through a developmental decision depends on the state of the aforementioned polysemous gene *eud-1*. I consider this computational and evolutionary application of the theoretical framework proposed so far as an essential part of my work, as I believe it may represent a further demonstration of the possible continuity between philosophical arguments and scientific modelling.

⁸²La Mantia, F. (2012). Che senso ha? Number 2 in Semiotica e filosofia del linguaggio. Mimesis, Milano, Udine.

⁸³Tronick, E. and Hunter, R. G. (2016). Waddington, dynamic systems, and epigenetics. *Frontiers in Behavioral Neuroscience*, 10.

Chapter 4

Understanding life through computer simulations: an agent-based approach

Introduction

The study of evolution is much more than a reconstruction of the history of life on Earth, although the latter is certainly a critical part of this area of biology. Indeed, since evolution may be basically regarded as a huge, uninterrupted process of probabilistic nature resulting from a combination of chance and several mechanisms with a well-defined functioning, its fundamental dynamics may also be isolated and replicated *outside* their original context of application.

This is the reason why, despite their complexity, evolutionary events may be considered as manifestations of a sound mechanistic core. Both empirical and theoretical knowledge acquired on this core may be used to build computational models to *simulate* evolution digitally. One of the most fruitful approaches in computational biology is to programme some individual components with an automatic behaviour, which is based on the real behaviour of their biological counterparts (e.g., molecules, cells, or full organisms), and let them interact with each other spontaneously within a virtual environment.

These are the basic principles underlying *agent-based modelling*, a technique that allows to deal with *complex systems* and replicate evolutionary dynamics efficiently. Since one of the aims of this work is to represent the change over time of plastic phenotypic responses computationally, it is now of crucial importance to provide an overview of the general aspects of this kind of modelling. Indeed, this particular method of investigation is extensively applied

throughout the entire Chapter 5, that is a purely experimental chapter.

In Section 4.1, I briefly introduce the basic characteristics of complex systems and the pivotal concept of *emergence*, focusing on the application of these ideas in biology and, more specifically, in evolutionary research.

In Section 4.2, I introduce the fundamental concept of *algorithm*, then I provide a concise historical background of the ideas underlying the fields of *artificial life* and agent-based modelling, starting from some of the main philosophical concepts embedded in these areas. I also introduce for the first time NetLogo, a software that was specifically designed to implement agent-based models and that I use for my simulations, and *agents*, which are NetLogo interacting entities.

In Section 4.3, I describe NetLogo features in a more detailed way, ranging from the elements of the Interface, that is used to interact with models, to some hints on its programming language.

In Section 4.4, I introduce my case study, that is based on preexisting information on a nematode worm species showing genetically inherited phenotypic plasticity, both morphological and behavioural.

4.1 Complex evolutionary systems

One of the main assumptions of computational biology is that the relevant dynamics of biological systems may be simulated by computers. In other terms, it is possible to represent nature by means of models whose functioning is based on a number of code lines, then perform simulations to grasp something about the modelled phenomenon, drawing remarkable benefits from increased control over the system and capacity to repeat each experiment numerous times (Taylor and Jefferson, 1993)¹.

What is most important about these simulated systems is that they isolate and preserve, with some degree of fidelity, the main characteristics of their real counterparts. Obviously, there is no way a computational model may reproduce every detail of reality, which is not a problem at all. Indeed, that would not be really useful from

¹Taylor, C. and Jefferson, D. (1993). Artificial life as a tool for biological inquiry. *Artificial Life*, 1:1–13.

a scientific point of view, while it is often much more valuable to have a simplified and scaled version of the considered system. One of the most suitable approaches for building such models is that of simulating the activity of organisms and other biological entities by making them to co-execute some sets of commands, and letting them “free” to generate systemic outcomes from bottom up, i.e., from a lower to a higher level of organisation.

Since biology is the perfect case of a *multilevel* science, where many interacting units contribute to determining aggregated phenomena and properties, it follows that biology is highly compatible with this particular type of computational treatment. As a matter of fact, there are at least four levels of biological organisation: the molecular level, the cellular level, the organism level, and the population-ecosystem level (Taylor and Jefferson, 1993)². This suggests that an all-encompassing understanding of life is strongly dependent on the investigation of the intricate interplay generated by heterogeneous sub-units operating at each of these levels. For this reason, life itself may be seen as a combination of a huge amount of nested *complex systems*.

What has just been said about the layered structure of life, i.e., the importance of the simultaneous and collective activity of multiple, different sub-units, is one of the defining traits of all complex systems. Regardless of whether they are biological systems or not, they are all the result of interactions among their components. Nevertheless, the fulfillment of this essential requirement is technically not sufficient to define a complex system. Indeed, even a desert is the product of the interaction of many parts (i.e., the grains of sand), but it would not be regarded as a good example of a complex system by researchers in this broad area, because the interaction among grains is trivial: each of them is just physically stacked on top of each other (Gandolfi, 1999, p.17)³. A real complex system is much more than that.

In the first place, it should have the capacity for self-regulation through *feedback* mechanisms, which means that some events resulting from the activity of the system can change the overall behaviour of the system itself dynamically, either reinforcing or repressing its

²Taylor, C. and Jefferson, D. (1993). Artificial life as a tool for biological inquiry. *Artificial Life*, 1:1–13.

³Gandolfi, A. (1999). *Formicai, imperi, cervelli. Introduzione alla scienza della complessità*. Bollati Boringhieri.

current activity. The self-regulatory capacity of complex systems is also expressed by their *robustness*, that is the property of restoring, at least partially, a previous state following perturbations. Furthermore, although the behaviour of these systems is influenced by a significant amount of *stochasticity*, they are also capable of generating some clear regularities and trends, then they also show a higher-level *order*, that is reached without any form of *centralised control* directing the system regardless of the activity of its individual parts. Another common characteristic of these systems is the presence of *non-linear* phenomena, that is, when variations in some variables regulating the system are not proportional to the effects induced by such variations. Above all, the process of *emergence* is of critical importance for all complex systems. Emergence is their fundamental property that is observed when new, collective behaviours (i.e., *emerging patterns*) are spontaneously generated by the interaction among multiple elements of the system (Zheng, 2020)⁴. In other words, the fact that each individual, local activity performed by these multiple sub-units can result in remarkable global changes at the system level is a determining factor in the evolution of complex systems (Prigogine and Stengers, 1988, p.203)⁵.

Although this kind of systems, on closer inspection, are found everywhere in nature, ranging from atomic to astronomical systems, biology is particularly full of cases that satisfy all conditions listed above. Any level contains a lot of plausible examples of that. Just to mention a few: the process of polymerisation of proteins, animal mating systems, cell osmosis, or the equilibrium of allele frequencies over time in a population. In the context of this work, complex systems dealing with evolutionary processes are clearly the main focus, and there are several good reasons to stress the great importance of modelling these processes computationally in order to understand their emerging dynamics and regularities. Other than the aspects mentioned at the beginning of this section (i.e., computer simulations are easily repeatable, manipulable, and subject to experimental control), there is a point that is specifically applicable to evolutionary complex systems.

Except for some case studies of simple organisms with very short life cycles and asexual reproduction, like *E. coli* bacteria (Lenski,

⁴Zheng, Z. (2020). An Introduction to Emergence Dynamics in Complex Systems, pages 133–196. Springer Singapore.

⁵Prigogine, I. and Stengers, I. (1988). Order out of chaos. Bantam Books, Toronto.

2017)⁶, evolution is usually an *extremely* slow process, too slow to allow human observation and experimentation (Ofria and Wilke, 2004)⁷. So, scientists who want to make predictions in this field *need* to use some models. In particular, some computational techniques based on modelling the interaction among organisms and their genetic, environmental, or behavioural variables through *algorithms* are of huge usefulness for biologists, because they allow to replicate most key features of complex evolutionary systems in a relatively simple, but still highly valuable way.

4.2 Algorithms and biological machines from antiquity to agent-based models: an overview

Since algorithms are the building blocks required for the conversion of any natural complex system into a computational model, they must be clearly defined first. A possible definition is the following: an algorithm is an ordered, finite sequence of steps aimed at solving a class of problems. More specifically, it is useful to provide a criterion to recognise algorithms that is based on the Church-Turing thesis: an algorithm is anything that can be implemented with a Turing machine. In other terms, algorithms are very precise instructions which could be executed automatically by a machine. Indeed, computer *programs* are algorithms written in a specific *programming language*, that is a formal language with its own syntactic rules. However, algorithms can be executed by humans as well, which is clear when considering their earliest occurrences in the form of mathematical “recipes”, written on stone by the ancient Babylonians and used to solve arithmetical problems, or simply by looking at any child who learns how to calculate the result of additions or subtractions in column. These basic examples give an idea of how old and widespread the use of algorithms is.

The modelling approach based on the implementation of algorithmic organisms as computational units for simulations underlies two long-lasting philosophical ideas of huge relevance: the Cartesian perspective considering living beings (except for humans, at

⁶Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, 11(10):2181–2194.

⁷Ofria, C. and Wilke, C. O. (2004). Avida: A software platform for research in computational evolutionary biology. *Artificial Life*, 10(2):191–229.

least for what concerns their mind) as machines whose functioning depends only on an automated behaviour (Descartes, 1909, pp.200-202)⁸, and Leibniz’s dream of building some calculators which could replace men’s work in automatic activities. The combination of these famous concepts strongly recalls the theoretical foundations of several present day areas of investigation. Currently, the most important ones are *artificial life* and *agent-based modelling*, which are both of critical importance for this work and are described more in depth next in this section. However, these two fields are relatively recent, and are preceded by some prominent conceptual ancestors.

The first remarkable application of algorithms as instructions encoding behaviour of computational units is undoubtedly represented by *cellular automata* (from here on, CA). The original intention of one of the creators of CA, the Hungarian mathematician John von Neumann, was that of producing some machines that would be capable of *self-replicating*, in order to benefit from their exponential growth in some hypothetical large-scale spatial mining missions (Wilensky and Rand, 2015, p.433)⁹. Together with his colleague Stanislaw Ulam at the Los Alamos National Laboratory, von Neumann invented a simplified, two-dimensional system composed by self-replicating entities. These entities were cells on a lattice, and the rules for their self-replication were implemented through algorithms. One of the simplest, yet still admirable applications of CA is John Conway’s Game of Life, that was developed in 1970 (Wilensky and Rand, 2015, pp.433-434)¹⁰.

Like von Neumann and Ulam’s CA, Conway’s CA are two-dimensional square cells that are part of a grid. Each cell has exactly eight other cells in its immediate surroundings, which are called *neighbours*. Each cell of the grid may be either on (i.e., alive) or off (i.e., dead). The instructions describing any possible interaction between a cell and its neighbours from each cell’s point of view are the following:

1. if you count exactly three neighbours which are on, turn on;
2. if you are on and you count two or three neighbours which are

⁸Descartes, R. (1909). Oeuvres de Descartes. Leopold Cerf, Imprimeur-Editeur.

⁹Wilensky, U. and Rand, W. (2015). Introduction to agent-based modeling. The MIT Press, Cambridge, Massachusetts.

¹⁰Wilensky, U. and Rand, W. (2015). Introduction to agent-based modeling. The MIT Press, Cambridge, Massachusetts.

on, stay on;

3. in all other possible situations, turn off.

The most surprising thing about this simple computational system is its capacity of generating several unexpected emerging patterns (Wilensky and Rand, 2015, p.434)¹¹. Sequential generations of cells produced by the system and its algorithmic rules give rise to a number of different, yet recurring configurations (see Figure 4.1) with a variable degree of complexity, that give the impression of moving on the lattice. Higher-level regularities and suggestive global dynamics based on a minimal set of individual instructions are a significant, distinguishing aspect of this seminal model.

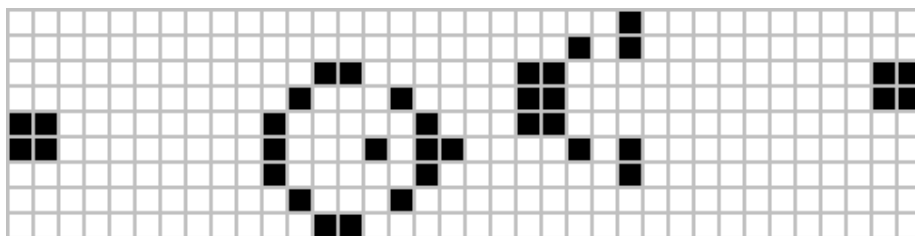


FIGURE 4.1: One of the recurring patterns in Conway's Game of Life.

The same principles have also been applied to entities more similar to “real” organisms than two-dimensional cells. A remarkable example of this approach is Richard Dawkins’ computer program named *The Blind Watchmaker* (Dawkins, 2003)¹². The program was developed to simulate the fundamental processes of Darwinian evolution, producing generations of animal-like computational individuals with surrealistic shapes by using simple algorithmic procedures which describe reproduction (i.e., genetic inheritance, including some probability of mutation) and development, and leaving the user free of selecting the proposed morphs according to his aesthetic taste, that is a form of artificial selection. The outcomes of several iterations of these procedures are hordes of very evocative *biomorphs*, following their author’s terminology.

¹¹Wilensky, U. and Rand, W. (2015). Introduction to agent-based modeling. The MIT Press, Cambridge, Massachusetts.

¹²Dawkins, R. (2003). The evolution of evolvability, pages 239–255. Elsevier.

Now, Dawkins' attempt of modelling life through algorithms is more like a basic representation of evolution for didactic aims, but that is just one of the possibilities. Indeed, similar methods may be applied to evolutionary research as well, as shown by the development of Avida in 1993. Avida is a software platform that was specifically designed both to perform evolutionary experiments involving populations of self-replicating computer programs, and to analyse the experimental outcomes accurately, including some tools for statistics and for examining lines of descent. In Avida, each organism has its own digital genome (i.e., a computer program) and interacts with an environment (Ofria and Wilke, 2004)¹³, so the underlying approach is very close to the most appropriate for this research.

Generally speaking, all case studies considered so far, that is, CA, Game of Life, biomorphs, and Avida experiments, are strictly related to the aforementioned area of artificial life (or ALife). However, the origin of ALife as a coherent and unified field of investigation dates back to a multidisciplinary workshop organised by theoretical biologist Christopher Langton in 1989, which is later than some of the proposed examples. The first, significant developments of this wide discipline were catalysed by the introduction of personal computers, that made simpler and convenient using computing for scientific aims. In Langton's intention, the key feature of ALife is not just that of replicating real biological systems (which is extremely useful to study topics like the origins of life and sex, adaptation or ecosystems dynamics), since it may be useful as well to understand life as it *could* be, focusing on its functioning regardless of its medium (Taylor and Jefferson, 1993; Macal, 2009)¹⁴¹⁵.

While having its roots in CA, the field of ALife shares much of its assumptions with agent-based modelling (from here on, ABM), to the point that the latter may be regarded as the computational arm of the former (Macal, 2009)¹⁶. Indeed, many of ALife principles are practically implemented in the form of computational models by

¹³Ofria, C. and Wilke, C. O. (2004). Avida: A software platform for research in computational evolutionary biology. *Artificial Life*, 10(2):191–229.

¹⁴Taylor, C. and Jefferson, D. (1993). Artificial life as a tool for biological inquiry. *Artificial Life*, 1:1–13.

¹⁵Macal, C. M. (2009). *Agent Based Modeling and Artificial Life*, pages 112–131. Springer New York.

¹⁶Macal, C. M. (2009). *Agent Based Modeling and Artificial Life*, pages 112–131. Springer New York.

ABM: both of them consider individual entities with a simple, algorithmic behaviour, whose spontaneous interaction generates higher-level order and emerging patterns. Thus, both ALife and ABM may be placed in the context of self-organising systems with a bottom-up determination of outcomes. However, they are not perfectly overlapping, since ABM is a more general modelling approach that does not deal necessarily with lifelike objects as ALife does (Wilensky and Rand, 2015, p.445)¹⁷.

Although ABM is a relatively young area, some models sharing analogous techniques can be found since 1970, especially for the study of social processes. For instance, as for this area of early ABM, a very well-known case study is undoubtedly Schelling’s model of housing segregation (Macal, 2009)¹⁸. Currently, agent-based models are widespread in almost every field, ranging from physics to epidemiology, and are particularly suited to replicating the dynamics of complex systems, since their functioning relies on a methodical “translation” of all principles of complex systems (see Section 4.1) into a computer program.

Apart from the already mentioned aim of generating aggregated behaviours exclusively from the interaction among computational units, the common feature shared by all these models is, indeed, the concept of *agent* itself. Since it might appear controversial, I will now clarify what an agent is in detail. An agent is a computational individual or, in other words, a digital entity obeying some algorithms and showing the following characteristics:

- it is *autonomous*, meaning that its behaviour is only determined by the algorithms assigned to it by its programmer, and not by a common, central control managing the whole system in real time;
- it is *reactive*, that is, it can change its own behaviour or state in response to some interactions with other agents or the environment, as well as induce analogous changes in the latter;
- it is *proactive*, in the sense that it continuously tries to achieve one or more aims programmed by the programmer.

¹⁷Wilensky, U. and Rand, W. (2015). Introduction to agent-based modeling. The MIT Press, Cambridge, Massachusetts.

¹⁸Macal, C. M. (2009). Agent Based Modeling and Artificial Life, pages 112–131. Springer New York.

Of course, the fact that each digital agent follows its instructions all the time during simulations implies that, despite its philosophically evocative name, it is just a component of a calculator and does not show any sign of independence or intentionality.

Agents can replicate all relevant aspects of the behaviour of a wide variety of real world entities, for instance, molecules, bacteria, people infected with viruses, and many others. Due to their high versatility, agent-based models are useful both for educational and research purposes. As a matter of fact, they may be used to carry out virtual experiments, test hypotheses, find analogies between different phenomena by isolating their salient features, or communicate ideas in a more intuitive way.

Currently, there are several available tools which have been designed to create agent-based models and run simulations with them. NetLogo, that was developed by professor of Learning Sciences, Computer Science, and Complex Systems Uri Wilensky (1999)¹⁹ at Northwestern University, is probably the one that can be mastered most easily by experts from different areas of investigation, even without already having a considerable programming background. It is also particularly well-suited to implement digital models which would replicate the fundamental dynamics of complex evolutionary systems, that is a good reason to discuss some of its features in detail in the next section.

4.3 Some basic facts about NetLogo and its functioning

NetLogo is a user-friendly programmable modelling environment that was designed for the implementation of agent-based models. Although it was developed using Scala and Java languages, NetLogo itself is also an original programming language, with its own terms and rules.

When a user downloads NetLogo and opens the relative toolkit, he can interact with three different tabs. The *Interface* tab is used to interact with models and manage simulations. In fact, this tab contains NetLogo world, that is a box where simulations are performed, a multitude of interactive elements like buttons, sliders,

¹⁹Wilensky, U. (1999). Netlogo. Northwestern University, Evanston, IL Center for Connected Learning and Computer-Based Modeling, url = <http://ccl.northwestern.edu/netlogo/>

switches, monitors, and plots, a counter of ticks (i.e., discrete units of time), and several other functions and adjustable settings. The *Info* tab is used by the author of the model (or by any other user) to provide and read information about the modelled phenomenon, the functioning of the model, its relevant features (including some suggestions on interesting simulations to run), references and credits. Finally, the *Code* tab is the real heart of any NetLogo model. It is used by the programmer to write all algorithms that are assigned to agents, open specific packages with peculiar functions, or create variables which can be recalled by name when necessary (e.g., to report some calculated values that may also be used to plot graphs in the Interface).

NetLogo is also characterised by heterogeneous categories of agents, each of them has unique peculiarities and reserved terms. More specifically, there are four different types of agents:

- *patches* are fixed agents, corresponding to square cells on a grid and defined by Cartesian coordinates;
- *turtles* are agents which can move around NetLogo world and are located above patches;
- *links* are agents which are used to represent connections between other types of agents, when considering models with a network structure;
- the *observer* is a sort of a counterpart of the programmer of the model, since it sees all events in NetLogo world and assigns instructions to all other classes of agents.

NetLogo language is relatively simple to learn and use. It consists of *primitive* terms of different kind which, once combined correctly by respecting NetLogo rules of syntax, may be used to create new, *derivative* terms for all modelling needs. All information on primitives and their syntactic characteristics are reported in a Dictionary, that can be easily accessed through NetLogo website. There are four types of primitive terms:

- *commands* have a blue colour and are used to give orders to agents, as well as to indicate plots what to draw and to open some files (e.g., images);

- *reporters* have a violet colour and are used for computing values. They usually correspond to agents characteristics like shape, size, position in the world, identification number, and others, and are also used to make mathematical operations;
- *keywords* have a green colour and are used at the beginning of each code to define agent subsets and specify their characteristics, as well as to list all variables assigned to them or to the whole system. Keywords are also used to begin and end each well-defined block of instructions (i.e., a *procedure*);
- *inputs* have a dark orange colour and are used to insert some numerical values or, when put in inverted commas, to set literal values or labels created by the user.

All other terms, such as derivative ones or names of procedures, have a black colour, except for *comments*. Comments are grey, are always preceded by semicolons, and may be used to explain the functioning of some parts of the code or prevent some lines from being executed (without deleting them).

As for simulations, they may be run either in a discrete mode, that is, executing all code lines just one time, then stopping at each tick, or in a continuous mode, constantly iterating new cycles (unless the user interrupts them or a stop condition written in the code is executed). Other than that, NetLogo integrated tool *BehaviorSpace* was conceived to run multiple simulations, which are of critical importance when the user wants to extract some statistically informative outputs. Indeed, due to the stochastic nature of the simulated systems, it is very unlikely to have identical simulations by running the same model multiple times. Through BehaviorSpace, users can design and perform even huge sets of simulations automatically and obtain a final synthesis of the outcomes of interest in the form of a CSV file, that is readable by spreadsheet programs like Microsoft Excel or other related programs.

Finally, another NetLogo section of some relevance is the Models Library, that is a set of selected models uploaded and tested by NetLogo's developers. All these models are divided by area of investigation, except when they are extracted from specific didactic experiences. However, as a proof of the vitality of ABM, the vast majority of highly reliable models implemented with NetLogo are found directly in the scientific literature of the most diverse fields.

4.4 Modelling the evolution of phenotypic plasticity with NetLogo

As I have tried to make clear throughout this chapter, ABM is an approach of huge usefulness and interest when it comes to simulate complex systems. More specifically, biological evolution is the perfect subject for such a computational treatment, and NetLogo is an appropriate tool to carry out an evolutionary study which is based on complex systems principles.

As might be guessed from these premises, the next chapter has an experimental connotation. Indeed, it is focused on the implementation of an agent-based model involving digital populations of agents whose evolutionary trajectories are studied by performing and analysing large sets of simulations with some simple descriptive statistics. The context is that of the evolutionary interplay between a genotype expressing only one phenotype regardless of environmental factors and a plastic genotype, with a focus on the change in frequency over time of the latter “polysemous” variant.

Although this research is based on a particular species of nematode worm, some of the final results of the investigation could eventually be useful for a better understanding of phenotypic plasticity as a whole. This possibility seems reasonable when considering that this species is more and more recognised as a new model organism in this area of evolutionary biology, joining other famous “colleagues”, like rats or fruit flies, that have often led to great discoveries of general interest.

Chapter 5

Key parameters regulating the evolutionary interplay between plastic and non-plastic strains of the nematode worm *Pristionchus pacificus*

Introduction

Evolutionary change affecting the modulation of sensitivity to environmental stimuli

One of the most widespread abilities of organisms is that of being able to change their appearance, activity, or behaviour in response to variations in environmental conditions. Since all ecosystems are characterised by fluctuations, especially considering the drastic effects of climate change and human impact, the capacity of wild populations to experience rapid phenotypic changes induced by environmental stimuli is of outstanding relevance to decrease extinction risk. Furthermore, this ability may also facilitate adaptation, following a path which is complementary to the slower process of genetic change in response to natural selection (Chevin et al., 2013; Dardiry et al., 2023)¹².

Just to mention a few cases of environmental responsiveness from different kingdoms, *Ganoderma sinense* mushrooms show a

¹Chevin, L.-M., Gallet, R., Gomulkiewicz, R., Holt, R. D., and Fellous, S. (2013). Phenotypic plasticity in evolutionary rescue experiments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1610):20120089.

²Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

remarkable morphological diversity in relation to changes in air pressure, humidity, pH, and other extrinsic factors (Hapuarachchi, 2019)³, some algal species of the genus *Volvox* react to a low-nutrient medium by increasing the colony radius, flagellar length, and collective flagellar beating (Solari et al., 2011)⁴, and eggs from different populations of Port Jackson shark (*Heterodontus portusjacksoni*) reared under elevated temperature regimes produce either individuals with reduced maximum swimming activity or with highly increased resting oxygen consumption rate (Gervais et al., 2020)⁵.

These are all cases of *phenotypic plasticity*. The idea of phenotypic plasticity is at least as old as the distinction between genotype and phenotype itself, which began to be formalised in the early years of the 20th century. In the present days, this pivotal concept is widely recognised by biologists and may be regarded as a relevant component to pursue a greater understanding of evolution and possibly extend the scope of evolutionary theory (Pigliucci, 2010b; Futuyma, 2021; Gomulkiewicz and Stinchcombe, 2022)^{6,7,8}. Phenotypic plasticity is defined as the property of a genotype to produce different phenotypes in response to environmental cues, for instance, by changing developmental trajectories (Pigliucci, 2001; Fusco and Minelli, 2010, p.1)^{9,10}.

This basic definition has at least two fundamental implications: in the first place, genotype may be conceived as a spectrum of multiple, potential developmental outcomes which are environmentally dependent (Sultan, 2017)¹¹. Secondly, as already noticed even by

³Hapuarachchi, K. (2019). High phenotypic plasticity of *ganoderma sinense* (ganodermataceae, polyporales) in china. *Asian Journal of Mycology*, 2(1):1–47.

⁴Solari, C. A., Drescher, K., Ganguly, S., Kessler, J. O., Michod, R. E., and Goldstein, R. E. (2011). Flagellar phenotypic plasticity in volvocalean algae correlates with p’eclet number. *Journal of The Royal Society Interface*, 8(63):1409–1417.

⁵Gervais, C. R., Huveneers, C., Rummer, J. L., and Brown, C. (2020). Population variation in the thermal response to climate change reveals differing sensitivity in a benthic shark. *Global Change Biology*, 27(1):108–120.

⁶Pigliucci, M. (2010). *Evolution, the Extended Synthesis*. The MIT Press Ser. MIT Press, Cambridge.

⁷Futuyma, D. J. (2021). How Does Phenotypic Plasticity Fit into Evolutionary Theory?, pages 349–366. CRC Press.

⁸Gomulkiewicz, R. and Stinchcombe, J. R. (2022). Phenotypic plasticity made simple, but not too simple. *American Journal of Botany*, 109(10):1519–1524.

⁹Pigliucci, M. (2001). *Phenotypic plasticity. Syntheses in ecology and evolution*. Johns Hopkins University Press, Baltimore, Md.

¹⁰Fusco, G. and Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1540):547–556.

¹¹Sultan, S. E. (2017). Developmental plasticity: re-conceiving the genotype. *Interface*

A. D. Bradshaw more than half a century ago, plasticity has a genetic basis, thus it is subject to change over generations and this change can be driven by mechanisms like natural selection or genetic drift (Bradshaw, 1965; Chevin et al., 2013)^{12,13}. This fact is also strongly supported by experimental evidence, as shown by the case of the grass *Sorghum bicolor*, whose plasticity in height is controlled by three genetic loci which are sensitive to variation in the diurnal temperature range (Mu et al., 2021)¹⁴, and by the nematode worm *Caenorhabditis elegans*, where a single-nucleotide substitution in *kcnl-1* gene induces matricidal hatching by repressing responsiveness to egg-laying-related environmental stimuli. In particular, the latter mutation was shown to be maintained in contexts characterised by fluctuations in the availability of food (Vigne et al., 2021)¹⁵.

A consequence of these assertions and findings is that basic evolutionary processes affecting gene frequencies can act as modulators of phenotypic plasticity, because they may reduce, maintain, or enhance sensitivity to environmental variables, depending on the surrounding conditions. In other words, evolutionary processes can modify the range of potential developmental outcomes expressed by a genotype. These modifications happen because, as any other trait, trait plasticity affects fitness, so it may be either locally maladaptive, neutral, or adaptive (Acasuso-Rivero et al., 2019; Scheiner and Levis, 2021; Lee et al., 2022)^{16,17,18}. Assuming that the environmental cues are reliable, adaptive plasticity is a likely outcome in frequently changing environments. In these situations, since the plastic geno-

Focus, 7(5):20170009.

¹²Bradshaw, A. (1965). Evolutionary Significance of Phenotypic Plasticity in Plants, pages 115–155. Elsevier.

¹³Chevin, L.-M., Gallet, R., Gomulkiewicz, R., Holt, R. D., and Fellous, S. (2013). Phenotypic plasticity in evolutionary rescue experiments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1610):20120089.

¹⁴Mu, Q., Guo, T., Li, X., and Yu, J. (2021). Phenotypic plasticity in plant height shaped by interaction between genetic loci and diurnal temperature range. *New Phytologist*, 233(4):1768–1779.

¹⁵Vigne, P., Gimond, C., Ferrari, C., Vielle, A., Hallin, J., Pino- Querido, A., El Mouridi, S., Mignerot, L., Frøkjær-Jensen, C., Boulin, T., Teotonio, H., and Braendle, C. (2021). A singlenucleotide change underlies the genetic assimilation of a plastic trait. *Science Advances*, 7(6).

¹⁶Acasuso-Rivero, C., Murren, C. J., Schlichting, C. D., and Steiner, U. K. (2019). Adaptive phenotypic plasticity for life-history and less fitness-related traits. *Proceedings of the Royal Society B: Biological Sciences*, 286(1904):20190653.

¹⁷Scheiner, S. M. and Levis, N. A. (2021). The Loss of Phenotypic Plasticity Via Natural Selection: Genetic Assimilation, pages 161– 181. CRC Press.

¹⁸Lee, U., Mortola, E. N., Kim, E.-j., and Long, M. (2022). Evolution and maintenance of phenotypic plasticity. *Biosystems*, 222:104791.

type has a higher fitness across fluctuating conditions, there is a strong selection pressure in favour of plasticity (Ghalambor et al., 2007; Acasuso-Rivero et al., 2019)¹⁹²⁰. Conversely, environments with infrequent variation or unpredictable patterns of variation select for genetically fixed, constitutive traits. As a matter of fact, a reduction in the degree of plasticity by genetic mitigation of the environmental sensitivity may be beneficial when plasticity is maladaptive, i.e., when changes induced by the environment move the mean phenotype further from the optimum (Acasuso-Rivero et al., 2019; Storz and Scott, 2021)²¹²². Nevertheless, selection may also reinforce, rather than repress, a plastic response. This happens when a genetic change in the same direction of the environmentally induced change is selected, a scenario in which plasticity precedes and facilitates adaptation (although this is not common in the case of adaptation to a new environment) (Ho and Zhang, 2018)²³. Finally, plasticity may have no effects on fitness, so its evolutionary path is likely to depend on stochastic mechanisms such as genetic drift (Gomulkiewicz and Stinchcombe, 2022)²⁴. Therefore, biological systems involving the evolution and modulation of plastic responses may produce a wide range of alternative outcomes, that can only be explained by considering the intricate interplay between environmental cues, developmental pathways, and genetic loci with different degrees of sensitivity to contextual factors. For a more in-depth introduction on phenotypic plasticity, see Chapter 2.

¹⁹Ghalambor, C. K., McKay, J. K., Carroll, S. P., and Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21(3):394–407.

²⁰Acasuso-Rivero, C., Murren, C. J., Schlichting, C. D., and Steiner, U. K. (2019). Adaptive phenotypic plasticity for life-history and less fitness-related traits. *Proceedings of the Royal Society B: Biological Sciences*, 286(1904):20190653.

²¹Acasuso-Rivero, C., Murren, C. J., Schlichting, C. D., and Steiner, U. K. (2019). Adaptive phenotypic plasticity for life-history and less fitness-related traits. *Proceedings of the Royal Society B: Biological Sciences*, 286(1904):20190653.

²²Storz, J. F. and Scott, G. R. (2021). Phenotypic plasticity, genetic assimilation, and genetic compensation in hypoxia adaptation of high-altitude vertebrates. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 253:110865.

²³Ho, W.-C. and Zhang, J. (2018). Evolutionary adaptations to new environments generally reverse plastic phenotypic changes. *Nature Communications*, 9(1).

²⁴Gomulkiewicz, R. and Stinchcombe, J. R. (2022). Phenotypic plasticity made simple, but not too simple. *American Journal of Botany*, 109(10):1519–1524.

Simulating the evolution of plastic responses

One of the possibilities to study these complex evolutionary systems, which are often difficult to model using equations and are usually rich of non-linear behaviours, is the use of a computational, simulation-based approach. Indeed, this approach enables the simultaneous implementation of all environmental, developmental, and genetic variables by modelling populations as sets of co-executing programs (Taylor and Jefferson, 1993)²⁵. Computer simulations are also an ideal solution when it comes to study hardly predictable phenomena, such as those involving multiple evolutionary forces and dynamics, including the effects of oscillating environments. In these cases, simulations may be used to generate *in silico* data sets of changes in frequency of genetic polymorphisms under specific scenarios, preserving the stochasticity of the related phenomena by running large sets of replicas (Hoban et al., 2012)²⁶. Furthermore, simulated evolution allows some limitations of classic experimental evolution to be overcome. In the first place, it enables the possibility of replication of experiments in a virtually unlimited way, that is crucial because the use of multiple runs can prevent biases due to random events (Fry, 2009)²⁷. Secondly, it speeds up in a dramatic way phenomena that would otherwise be very slow. Even with very suitable subjects like *C. elegans*, researchers can keep track of a maximum of several hundred generations (Teotónio et al., 2017)²⁸ whereas simulations, in principle, could make possible to give the experiments a scope comparable to that of Richard Lenski’s microbial long-term evolution experiment (Lenski, 2017)²⁹, with any organism and much less time required to obtain some outcomes. Finally, the simulation approach facilitates experimental design, since it is not required to procure and/or rear any subject, and even a large number of variables may be easily and instantly manipulated and controlled by researchers. Collectively, all these issues are usu-

²⁵Taylor, C. and Jefferson, D. (1993). Artificial life as a tool for biological inquiry. *Artificial Life*, 1:1–13.

²⁶Hoban, S., Bertorelle, G., and Gaggiotti, O. E. (2012). Computer simulations: tools for population and evolutionary genetics. *Nature Reviews Genetics*, 13(2):110–122.

²⁷Fry, J. D. (2009). *Laboratory Experiments on Speciation*, pages 631– 656. University of California Press.

²⁸Teotónio, H., Estes, S., Phillips, P. C., and Baer, C. F. (2017). Experimental evolution with *Caenorhabditis* nematodes. *Genetics*, 206(2):691–716.

²⁹Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, 11(10):2181–2194.

ally time-consuming and challenging in the case of real experimental evolution (Kawecki et al., 2012; Teotónio et al., 2017)^{30,31}.

Several studies using computer simulations and dealing with the evolution of plastic responses can already be found in the literature. For instance, some authors adopted this approach to investigate the conditions and selective pressures leading to adaptive plasticity (Lalejini and Ofria, 2016)³². In another study, digital evolution showed the prevalence of plasticity in artificial systems that can imitate the development of organisms. Moreover, the author showed that it was possible to quantify the contribution of both genome and the environment in the phenotype of a digital organism, as well as the fitness costs associated with plasticity (Fortuna, 2022)³³. Finally, some other researchers used simulations to explore the conditions inducing either adaptive plasticity or matching habitat choice as possible solutions to environmental heterogeneity (Edelaar et al., 2017)³⁴. The latter study is of particular interest in this context, since the outcomes were generated by an *agent-based model* that was implemented with NetLogo (Wilensky, 1999)³⁵, that is the simulation platform I used in this work.

Agent-based modelling is an efficient way of developing a virtual complex system whose evolution depends on computational individuals (i.e., the agents) which have specific properties assigned by the programmer and whose behaviour is described by algorithms (Macal, 2009)³⁶. All global dynamics of the system, or emerging patterns, are spontaneously generated by multiple interactions between agents (Chiacchio et al., 2014)³⁷. This approach is particu-

³⁰Kawecki, T. J., Lenski, R. E., Ebert, D., Hollis, B., Olivieri, I., and Whitlock, M. C. (2012). Experimental evolution. *Trends in Ecology & Evolution*, 27(10):547–560.

³¹Teotónio, H., Estes, S., Phillips, P. C., and Baer, C. F. (2017). Experimental evolution with *Caenorhabditis nematodes*. *Genetics*, 206(2):691–716.

³²Lalejini, A. and Ofria, C. (2016). The evolutionary origins of phenotypic plasticity. In *Proceedings of the Artificial Life Conference 2016, ALIFE 2016*. MIT Press.

³³Fortuna, M. A. (2022). The phenotypic plasticity of an evolving digital organism. *Royal Society Open Science*, 9(9).

³⁴Edelaar, P., Jovani, R., and Gomez-Mestre, I. (2017). Should i change or should i go? phenotypic plasticity and matching habitat choice in the adaptation to environmental heterogeneity. *The American Naturalist*, 190(4):506–520.

³⁵Wilensky, U. (1999). *Netlogo*. Northwestern University, Evanston, IL Center for Connected Learning and Computer-Based Modeling, url = <http://ccl.northwestern.edu/netlogo/>

³⁶Macal, C. M. (2009). *Agent Based Modeling and Artificial Life*, pages 112–131. Springer New York.

³⁷Chiacchio, F., Pennisi, M., Russo, G., Motta, S., and Pappalardo, F. (2014). Agent-based modeling of the immune system: Netlogo, a promising framework. *BioMed Research International*, 2014:1–6.

larly suitable for ecological and evolutionary systems (Murphy et al., 2020)³⁸, where change in the gene pool of a population may be seen as a collective phenomenon produced by a variable number of organisms following a given set of hereditary rules (Tarantino et al., 2023)³⁹. NetLogo (Wilensky, 1999)⁴⁰ is both a programming language and a platform used to implement agent-based models and run dynamic, interactive simulations. Because of its simple syntax, possibility to generate data from large sets of simulations, and ease of replication and observation of non-linear behaviours, NetLogo is a well-acknowledged tool in current biological research Chiacchio et al. (2014); Dalle Nogare and Chitnis (2020); Rigato and Fusco (2020)^{41,42,43}.

In this study, NetLogo is used to simulate the evolution of digital populations belonging to an exemplary nematode species involved in research on phenotypic plasticity, *Pristionchus pacificus*. More specifically, my primary objective is to investigate the role and combined effect of several variables, such as the timescales of environmental change, the magnitude of the plastic response, and the intrinsic costs of plasticity (see Methods) on the genetic composition of the population.

To achieve the maximum performance and biological reliability from my simulations, it was crucial to combine agent-based modelling with an accurate choice of the model organism. Although the use of a single species may be regarded as a limitation when considering the generality of previous computational research on plasticity, this option enables a much deeper realism of the modelled system, due to the high availability of real-world data. Moreover, this ap-

³⁸Murphy, K. J., Ciuti, S., and Kane, A. (2020). An introduction to agent-based models as an accessible surrogate to field-based research and teaching. *Ecology and Evolution*, 10(22):12482–12498.

³⁹Tarantino, R., Panunzi, G., and Romano, V. (2023). Modeling of Hardy-Weinberg Equilibrium Using Dynamic Random Networks in an ABM Framework, pages 241–250. Springer International Publishing.

⁴⁰Wilensky, U. (1999). Netlogo. Northwestern University, Evanston, IL Center for Connected Learning and Computer-Based Modeling, url = <http://ccl.northwestern.edu/netlogo/>

⁴¹Chiacchio, F., Pennisi, M., Russo, G., Motta, S., and Pappalardo, F. (2014). Agent-based modeling of the immune system: Netlogo, a promising framework. *BioMed Research International*, 2014:1–6.

⁴²Dalle Nogare, D. and Chitnis, A. B. (2020). Netlogo agent-based models as tools for understanding the self-organization of cell fate, morphogenesis and collective migration of the zebrafish posterior lateral line primordium. *Seminars in Cell & Developmental Biology*, 100:186–198.

⁴³Rigato, E. and Fusco, G. (2020). A heuristic model of the effects of phenotypic robustness in adaptive evolution. *Theoretical Population Biology*, 136:22–30.

proach is fully in line with a recent study (Kalirad and Sommer, 2024a)⁴⁴ where authors presented an agent-based model aimed at investigating the competition and coexistence between alternative strains of *P. pacificus*, that is, strains developing two alternative phenotypes depending on environmental cues vs strains developing a single phenotype regardless of surrounding conditions. The hope is that the insights suggested by these models can not only provide relevant information on this exemplary species, but can also be generalised to some related case studies and add some pieces in the big picture underlying phenotypic plasticity and its evolution.

***Pristionchus pacificus* as a digital agent to explore the interplay between plastic and non-plastic strains**

P. pacificus nematodes are the main agents of my agent-based model. After its discovery in Pasadena, CA in 1988 and its description as a novel species some years later (Sommer et al., 1996)⁴⁵, this animal has been used as a comparative species to *C. elegans*, and its genomic information has been considered an important element to fill the gap between *C. elegans* and the diversity of nematodes. In other words, it has been conceived as a satellite species (Hong and Sommer, 2006; Sommer, 2006; Srinivasan and Sternberg, 2008; Schroeder, 2021)⁴⁶⁴⁷⁴⁸⁴⁹. However, in recent years, *P. pacificus* has become an established, successful model organism in evolutionary biology, especially for the investigation of phenotypic plasticity (Gilarte et al., 2015; Dardiry et al., 2023)⁵⁰⁵¹. I list below some of the general characteristics of this species, focusing on those that are rel-

⁴⁴Kalirad, A. and Sommer, R. J. (2024a). The role of plasticity and stochasticity in coexistence. *Ecology Letters*, 27(2).

⁴⁵Sommer, R., Carta, L., Kim, S.-Y., and Sternberg, P. (1996). Morphological, genetic and molecular description of *pristionchus pacificus* sp. n. (nematoda: Neodiplogastridae). *Fundamental and Applied Nematology*, 19:511–521.

⁴⁶Hong, R. L. and Sommer, R. J. (2006). *Pristionchus pacificus*: a well-rounded nematode. *BioEssays*, 28(6):651–659.

⁴⁷Sommer, R. (2006). *Pristionchus pacificus*. *WormBook*.

⁴⁸Srinivasan, J. and Sternberg, P. W. (2008). *Pristionchus pacificus*: an appropriate fondness for beetles. *Nature Genetics*, 40(10):1146–1147.

⁴⁹Schroeder, N. E. (2021). Introduction to *pristionchus pacificus* anatomy. *Journal of Nematology*, 53(1):1–9.

⁵⁰Gilarte, P., Kreuzinger-Janik, B., Majdi, N., and Traunspurger, W. (2015). Life-history traits of the model organism *pristionchus pacificus* recorded using the hanging drop method: Comparison with *caenorhabditis elegans*. *PLOS ONE*, 10(8):e0134105.

⁵¹Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

evant to this study. Further details are provided in the Methods, as empirical support of the features implemented in the agent-based model.

P. pacificus (family: Diplogasteridae) is a cosmopolitan nematode species that lives preferentially on scarab beetles and whose size at the adult stage is about 1 mm (Mitreva et al., 2005; Sommer et al., 2017)⁵²⁵³. It can be easily reared on bacterial cultures in the laboratory (Schroeder, 2021)⁵⁴, mainly due to its 4-day life cycle at 20°C (Sommer, 2006)⁵⁵. Before reaching the adulthood, it experiences four different juvenile stages (J1, J2, J3, and J4), each of which ends with a moulting event. In particular, J1 larvae moult to J2 before hatching the egg (Hong and Sommer, 2006; Schroeder, 2021)⁵⁶⁵⁷. *P. pacificus* worms have an androdioecious mating system, where populations are composed by self-fertilising hermaphrodites (XX) and a small percentage of males (XO), whose rates of production have been proven to be temperature-dependent (Pires-daSilva and Sommer, 2004; Morgan et al., 2017)⁵⁸⁵⁹. Since selfing is a strong type of inbreeding, the prevalence of hermaphroditic individuals can lead to a clonal population (Avisé, 2011)⁶⁰, that is a feature of critical importance for experimental aims.

In the context of this work, the most relevant characteristics of *P. pacificus* are its buccal and behavioural phenotypes, including the complex developmental processes leading to the final outcomes and how these processes can be influenced by the environment. Even when the environmental conditions are fixed, adults with the same genetic background consistently exhibit two alterna-

⁵²Mitreva, M., Blaxter, M. L., Bird, D. M., and McCarter, J. P. (2005). Comparative genomics of nematodes. *Trends in Genetics*, 21(10):573–581.

⁵³Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

⁵⁴Schroeder, N. E. (2021). Introduction to *pristionchus pacificus* anatomy. *Journal of Nematology*, 53(1):1–9.

⁵⁵Sommer, R. (2006). *Pristionchus pacificus*. *WormBook*.

⁵⁶Hong, R. L. and Sommer, R. J. (2006). *Pristionchus pacificus*: a well-rounded nematode. *BioEssays*, 28(6):651–659.

⁵⁷Schroeder, N. E. (2021). Introduction to *pristionchus pacificus* anatomy. *Journal of Nematology*, 53(1):1–9.

⁵⁸Pires-daSilva, A. and Sommer, R. J. (2004). Conservation of the global sex determination gene *tra-1* in distantly related nematodes. *Genes & Development*, 18(10):1198–1208.

⁵⁹Morgan, K., McGaughran, A., Rodelsperger, C., and Sommer, R. J. (2017). Variation in rates of spontaneous male production within the nematode species *pristionchus pacificus* supports an adaptive role for males and outcrossing. *BMC Evolutionary Biology*, 17(1).

⁶⁰Avisé, J. (2011). Clones, hermaphrodites and pregnancies: nature’s oddities offer evolutionary lessons on reproduction. *Journal of Zoology*, 286(1):1–14.

tive mouth forms, that is, *eurystomatous* (EU) and *stenostomatous* (ST), with typical, non-identical, and fluctuating ratios observed between different wild isolates (i.e., the EU/ST dimorphism is regulated stochastically) (Ragsdale et al., 2013; Susoy and Sommer, 2016; Sommer et al., 2017)^{61,62,63}. Among the main divergences between the two morphs, EU worms have a broad buccal cavity, a claw-like dorsal tooth and an opposing subventral tooth, whereas ST worms have a narrow buccal cavity and a single, flint-like dorsal tooth. These differences in morphology were shown to lead to alternative dietary behaviours, with EU worms being facultative predators and ST worms exclusively microbe-feeders (Sommer, 2006; Seroby et al., 2013; Sommer et al., 2017; Sieriebriennikov et al., 2018)^{64,65,66,67}.

The dimorphism is related to a binary, irreversible developmental decision made during larval stages and induced by a gene acting as a switch, the sulfatase-encoding gene *eud-1*, that directs a gene regulatory network resulting in a set of proteins which affect mouth formation. Interestingly, the expression of this switch is regulated by several environmental inputs, that may cause the EU/ST ratio to deviate from its typical values. So, this species shows a fascinating combination of both stochastic and conditional regulation of phenotypic plasticity (Sommer et al., 2017)⁶⁸. In particular, *P. pacificus* dimorphism is a polyphenism, that is, a kind of plasticity where organisms show discrete, distinct developmental outputs rather than a

⁶¹Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

⁶²Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

⁶³Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

⁶⁴Sommer, R. (2006). *Pristionchus pacificus*. *WormBook*.

⁶⁵Seroby et al., V., Ragsdale, E. J., Muller, M. R., and Sommer, R. J. (2013). Feeding plasticity in the nematode *pristionchus pacificus* is influenced by sex and social context and is linked to developmental speed. *Evolution & Development*, 15(3):161–170.

⁶⁶Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

⁶⁷Sieriebriennikov, B., Prabh, N., Dardiry, M., Witte, H., Roseler, W., Kieninger, M. R., Rodelsperger, C., and Sommer, R. J. (2018). A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. *Cell Reports*, 23(10):2835–2843.e4.

⁶⁸Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

continuous range of responses (Bento et al., 2010; Fusco and Minelli, 2010; Yang and Pospisilik, 2019)⁶⁹⁷⁰⁷¹.

Environmental factors regulating mouth polyphenism include nutrient availability, temperature, and crowding (Sommer et al., 2017; Sieriebriennikov et al., 2018; Dardiry et al., 2023)⁷²⁷³⁷⁴. It was also shown that both mouth phenotypes are adaptive, each one under specific surrounding conditions: ST nematodes develop faster in contexts with abundant microbial food, whereas EU nematodes can access alternative resources when bacteria are scarce (Susoy and Sommer, 2016)⁷⁵. Finally, it is now well-acknowledged that both mouth-form ratio and environmentally-induced changes in this ratio can be altered by genetic changes. In particular, some dominant mutations affecting the *eud-1* gene were shown to determine a constitutive expression of the ST phenotype in the lab, whereas additions of *eud-1* copies or overexpression of *eud-1* resulted in the exclusive expression of the EU phenotype (Susoy and Sommer, 2016; Sommer et al., 2017; Sieriebriennikov et al., 2018)⁷⁶⁷⁷⁷⁸. This means that, in principle, it is possible to conceive experimental scenarios involving both plastic and non-plastic (or dimorphic and monomorphic) strains/genotypes of *P. pacificus*, depending on the state of *eud-1*.

⁶⁹Bento, G., Ogawa, A., and Sommer, R. J. (2010). Co-option of the hormone-signalling module dafachronic acid-daf-12 in nematode evolution. *Nature*, 466(7305):494–497.

⁷⁰Fusco, G. and Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1540):547– 556.

⁷¹Yang, C.-H. and Pospisilik, J. (2019). Polyphenism – a window into gene-environment interactions and phenotypic plasticity. *Frontiers in Genetics*, 10.

⁷²Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

⁷³Sieriebriennikov, B., Prabh, N., Dardiry, M., Witte, H., Roseler, W., Kieninger, M. R., Rodelsperger, C., and Sommer, R. J. (2018). A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. *Cell Reports*, 23(10):2835–2843.e4

⁷⁴Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

⁷⁵Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

⁷⁶Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

⁷⁷Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

⁷⁸Sieriebriennikov, B., Prabh, N., Dardiry, M., Witte, H., Roseler, W., Kieninger, M. R., Rodelsperger, C., and Sommer, R. J. (2018). A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. *Cell Reports*, 23(10):2835–2843.e4

The model presented here, which I named *PhePlastiComp*, is used as a platform to explore the evolutionary interplay between these alternative strains under different scenarios, including genetic and environmental changes. Differently from previous research, in this study non-plastic strains are produced by mutations affecting the *eud-1* gene. However, I do not consider known mutations disrupting the polyphenism: in the model, non-plastic strains include mutants resulting from any hypothetical mutation that affects *eud-1* and has the exclusive effect of suppressing mouth dimorphism (which is only experienced by plastic strains). This choice was made to keep the generality of the model broader and because laboratory mutants with induced *eud-1* mutations might show reduced fitness when compared to wild-type worms. Future field studies could support the presence of strains carrying *eud-1* mutations with no negative effects on fitness, that could eventually provide further empirical data for comparisons with the outcomes of my simulations.

The main aim of the scenarios included in this chapter (see Methods) is to give a general evaluation of the complex interplay between three key variables of the system, more specifically: i) the *degree of plasticity*, here defined as the magnitude of changes in the EU/ST ratio experienced by plastic strains in response to environmental variation, ii) the *timescales of environmental change* in terms of availability of alternative food sources, and iii) the *cost in fecundity* due to the production and maintenance of plasticity in plastic strains. Hopefully, the chosen reference system could suggest new insights and details about the balance between benefits and costs of plasticity, as well as about competition (or coexistence) between plastic and non-plastic genotypes of *P. pacificus*. Furthermore, it could also shed light on population dynamics of similar nematode systems (e.g., *Allodiplogaster sudhausi* (Wighard et al., 2024)⁷⁹) and other species showing polyphenism and the presence of developmental switch genes.

⁷⁹Wighard, S., Witte, H., and Sommer, R. J. (2024). Conserved switch genes that arose via whole-genome duplication regulate a cannibalistic nematode morph. *Science Advances*, 10(15).

5.1 Methods

Model overview

The agent-based model *PhePlastiComp* was implemented using NetLogo version 6.2.0 (Wilensky, 1999)⁸⁰. In this context, the NetLogo lattice is a virtual representation of a culture plate or a patchy environment. The main agents are *P. pacificus* nematodes whose characteristics are based on empirical data coming from the original Californian strain PS312. Each nematode has a specific genotype, that is, wild-type or mutant. The basic conditions of culturing simulated in the experiments correspond to the standard laboratory protocol used for this species, where worms are kept on nematode growth medium agar plates containing *E. coli* strain OP50 at a fixed temperature of 20 °C and without overcrowding (Sommer et al., 1996; Sommer, 2006; Serobyán et al., 2013; Susoy and Sommer, 2016)⁸¹⁸²⁸³⁸⁴. A screenshot reporting the Interface of the model is shown in Figure 5.1.

⁸⁰Wilensky, U. (1999). Netlogo.

⁸¹Sommer, R., Carta, L., Kim, S.-Y., and Sternberg, P. (1996). Morphological, genetic and molecular description of *pristionchus pacificus* sp. n. (nematoda: Neodiplogastridae). *Fundamental and Applied Nematology*, 19:511–521.

⁸²Sommer, R. (2006). *Pristionchus pacificus*. WormBook.

⁸³Serobyán, V., Ragsdale, E. J., Muller, M. R., and Sommer, R. J. (2013). Feeding plasticity in the nematode *pristionchus pacificus* is influenced by sex and social context and is linked to developmental speed. *Evolution & Development*, 15(3):161–170.

⁸⁴Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

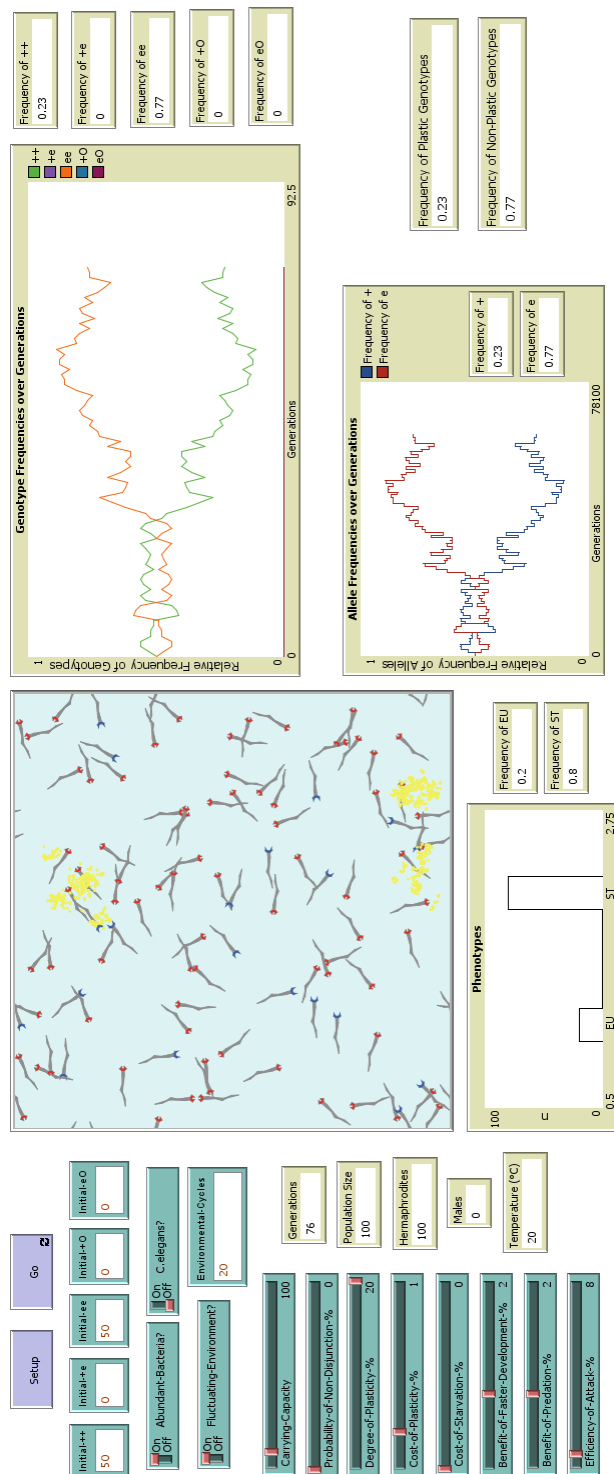


FIGURE 5.1: Interface of the NetLogo model.

P. pacificus individuals with different genotypes and other agents used as food sources, namely *E. coli* bacteria and *C. elegans* larvae, are introduced at the beginning of each simulation. In particular, *E. coli* bacteria are either abundant or scarce (but never absent), whereas *C. elegans* larvae, that are preys of *P. pacificus* worms developing the EU phenotype, are either present or absent. In the model, the succession of these two alternative conditions (i.e., abundant bacteria/no larvae and low bacteria/larvae) is periodic and is regulated by the parameter *Environmental-Cycles*, that indicates the timescale of environmental change in terms of non-overlapping generations of *P. pacificus*. For instance, if bacteria are initially abundant, preys are absent, and *Environmental-Cycles* = 10, the system will take 10 generations of *P. pacificus* before reversing the proportions of the two food sources. This is clearly feasible in the lab, but I argue it could also be plausible in the wild. Although the habitat of *P. pacificus* is heterogeneous (e.g., soil, scarab bodies and corpses, decomposing vegetal matter (Susoy and Sommer, 2016; Félix et al., 2018)⁸⁵⁸⁶), and several environmental changes may happen even during the lifetime of each individual with a rhythm that might be highly unpredictable (Susoy and Sommer, 2016; Sieriebriennikov and Sommer, 2018)⁸⁷⁸⁸, it could be assumed that, in some cases, a specific nutrient source may be more prevalent than the other (on average) for a long time interval.

P. pacificus nematodes are either hermaphrodites (XX) or hemizygous males (XO). Males may be introduced from the start of simulations, as well as being generated during simulations due to meiotic non-disjunction, with a given probability per reproductive event which is regulated by the parameter *Probability-of-Non-Disjunction-%*. This spontaneous arise of new males is very infrequent both in reality, where males are usually less than 1% of the popula-

⁸⁵Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

⁸⁶Félix, M.-A., Ailion, M., Hsu, J.-C., Richaud, A., and Wang, J. (2018). *Pristionchus* nematodes occur frequently in diverse rotting vegetal substrates and are not exclusively necromenic, while *panagrellus redivivoides* is found specifically in rotting fruits. *PLOS ONE*, 13(8):e0200851.

⁸⁷Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

⁸⁸Sieriebriennikov, B. and Sommer, R. J. (2018). Developmental plasticity and robustness of a nematode mouth-form polyphenism. *Frontiers in Genetics*, 9.

tion (Hong and Sommer, 2006; Schroeder, 2021)⁸⁹⁹⁰, and in simulations. Almost all reproductive events occurring in the following experimental scenarios consist of selfing (because most nematodes are hermaphrodites), with only a small fraction of crosses between hermaphrodites and hemizygous males.

Each simulation replicates a sequence of non-overlapping generations of *P. pacificus*. Every generation encompasses the whole life cycle of nematodes, and each 10 ticks (i.e., NetLogo chronological unit) correspond to 1 hour in the real world. Worms of each generation start as eggs, then go through all developmental phases typical of this species, with a timing approximately corresponding to that observed in reality. More specifically, all *P. pacificus* nematodes reach the J1 (i.e., Juvenile 1) stage 150 ticks (i.e., 15 hours) after oviposition (of their parents), then undergo the first moult while they are still inside the eggshell, 200 ticks after oviposition, reaching the J2 stage. Hatching takes place 240 ticks after oviposition. The following steps are the reaching of the J3 and J4 stages, 400 ticks and 480 ticks after oviposition, respectively. At the end of the J4 phase, after the 4th moult (700 ticks after oviposition), the adult age is reached and all nematodes develop their mouth form irreversibly (Bento et al., 2010; Ragsdale et al., 2013)⁹¹⁹², so now they can eat bacteria and/or *C. elegans* larvae. Furthermore, each male (if present) can copulate with a single, randomly chosen hermaphrodite, while all other hermaphrodites perform selfing. 820 ticks after the previous cycle of oviposition, all hermaphrodites produce their offspring as new eggs (Hong and Sommer, 2006; Sommer et al., 2017)⁹³⁹⁴. For simplicity, oviposition and fertilisation are considered simultaneous in the model. The total number of offspring generated by each hermaphrodite and the genotype ratio of offspring

⁸⁹Hong, R. L. and Sommer, R. J. (2006). *Pristionchus pacificus*: a well-rounded nematode. *BioEssays*, 28(6):651–659.

⁹⁰Schroeder, N. E. (2021). Introduction to *pristionchus pacificus* anatomy. *Journal of Nematology*, 53(1):1–9.

⁹¹Bento, G., Ogawa, A., and Sommer, R. J. (2010). Co-option of the hormone-signalling module *dafachronic acid-daf-12* in nematode evolution. *Nature*, 466(7305):494–497.

⁹²Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

⁹³Hong, R. L. and Sommer, R. J. (2006). *Pristionchus pacificus*: a well-rounded nematode. *BioEssays*, 28(6):651–659.

⁹⁴Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

are calculated considering genotype(s) and phenotype(s) of the parent(s), as well as the environmental conditions experienced by the parent(s) (see section “Reproductive rules and calculation of the offspring”). A random extrinsic mortality, whose magnitude is proportional to the selected value of the parameter *Carrying-Capacity*, is then applied to the offspring and all parents die immediately after oviposition. The whole cycle is reiterated for each new generation.

Mouth-form and feeding plasticity

Mouth dimorphism and the related behavioural plasticity in dietary habits are critical characteristics of the *P. pacificus* nematodes implemented in the model. In a few words, EU nematodes (blue colour) have large mouths and can feed both on bacteria and other nematodes, whereas ST nematodes (red colour) have narrow mouths and can only feed on bacteria. During simulations, some nematodes can express either one or the other form (i.e., they have a plastic genotype), while others express only one form constitutively (i.e., they have a non-plastic genotype).

In real worms, one of the main regulators of the plastic response is the sulfatase-encoding *eud-1* gene, a dosage-dependent developmental switch that is sensitive to several environmental factors and is located on the X chromosome (Ragsdale et al., 2013; Sommer et al., 2017; Werner et al., 2017; Sieriebriennikov et al., 2018; Dardiry et al., 2023)^{95,96,97,98,99}. While wild-type individuals of the reference strain PS312 (*++*) express both mouth forms (on average, 80% EU and 20% ST under the laboratory conditions described above), mutations affecting this key gene cause changes in pheno-

⁹⁵Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

⁹⁶Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

⁹⁷Werner, M. S., Sieriebriennikov, B., Loschko, T., Namdeo, S., Lenuzzi, M., Dardiry, M., Renahan, T., Sharma, D. R., and Sommer, R. J. (2017). Environmental influence on *pristionchus pacificus* mouth form through different culture methods. *Scientific Reports*, 7(1).

⁹⁸Sieriebriennikov, B., Prabh, N., Dardiry, M., Witte, H., Roseler, W., Kieninger, M. R., Rodelsperger, C., and Sommer, R. J. (2018). A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. *Cell Reports*, 23(10):2835–2843.e4.

⁹⁹Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

type ratios (Susoy and Sommer, 2016)¹⁰⁰. This has been observed with loss-of-function mutations producing dominant *eud-1* (in the model, *e*) alleles, that induce a shift towards ST-biased outputs to the point of making mutants completely deficient in developing the EU form (Sommer et al., 2017; Casasa et al., 2023)¹⁰¹¹⁰². In particular, in the equivalent strain of PS312 named RS2333 (Serobyany et al., 2013; Lenuzzi et al., 2021)¹⁰³¹⁰⁴, *ee* homozygotes (and *eO* males, in the model) lose their ability to express plastic responses, since they can only develop the ST morph, whereas *+e* heterozygotes show incomplete dominance and are still plastic, as well as *+O* males (Ragsdale et al., 2013; Sommer et al., 2017)¹⁰⁵¹⁰⁶. As for *+e* and *+O* worms, they are roughly 10-40% EU in the lab (Ragsdale et al., 2013)¹⁰⁷.

All phenotype ratios shown by agents of the NetLogo model under the standard protocol are similar to those observed in the lab under the same conditions, including some stochastic fluctuations which are also consistent with real observations. The simultaneous expression of both morphs in isogenic populations carrying at least one copy of *+* and living in a stable environment is an example of

¹⁰⁰Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹⁰¹Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

¹⁰²Casasa, S., Katsougia, E., and Ragsdale, E. J. (2023). A mediator subunit imparts robustness to a polyphenism decision. *Proceedings of the National Academy of Sciences*, 120(32).

¹⁰³Serobyany, V., Ragsdale, E. J., Muller, M. R., and Sommer, R. J. (2013). Feeding plasticity in the nematode *pristionchus pacificus* is influenced by sex and social context and is linked to developmental speed. *Evolution & Development*, 15(3):161–170.

¹⁰⁴Lenuzzi, M., Witte, H., Riebesell, M., Rodelsperger, C., Hong, R. L., and Sommer, R. J. (2021). Influence of environmental temperature on mouth-form plasticity in *pristionchus pacificus* acts through *daf-11*-dependent *cgmp* signaling. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 340(2):214–224.

¹⁰⁵Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

¹⁰⁶Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

¹⁰⁷Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

stochastic regulation of plasticity, that is typical of this species (Susoy and Sommer, 2016; Sieriebriennikov and Sommer, 2018)^{108,109}. Both in the model and in reality, mutants lacking the $+$ allele are unable to express the dimorphism. In particular, it should be recalled that the *eud-1* mutants of the model are produced by any hypothetical mutation affecting that gene and having exclusive effects in the reduced (i.e., $+e$, $+O$) or null (i.e., ee , eO) expression of the EU phenotype.

Other than genotypes and stochasticity, mouth-form ratios are also affected by changes in environmental conditions. Indeed, mouth dimorphism is regulated by environmental factors, the most relevant of which are starvation, temperature, and crowding (Susoy and Sommer, 2016; Lenuzzi et al., 2021)^{110,111}. For instance, temperatures below 15°C reduce the percentage of worms developing the EU morph in PS312 (Lenuzzi et al., 2021)¹¹², and crowding is positively related to a higher expression of the EU phenotype due to a specific pheromone signal (Bento et al., 2010; Susoy and Sommer, 2016)^{113,114}. However, my study considers neither the influence of temperature, that is kept fixed over time at 20°C, nor the effects of population density in mouth formation. In fact, it was found that the pheromones inducing shifts towards more EU-biased ratios in juvenile individuals are stage specific and come from adults (Werner et al., 2018)¹¹⁵. Since generations are rigorously non-overlapping in the model, juvenile *P. pacificus* worms are never in contact with

¹⁰⁸Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹⁰⁹Sieriebriennikov, B. and Sommer, R. J. (2018). Developmental plasticity and robustness of a nematode mouth-form polyphenism. *Frontiers in Genetics*, 9.

¹¹⁰Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹¹¹Lenuzzi, M., Witte, H., Riebesell, M., Rodelsperger, C., Hong, R. L., and Sommer, R. J. (2021). Influence of environmental temperature on mouth-form plasticity in *pristionchus pacificus* acts through *daf-11*-dependent *cgmp* signaling. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 340(2):214–224.

¹¹²Lenuzzi, M., Witte, H., Riebesell, M., Rodelsperger, C., Hong, R. L., and Sommer, R. J. (2021). Influence of environmental temperature on mouth-form plasticity in *pristionchus pacificus* acts through *daf-11*-dependent *cgmp* signaling. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 340(2):214–224.

¹¹³Bento, G., Ogawa, A., and Sommer, R. J. (2010). Co-option of the hormone-signalling module *dafachronic acid-daf-12* in nematode evolution. *Nature*, 466(7305):494–497.

¹¹⁴Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹¹⁵Werner, M. S., Claaßen, M. H., Renahan, T., Dardiry, M., and Sommer, R. J. (2018). Adult influence on juvenile phenotypes by stage-specific pheromone production. *iScience*, 10:123–134.

adults, so adults' pheromones cannot influence the irreversible developmental decision of younger nematodes.

Consequently, this model was designed to apply environmentally-induced shifts in phenotype ratios only in response to dietary variation. A reduced presence of bacteria in the simulation grid induces an increase (*Degree-of-Plasticity-%*) in the average frequency of EU nematodes for each plastic genotype, whereas non-plastic genotypes are not responsive when subject to changes in food availability. In other words, the parameter *Degree-of-Plasticity-%* represents the level of conditional plasticity, as it is the range of variation in the EU/ST ratio induced by changes in food availability (for further details on the level of plasticity of switch-like reaction norms, see Crowther et al. (2023)¹¹⁶). It is an important variable because it enables the investigation of different levels of environmental responsiveness, including the extreme case (0%) where the regulation of plasticity is only stochastic and *not* conditional.

The increase in EU frequency in plastic strains in response to low bacterial food is well-acknowledged (Susoy and Sommer, 2016)¹¹⁷ and represents a case of adaptive plasticity. Indeed, the ability to express the EU morph enables the exploitation of alternative food sources, such as nematodes of other species (in the model, *C. elegans* larvae), resulting in greater fitness for that environmentally-induced phenotype under such conditions (Serobyán et al., 2014; Susoy and Sommer, 2016)¹¹⁸¹¹⁹. As for the possible formation of stress-resistant dauer larvae due to prolonged periods of food deprivation (Werner et al., 2018)¹²⁰, I excluded this developmental arrest from the model by allowing the presence of reduced quantities of bacteria in all simulations, which may produce dauer-inhibitor chemical(s) that antagonise this process (Ogawa and Brown, 2015)¹²¹.

¹¹⁶Crowther, C., Bonser, S. P., and Schwanz, L. E. (2023). Plasticity and the adaptive evolution of switchlike reaction norms under environmental change. *Evolution Letters*, 8(1):64–75

¹¹⁷Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹¹⁸Serobyán, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

¹¹⁹Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹²⁰Werner, M. S., Claßen, M. H., Renahan, T., Dardiry, M., and Sommer, R. J. (2018). Adult influence on juvenile phenotypes by stage-specific pheromone production. *iScience*, 10:123–134.

¹²¹Ogawa, A. and Brown, F. (2015). Dauer formation and dauerspecific behaviours in *Prionchus pacificus*, pages 257–299. BRILL.

TABLE 5.1: Mouth-form ratios in relation to genotype and variation in food sources.

Genotype	Frequency of EU with abundant bacteria	Frequency of ST with abundant bacteria	Frequency of EU with scarce bacteria	Frequency of ST with scarce bacteria
$++$ (wild-type homozygotes, plastic)	$\approx 80\%$	$\approx 20\%$	$\approx 80\% + \text{Degree-of-Plasticity-}\%$	$\approx 20\% - \text{Degree-of-Plasticity-}\%$
$+e$ (heterozygotes, plastic)	$\approx 25\%$	$\approx 75\%$	$\approx 25\% + \text{Degree-of-Plasticity-}\%$	$\approx 75\% - \text{Degree-of-Plasticity-}\%$
ee (mutant homozygotes, non-plastic)	0%	100%	0%	100%
$+O$ (wild-type males, plastic)	$\approx 25\%$	$\approx 75\%$	$\approx 25\% + \text{Degree-of-Plasticity-}\%$	$\approx 75\% - \text{Degree-of-Plasticity-}\%$
eO (mutant males, non-plastic)	0%	100%	0%	100%

All information about mouth-form ratios for each genotype and under each environmental context is summarised in Table 5.1.

□

Reproductive rules and calculation of the offspring

All selfing events and crosses are based on Mendelian rules and generate 100 zygotes if no benefits and costs in fecundity are applied (see details below). For instance, a $+e$ hermaphrodite performing selfing ($+e \times +e$) will generate 100 offspring with the following genotype ratio: $\frac{1}{4} ++ : \frac{1}{2} +e : \frac{1}{4} ee$, that is, 25 wild-type homozygotes, 50 heterozygotes, and 25 mutant homozygotes. Some of these zygotes can also be $+O$ or eO males, depending on the value of the parameter *Probability-of-Non-Disjunction-%*. Likewise, a $ee \times +O$ cross will respect the following ratio: $\frac{1}{2} +e : \frac{1}{2} +O$, that is, 50 heterozygotes, and 50 wild-type hemizygous males (for simplicity, non-disjunction events are not applied to crosses). However, most zygotes from each reproducing worm are eliminated immediately after fertilisation due

to random mortality and both benefits and costs in fecundity must be considered to obtain the real number and genotype ratio of offspring.

Mouth-form plasticity is preserved in *P. pacificus* due to context-dependent benefits in terms of fitness for each alternative morph (Serobyán et al., 2014)¹²². Together with some costs, these benefits are introduced in the model in terms of variation in fecundity, that is, by adding or subtracting a specific quantity from the total number of zygotes generated by each reproducing nematode. Variations in fecundity also depend on the genotype of the parent(s) and the environmental conditions experienced before reproduction. Let us consider in detail each parameter affecting the resulting number of offspring.

Cost-of-Plasticity-% is a reduction in fecundity applied to plastic genotypes only (i.e., $++$, $+e$, and $+O$), and refers to the number of random zygotes removed from the grid for each parent (or couple of parents) belonging to a plastic strain. This price is associated with the ability to express plasticity. For instance, it may be related to the maintenance of sensory or regulatory elements which are not required by non-plastic genotypes (DeWitt et al., 1998; Murren et al., 2015; Dardiry et al., 2023)¹²³¹²⁴¹²⁵. In crosses, this cost is applied two times if both partners belong to a plastic strain.

Cost-of-Starvation-% is a reduction in fecundity applied to all *P. pacificus* nematodes if microbial food is scarce, and refers to the number of random zygotes removed from the grid for each parent (or couple of parents) experiencing it. As a matter of fact, bacteria are by far the best source of nutrients for both morphs (Serobyán et al., 2014)¹²⁶ and it was ascertained that the complete absence

¹²²Serobyán, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

¹²³DeWitt, T. J., Sih, A., and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13(2):77–81.

¹²⁴Murren, C. J., Auld, J. R., Callahan, H., Ghalambor, C. K., Handelsman, C. A., Heskell, M. A., Kingsolver, J. G., Maclean, H. J., Masel, J., Maughan, H., Pfennig, D. W., Relyea, R. A., Seiter, S., Snell-Rood, E., Steiner, U. K., and Schlichting, C. D. (2015). Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity*, 115(4):293–301.

¹²⁵Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

¹²⁶Serobyán, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

of bacteria leads to slower development and delayed sexual maturation (Werner et al., 2017)¹²⁷. Although dietary restriction is not directly harmful for parents in other nematode species (under certain conditions), it was found that it can rather reduce the fitness of offspring (Mautz et al., 2019)¹²⁸. Thus, for simplicity, in the model this reduction in fitness is directly applied to parents experiencing bacterial starvation. As for crosses, this cost is applied two times, regardless of the genotype of the two partners.

Benefit-of-Faster-Development-% is an increase in fecundity (offspring number) applied each time to a different, random genotype among the zygotes produced by a ST nematode when bacteria are abundant. For example, if *Benefit-of-Faster-Development-%* is set at 2, microbial food is abundant, and we consider a ST nematode with *+e* genotype performing selfing, the additional number of offspring (2) will be applied either to *++*, *+e*, or *ee* zygotes from case to case. In crosses, *Benefit-of-Faster-Development-%* is applied only one time, and only if both partners are ST. In fact, since the fecundity advantage depends on the anticipation of sexual receptivity, this anticipation needs to be synchronised in both nematodes to be exploited. The increase in fitness for ST worms due to their faster developmental rate is reported in literature (Serobyany et al., 2014)¹²⁹. In the model, it was implemented as an increase in fecundity and not as a difference in lifespans to keep the sequence of generations simultaneous for both forms. The application of this parameter is also supported by comparisons with similar dynamics in other ecdysozoans, such as *D. melanogaster*, where the anticipation

¹²⁷Werner, M. S., Sieriebriennikov, B., Loschko, T., Namdeo, S., Lenuzzi, M., Dardiry, M., Renahan, T., Sharma, D. R., and Sommer, R. J. (2017). Environmental influence on *pristionchus pacificus* mouth form through different culture methods. *Scientific Reports*, 7(1).

¹²⁸Mautz, B. S., Lind, M. I., and Maklakov, A. A. (2019). Dietary restriction improves fitness of aging parents but reduces fitness of their offspring in nematodes. *The Journals of Gerontology: Series A*, 75(5):843–848.

¹²⁹Serobyany, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

of sexual maturation under certain dietary conditions may correspond to an early-life increase in the number of offspring (Dasgupta et al., 2022)¹³⁰.

Benefit-of-Predation-% is an increase in fecundity (offspring number) applied each time to a different, random genotype among the zygotes produced by an EU nematode when feeding on *C. elegans* larvae. This value must be multiplied by the sum of larvae killed by the EU nematode during its life cycle (up to a maximum of 5 larvae). The number of preys killed depends on the additional parameter *Efficiency-of-Attack-%*, that is the probability to kill a close larva. For instance, if *Benefit-of-Predation-%* is set at 2 and an EU nematode with *+e* genotype kills 2 preys, this additional number of offspring (i.e., $2 \times 2 = 4$) will be applied either to *++*, *+e*, or *ee* zygotes from case to case. In crosses with other EU nematodes, *Benefit-of-Predation-%* must be multiplied by the sum of all larvae killed by both partners during their life cycles. This increase in fecundity was also observed experimentally. Indeed, EU worms clearly showed their superiority in killing preys over the other form, supporting the hypothesis of an advantage in fitness on a mixed diet (Serobyán et al., 2014)¹³¹.

The following examples should make clearer the general functioning of the reproductive dynamics implemented in the model. For instance, consider a plate rich in bacteria and a ST hermaphrodite with a *+e* genotype performing selfing. If *Cost-of-Plasticity-%* = 2 and *Benefit-of-Faster-Development-%* = 3, the total number of zygotes produced will be: $100 - 2 + 3 = 101$, where the decrease (2) is applied in a completely random way to the offspring, whereas the increase (3) is randomly applied to one of the three genotypes of the offspring (i.e., either to *++*, *+e*, or *ee* zygotes). As a second example, consider a plate low in bacteria but containing *C. elegans* larvae, and a cross between two EU worms, a *++* and a *+O*. If *Cost-of-Starvation-%* = 1, *Cost-of-Plasticity-%* = 3, *Benefit-of-Predation-%* = 2, and if the first worm eats 2 preys and the second one eats 1 prey, the total number of zygotes produced by the hermaphrodite

¹³⁰Dasgupta, P., Halder, S., Dari, D., Nabeel, P., Vajja, S. S., and Nandy, B. (2022). Evolution of a novel female reproductive strategy in *Drosophila melanogaster* populations subjected to long-term protein restriction. *Evolution*, 76(8):1836–1848.

¹³¹Serobyán, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

will be: $100 - 1*2 - 3*2 + 2*(2 + 1) = 98$, where the decrease (8) is applied in a completely random way to the offspring and the increase (6) is randomly applied to one of the three genotypes of the offspring (i.e., either to $++$ or $+O$ zygotes).

All these and other parameters included in the model, as well as their description, range, and references are summarised in Table 5.2.

Simulation scenarios

Since agent-based models rely on large numbers of computational individuals whose interactions are regulated by probabilistic instructions, the final outcomes resulting from a single run are expected to differ from those generated by another run using the same settings (Hunter and Kelleher, 2020)¹³³. For this reason, all results for each scenario are extracted from many runs, more specifically, 100 runs for Symmetric Analyses (SAs) and 1000 runs for Invasibility Analyses (IAs). Symmetric Analyses start with an equal number of *P. pacificus* nematodes belonging to two alternative strains (i.e., plastic vs non-plastic), whereas Invasibility Analyses start with a single mutant or immigrant individual belonging to a different strain from that of the population to be invaded.

Some basic, preliminary scenarios are used to validate the overall behaviour of the system by comparison with real dynamics reported in the literature (see Table 5.3). All other scenarios are used to investigate the combined effects of several parameters, including the cost of plasticity, the benefits associated to each phenotype, the degree of plasticity, and the timescale of environmental fluctuations, on the evolutionary interplay between plastic and non-plastic strains (see Table 5.4). This interplay is monitored using the frequencies and average times to fixation (in terms of generations) of the alleles $+$ and e . Note that all simulations start with only hermaphrodite nematodes, that is, *Initial-+O* and *Initial-eO* are always set to 0 at tick = 0.

¹³²*The baseline number of offspring, i.e., the offspring produced by each hermaphrodite *P. pacificus* nematode without considering any costs or benefits, is 100.

¹³³Hunter, E. and Kelleher, J. D. (2020). A framework for validating and testing agent-based models: a case study from infectious diseases modelling.

TABLE 5.2: Parameters included in the NetLogo model, including a short description, and the range of selectable values.

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Parameter	Description	Range
<i>Initial-++</i>	Initial number of ++ nematodes	Any value
<i>Initial-+e</i>	Initial number of +e nematodes	Any value
<i>Initial-ee</i>	Initial number of ee nematodes	Any value
<i>Initial-+O</i>	Initial number of +O nematodes	Any value
<i>Initial-eO</i>	Initial number of eO nematodes	Any value
<i>Abundant-Bacteria?</i>	Quantity of bacteria (low/high quantity)	0-1
<i>C.elegans?</i>	Presence of <i>C. elegans</i> preys (not present/present)	0-1
<i>Fluctuating-Environment?</i>	Changeable dietary conditions over time (fixed/changeable conditions)	0-1
<i>Environmental-Cycles</i>	Number of <i>P. pacificus</i> generations at the end of which dietary conditions change	1-20
<i>Carrying-Capacity</i>	Maximum population size allowed (i.e., random extrinsic mortality removes exceeding <i>P. pacificus</i> nematodes)	0-1000
<i>Probability-of-Non-Disjunction-%</i>	Probability to generate a male offspring	0-100
<i>Degree-of-Plasticity-%</i>	Percentual increase/decrease of each mouth form in response to variation in the availability of food (for plastic strains)	0-20
<i>Cost-of-Plasticity-%*</i>	Cost in fecundity applied to the offspring of a parent (or a couple of parents) with a plastic genotype (i.e., ++, +e, or +O)	0-5
<i>Cost-of-Starvation-%*</i>	Cost in fecundity applied to the offspring of a parent (or a couple of parents) if bacteria are scarce	0-5
<i>Benefit-of-Faster-Development-%*</i>	Benefit in fecundity applied to the offspring of a parent (or a couple of parents) with a ST phenotype, if bacteria are abundant	0-5
<i>Benefit-of-Predation-%*</i>	Benefit in fecundity applied to the offspring of a parent (or a couple of parents) with an EU phenotype (the final outcome is proportional to the number of preys eaten)	0-5
<i>Efficiency-of-Attack-%</i>	Probability to kill a close prey. The number of killed preys (up to a maximum of 5) must be multiplied by <i>Benefit-of-Predation-%</i> to obtain the total benefit for that specific nematode	0-100

TABLE 5.3: List of all parameter values used for each experimental scenario (Validation).

	Initial- ++	Initial- +	Initial- ee	Abundant Bacteria?	<i>C. elegans</i> ?	Fluctua- tions- Environ- ment?	Environ- mental- Cycles	Carrying- Capacity	Probabi- lity-of- Non- Disjuncti- on-%	Degree- of- Plasticity -%	Cost-of- Plasticity -%	Cost-of- Starvation -%	Benefit- of- Faster- Develop- ment-%	Benefit- of- Predation -%	Efficiency -of- Attack- %
Decay of het- erozy- gosity	100	100	100	True	False	False	N/A	300	0	10	0	0	0	0	0
Neutral con- dition	150	0	150	True	False	False	N/A	300	0	10	0	0	0	0	0
Effects of <i>Benefit- of-Faster- Development</i> - %	150	0	150	True	False	False	N/A	300	0	10	0	0	1/2/3	0	0
Effects of <i>Benefit- of- Predation</i> - %	150	0	150	True	True	False	N/A	300	0	10	0	0	0	1/2/3	8
Effects of <i>Cost-of- Plasticity</i> - %	150	0	150	True	False	False	N/A	300	0	10	1/2/3	0	0	0	0

TABLE 5.4: List of all parameter values used for each experimental scenario (SA = Symmetric Analysis; IA = Invasibility Analysis).

	Initial- ++	Initial- +e	Initial- ee	Abundant - Bacteria?	<i>C. elegans</i> ?	Fluctua- ting- Environ- ment?	Environ- mental- Cycles	Carrying- Capacity	Probabi- lity-of- Non- Disjuncti- on-%	Degree- of- Plasticity -%	Cost-of- Plasticity -%	Cost-of- Starvation -%	Benefit- of- Faster- Develop- ment-%	Benefit- of- Predation -%	Efficiency -of- Attack- %
SA: Impact of environ- mental change and degree of plas- ticity	150	0	150	True/ False	False/ True	True	1/5/ 10/15/ 20	300	1	0/10/ 20	0/1	3	2	2	8
SA: Toler- ance of plastic strains towards the cost of plas- ticity	150	0	150	True/ False	False/ True	True	20	300	1	20	0/1/ 2/3	3	2	2	8
IA: Impact of cost of plas- ticity on the fixation of non- plastic strains	299	1	0	True	False	False	N/A	300	1	10	0/1/ 2/3	3	2	2	8
IA: Impact of dif- ferences between the two benefits	0/1	0/1	299	True/ False	False/ True	True	10	300	1	0/20	0/1	3	1	1/2/3	8

5.2 Results

Validation and preliminary runs

An expected, straightforward consequence of the overwhelming prevalence of selfing as a mode of reproduction is the abrupt collapse of the heterozygous genotype in a short time, leading to the exclusive presence of homozygous pure lines (Guzella et al., 2018; Cornetti et al., 2021)¹³⁴¹³⁵. The scenario “Decay of heterozygosity” informs that, using a population of 300 nematodes (initial composition: 100 $++$, 100 $+e$, and 100 ee) within a context devoid of costs, benefits, environmental variability, and non-disjunction events, and with *Carrying-Capacity* = 300, heterozygotes disappear very quickly from the simulation lattice in all runs: the average time to disappearance was 7.42 generations ($\sigma = 1.88$).

To test whether the two parameters *Benefit-of-Faster-Development-%* and *Benefit-of-Predation-%* were in line with empirical evidence suggesting adaptive advantages associated with faster sexual maturation of ST worms and with the ability of EU worms to exploit alternative food sources (Serobyany et al., 2014; Sommer et al., 2017)¹³⁶¹³⁷, respectively, I used the scenario “Neutral condition” as a baseline. Considering an initial symmetric condition (i.e., 150 $++$ and 150 ee , with *Carrying-Capacity* = 300) and excluding costs, benefits, environmental variability, and non-disjunction, an approximately equal frequency of fixation of both alleles was observed (relative f of fixation of $+$ = 52%, rel. f of fixation of e = 48%), which confirms their selective neutrality. Keeping in mind the mouth-form ratios associated with each genotype (see Table 5.1), benefits in fecundity favouring the ST phenotype are expected to increase the frequency of e , whereas benefits favouring the EU phenotype will mainly increase the frequency of $+$.

The scenario “Effects of *Benefit-of-Faster-Development-%*” dif-

¹³⁴Guzella, T. S., Dey, S., Chelo, I. M., Pino-Querido, A., Pereira, V. F., Proulx, S. R., and Teotonio, H. (2018). Slower environmental change hinders adaptation from standing genetic variation. *PLOS Genetics*, 14(11):e1007731.

¹³⁵Cornetti, L., Fields, P. D., and Ebert, D. (2021). Genomic characterization of selfing in the cyclic parthenogen *daphnia magna*. *Journal of Evolutionary Biology*, 34(5):792–802.

¹³⁶Serobyany, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

¹³⁷Sommer, R. J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B., and Werner, M. S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology*, 7(3):160332.

fers from the “Neutral condition” only for the introduction of a gradually increasing benefit in terms of fecundity for ST nematodes (1%, 2%, and 3%). Each of these benefits is associated with a set of 100 runs. In this case, *C. elegans* larvae are excluded from the simulation grid. The runs show that *Benefit-of-Faster-Development-%* can increase the frequency of fixation of e , while decreasing its average time to fixation (1%: rel. f of fixation of $e = 97\%$, mean time to fixation of $e = 280.32$, with $\sigma = 164.94$; 2%: rel. f of fixation of $e = 98\%$, mean time to fixation of $e = 209.81$, with $\sigma = 119.32$; 3%: rel. f of fixation of $e = 100\%$, mean time to fixation of $e = 148.6$, with $\sigma = 73.25$).

The scenario “Effects of *Benefit-of-Predation-%*” is also based on the “Neutral condition” above, this time considering a gradual increase of values of the parameter *Benefit-of-Predation-%* (1%, 2%, and 3%, 100 runs for each value). This benefit is only experienced by EU nematodes due to predation of *C. elegans* larvae, which in this case are present in the system, and is associated with a low capacity of killing each prey (*Efficiency-of-Attack-%* = 8%). This probability of killing preys was chosen because it led to outcomes that are comparable to those obtained in the previous scenario. This scenario confirms that the higher the increase in fecundity for EU worms, the greater the number of fixations of $+$, with decreasing average time to fixation of $+$ (1%: rel. f of fixation of $+$ = 94%, mean time to fixation of $+$ = 259.57, with $\sigma = 158.66$; 2%: rel. f of fixation of $+$ = 99%, mean time to fixation of $+$ = 192.33, with $\sigma = 113.41$; 3%: rel. f of fixation of $+$ = 99%, mean time to fixation of $+$ = 142.87, with $\sigma = 65.22$).

Finally, in the scenario named “Effects of *Cost-of-Plasticity-%*”, I tested whether increasing costs of plasticity (1%, 2%, and 3%) had a penalising impact on the fixation of $+$, that is an expected implication within a system where the ability to produce and maintain plastic responses affects fecundity in a negative way Dardiry et al. (2023). This impact was observed in all three experimental sets (1%: rel. f of fixation of $+$ = 3%, mean time to fixation of $+$ = 143.33; 2%: rel. f of fixation of $+$ = 1%, time to fixation of $+$ = 82; 3%: rel. f of fixation of $+$ = 0%. Except for the variation of the parameter *Cost-of-Plasticity-%*, this scenario is also identical to “Neutral condition”.

Effects of different timescales of environmental change and degree of plasticity on the fixation of plastic strains with no cost of plasticity

Symmetric Analyses were carried out using not very likely starting conditions, that is, equal numbers of individuals for both the alternative strains involved (Kalirad and Sommer, 2024a)¹³⁸ ($++$ and ee). However, this simplification makes it possible to identify several interesting population dynamics and explore the global impact of the main variables that were introduced into the system. All SAs include 100 runs for each different initial configuration of the model (i.e., the complete set of initial values used for the considered parameters).

The aim of the SA named “Impact of environmental change and degree of plasticity” is to compare the advantages of plastic strains with a different level of environmental responsiveness across several timescales of environmental fluctuation. To do this, I report the frequencies and mean times to fixation of the alleles $+$ and e extracted from runs performed with the following initial settings: *Initial-++* = 150, *Initial-ee* = 150, *Carrying-Capacity* = 300, alternate presence of abundant bacteria and *C. elegans* preys (i.e., the environment is fluctuating), *Environmental-Cycles* = 1, 5, 10, 15, or 20, *Probability-of-Non-Disjunction-%* = 1, *Degree-of-Plasticity-%* = 0, 10, or 20, *Cost-of-Plasticity-%* = 0 or 1, *Cost-of-Starvation-%* = 3, equal benefits in fecundity for both mouth phenotypes = 2 (i.e., *Benefit-of-Faster-Development-%* = *Benefit-of-Predation-%* = 2), and *Efficiency-of-Attack-%* = 8. In this section, I report the outcomes obtained with no costs associated with the production and maintenance of plastic responses (i.e., *Cost-of-Plasticity-%* = 0).

When conditional regulation of plasticity was absent (i.e., the degree of plasticity was null, so mouth-form ratios depended only on the stochastic variation around the typical values of PS312 observed under standard conditions), the rel. f of fixation of $+$ was 55%, 54%, 68%, 69%, and 60%, with timescales of environmental change (i.e., the duration of periods under each dietary regime) of 1, 5, 10, 15, and 20, respectively (see Figure 5.2). The latter values are the generations of *P. pacificus* it takes before there is a new reversal in the nutrient sources available within the NetLogo lattice

¹³⁸Kalirad, A. and Sommer, R. J. (2024a). The role of plasticity and stochasticity in coexistence. *Ecology Letters*, 27(2).

(*Environmental-Cycles*).

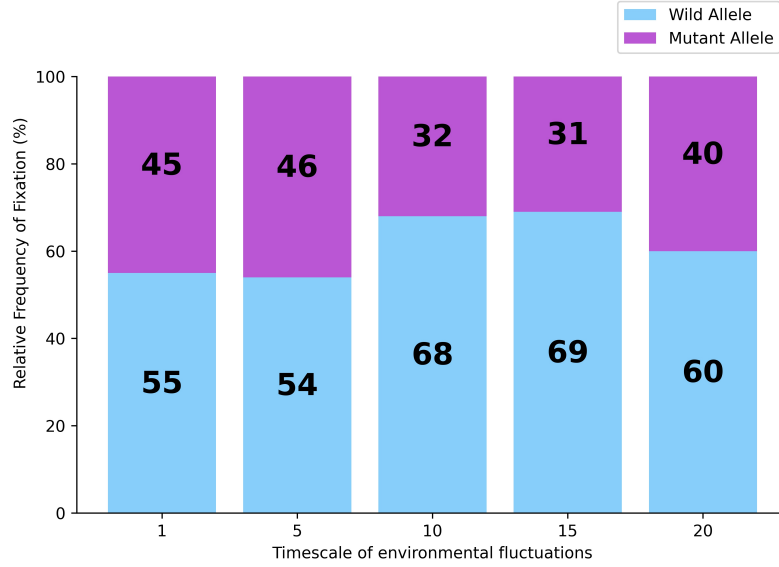


FIGURE 5.2: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on the relative frequencies of fixation of the two alleles (light blue bar = $+$, violet bar = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 0 and *Cost-of-Plasticity-%* = 0.

Since data on average times to fixation are not normally distributed and most sample sizes are small, I report the IQR as a measure of dispersion of data for this and most of the following scenarios. In this case, the mean times to fixation of the wild allele in terms of generations were: 370.98 (IQR = 305.5, with Q1 = 202, Q2 = 335, and Q3 = 507.5), 518.74 (IQR = 439.5, Q1 = 256.25, Q2 = 383, Q3 = 695.75), 467.84 (IQR = 317.25, Q1 = 230.5, Q2 = 354, Q3 = 547.75), 390.13 (IQR = 297, Q1 = 198, Q2 = 322, Q3 = 495), and 384.78 (IQR = 295, Q1 = 227.5, Q2 = 311.5, Q3 = 522.5), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively. As for the mutant allele, the mean times to fixation were: 401.42

(IQR = 374, Q1 = 170, Q2 = 295, Q3 = 544), 407.15 (IQR = 280, Q1 = 191.25, Q2 = 324, Q3 = 471.25), 436.59 (IQR = 335.25, Q1 = 203.75, Q2 = 345, Q3 = 539), 342.81 (IQR = 239.5, Q1 = 203, Q2 = 300, Q3 = 442.5), and 371.47 (IQR = 306, Q1 = 189.25, Q2 = 291, Q3 = 495.25), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively (Figure 5.3).

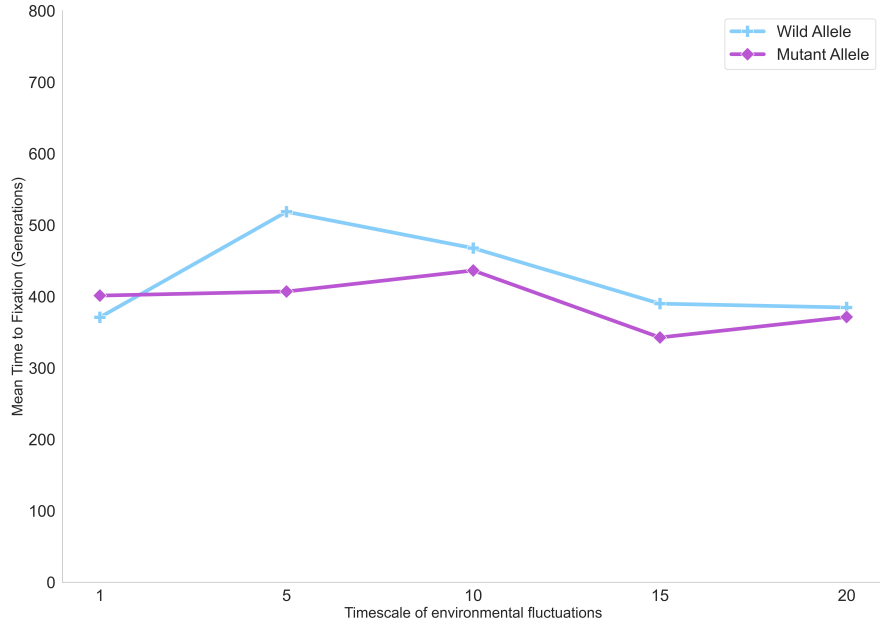


FIGURE 5.3: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on mean times to fixation of the two alleles (light blue line = $+$, violet line = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity*-% = 0 and *Cost-of-Plasticity*-% = 0.

When *Degree-of-Plasticity*-% = 10, that is a moderate environmentally induced shift in mouth-form ratio, the rel. f of fixation of $+$ was 63%, 60%, 68%, 77%, and 53%, with timescales of environmental change of 1, 5, 10, 15, and 20, respectively (Figure 5.4).

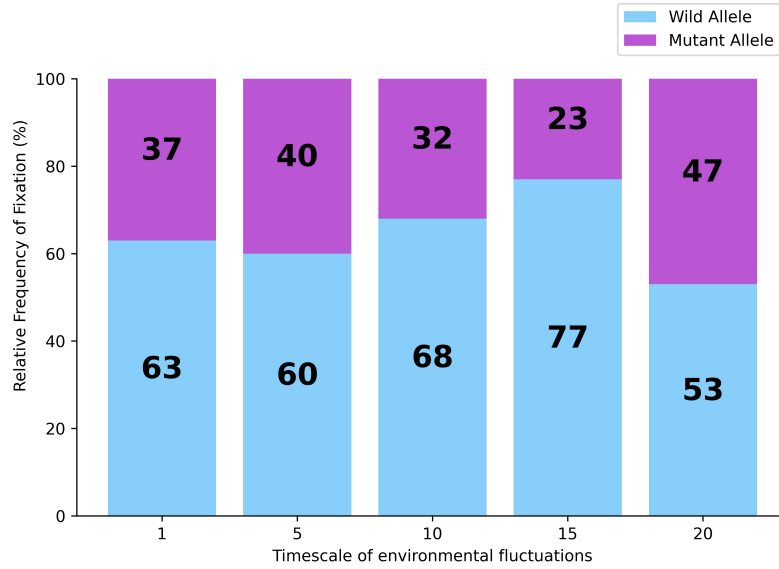


FIGURE 5.4: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on the relative frequencies of fixation of the two alleles (light blue bar = $+$, violet bar = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 10 and *Cost-of-Plasticity-%* = 0.

The mean times to fixation of the wild allele were: 444.06 (IQR = 299, Q1 = 222.5, Q2 = 331, Q3 = 521.5), 427.33 (IQR = 371.5, Q1 = 179, Q2 = 337.5, Q3 = 550.5), 435.16 (IQR = 410.75, Q1 = 199.25, Q2 = 412, Q3 = 610), 477.06 (IQR = 407, Q1 = 234, Q2 = 452, Q3 = 641), and 423.47 (IQR = 348, Q1 = 210, Q2 = 392, Q3 = 558), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively. The mean times to fixation of the mutant allele were: 429.81 (IQR = 412, Q1 = 198, Q2 = 337, Q3 = 610), 401.45 (IQR = 265.5, Q1 = 210.75, Q2 = 303, Q3 = 476.25), 407.59 (IQR = 316.75, Q1 = 249.25, Q2 = 335.5, Q3 = 566), 379 (IQR = 393.5, Q1 = 158, Q2 = 286, Q3 = 551.5), and 358.51 (IQR = 283, Q1 = 176, Q2 = 308, Q3 = 459), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively (Figure 5.5).

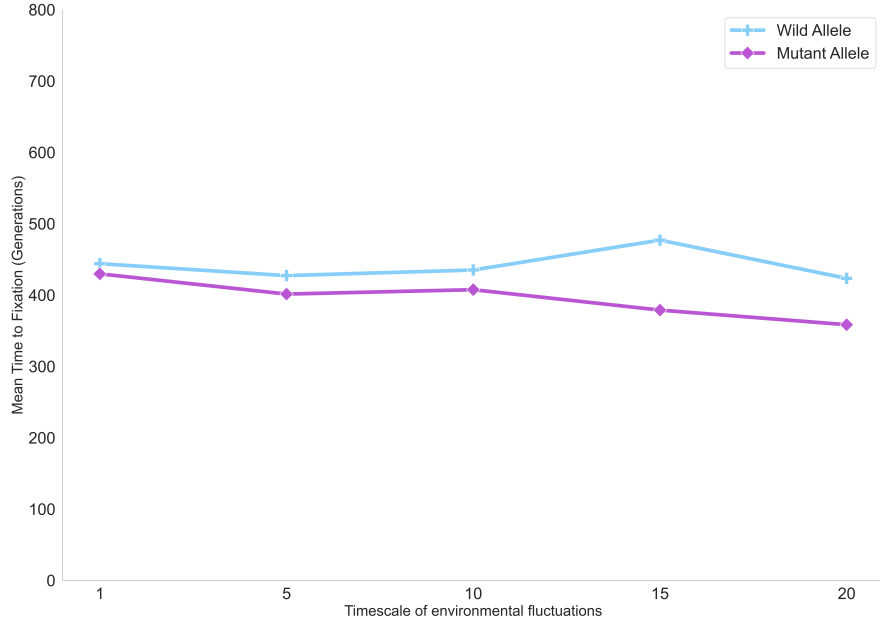


FIGURE 5.5: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on mean times to fixation of the two alleles (light blue line = $+$, violet line = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 10 and *Cost-of-Plasticity-%* = 0.

A *Degree-of-Plasticity-%* = 20 enables the highest possible environmentally induced departure from the mouth-form ratio shown by PS312 under standard laboratory conditions, because it makes the $++$ genotype fully penetrant in response to microbial starvation, with only EU nematodes developing when bacteria are scarce. With this value, the rel. f of fixation of $+$ was 71%, 74%, 62%, 77%, and 77%, with *Environmental-Cycles* = 1, 5, 10, 15, and 20, respectively (Figure 5.6).

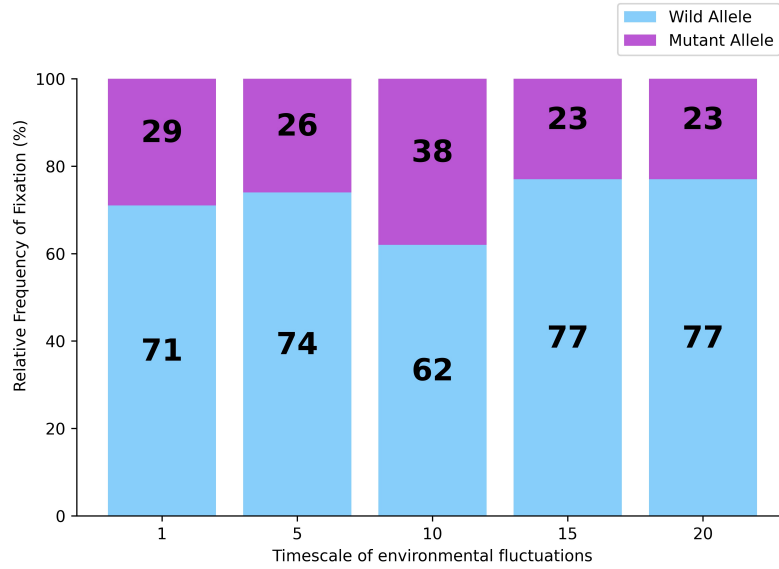


FIGURE 5.6: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on the relative frequencies of fixation of the two alleles (light blue bar = $+$, violet bar = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 20 and *Cost-of-Plasticity-%* = 0.

The mean times to fixation of the wild allele were: 353.42 (IQR = 206, Q1 = 177.5, Q2 = 235, Q3 = 383.5), 389.66 (IQR = 338.75, Q1 = 174.75, Q2 = 298.5, Q3 = 513.5), 428.52 (IQR = 279.25, Q1 = 248.25, Q2 = 344, Q3 = 527.5), 394.87 (IQR = 316, Q1 = 179, Q2 = 287, Q3 = 495), and 469.62 (IQR = 436, Q1 = 214, Q2 = 386, Q3 = 650), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively. The mean times to fixation of the mutant allele were: 257.45 (IQR = 204, Q1 = 136, Q2 = 211, Q3 = 340), 400.77 (IQR = 370.25, Q1 = 210, Q2 = 361.5, Q3 = 580.25), 410.87 (IQR = 281.5, Q1 = 226.5, Q2 = 333.5, Q3 = 508), 380.74 (IQR = 324.5, Q1 = 173, Q2 = 291, Q3 = 497.5), 469.74 (IQR = 436.5, Q1 = 201, Q2 = 365, Q3 = 637.5), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively (Figure 5.7).

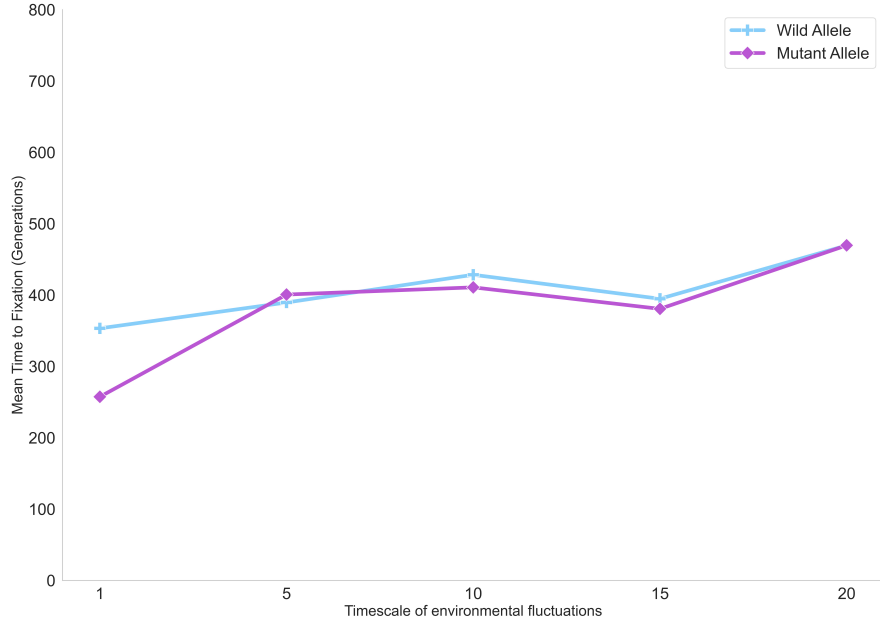


FIGURE 5.7: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on mean times to fixation of the two alleles (light blue line = $+$, violet line = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 20 and *Cost-of-Plasticity-%* = 0.

Collectively, these outcomes suggest a positive relation between the magnitude of the plastic response induced by diet and the frequency of fixation of the plastic strain. More generally, this SA shows that both the dimorphism itself and the shift in mouth-form ratios induced by conditional plasticity may be advantageous for plastic strains when the environment is fluctuating, leading to a higher frequency of fixation of $+$ than expected as a result of genetic drift alone. In other words, when it is not costly, mouth plasticity may be adaptive for *P. pacificus* both when its regulation is only stochastic and when it is stochastic and conditional simultaneously. Moreover, moderate-to-high values of *Environmental-Cycles* (i.e., when the alternation of food sources happens each 10-20 generations) seem to be more favourable for the wild-type allele.

Mean times to fixation of $+$ might decrease when using high values of *Degree-of-Plasticity-%*, whereas the timescale of environmental change seems to have no significant effect on such times. Interestingly, mean times to fixation of e seem to show a slightly positive relation with *Environmental-Cycles* when *Degree-of-Plasticity-%* = 20, with the highest average time to fixation for this allele reached in this scenario when *Environmental-Cycles* = 20 (469.74 generations).

Effects of timescales of environmental change and degree of plasticity on the fixation of plastic strains with low cost of plasticity

This section is also focused on the SA “Impact of environmental change and degree of plasticity”. In this case, I report the outcomes obtained with low costs of plasticity, that is, *Cost-of-Plasticity-%* is kept fixed at 1 for all runs.

When *Degree-of-Plasticity-%* = 0, the rel. f of fixation of $+$ was 3%, 7%, 7%, 9%, and 7%, with *Environmental-Cycles* = 1, 5, 10, 15, and 20, respectively (Figure 5.8).

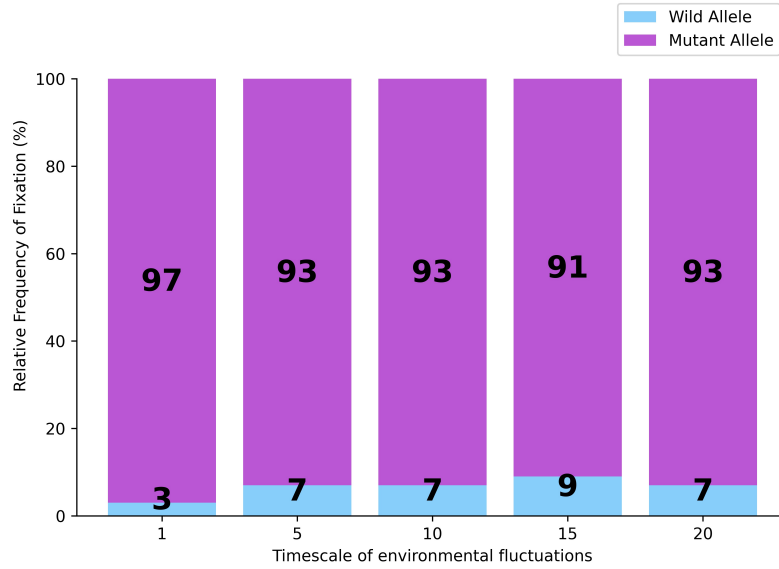


FIGURE 5.8: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on the relative frequencies of fixation of the two alleles (light blue bar = $+$, violet bar = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 0 and *Cost-of-Plasticity-%* = 1.

Mean times to fixation of the wild allele were: 378.67 (IQR = 294.5, Q1 = 207.5, Q2 = 283, Q3 = 502), 282.43 (IQR = 209.5, Q1 = 161, Q2 = 313, Q3 = 370.5), 273 (IQR = 177, Q1 = 174.5, Q2 = 262, Q3 = 351.5), 354.78 (IQR = 242, Q1 = 226, Q2 = 289, Q3 = 468), and 264.43 (IQR = 169, Q1 = 158.5, Q2 = 252, Q3 = 327.5), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively. As for the mutant allele, mean times to fixation were: 298.7 (IQR = 190, Q1 = 169, Q2 = 252, Q3 = 359), 262.81 (IQR = 187, Q1 = 124, Q2 = 223, Q3 = 311), 302.24 (IQR = 234, Q1 = 162, Q2 = 243, Q3 = 396), 314.57 (IQR = 225.5, Q1 = 165.5, Q2 = 233, Q3 = 391), 266.8 (IQR = 163, Q1 = 168, Q2 = 232, Q3 = 331) with *Environmental-Cycles* = 1, 5, 10, 15, and 20, respectively (Figure 5.9).

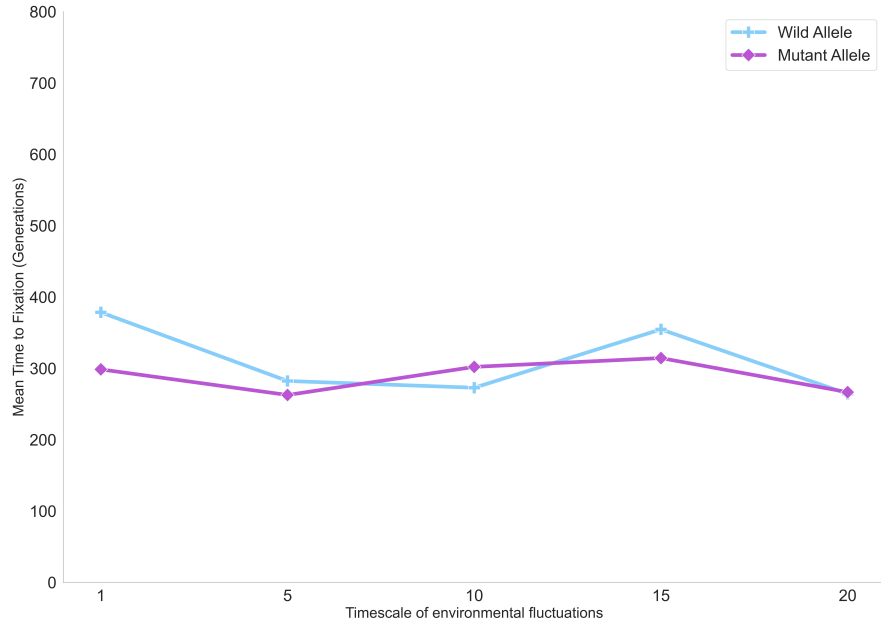


FIGURE 5.9: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on mean times to fixation of the two alleles (light blue line = $+$, violet line = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 0 and *Cost-of-Plasticity-%* = 1.

When *Degree-of-Plasticity-%* = 10, the rel. f of fixation of $+$ was 7%, 9%, 3%, 5%, and 6%, with *Environmental-Cycles* = 1, 5, 10, 15, and 20, respectively (Figure 5.10).

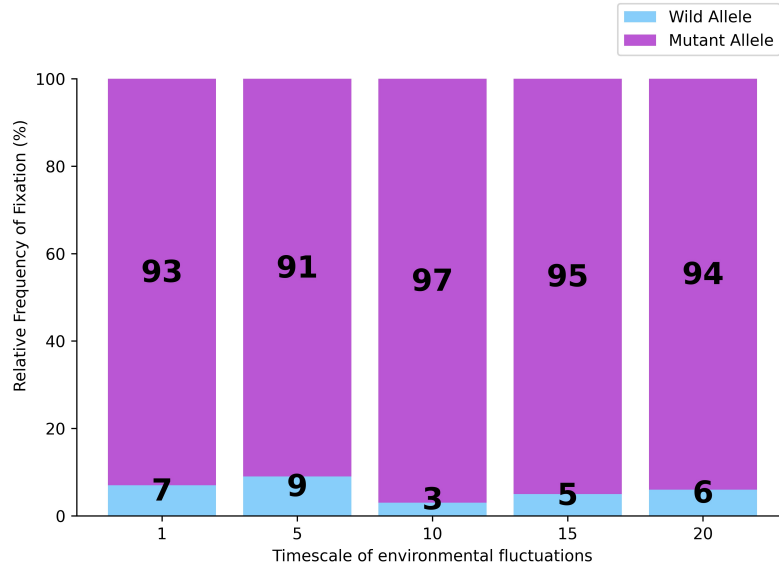


FIGURE 5.10: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on the relative frequencies of fixation of the two alleles (light blue bar = $+$, violet bar = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 10 and *Cost-of-Plasticity-%* = 1.

Mean times to fixation of the wild allele were: 362.57 (IQR = 260, Q1 = 224, Q2 = 283, Q3 = 484), 309.55 (IQR = 149, Q1 = 247, Q2 = 311, Q3 = 396), 356.67 (IQR = 101, Q1 = 306, Q2 = 356, Q3 = 407), 331 (IQR = 118, Q1 = 262, Q2 = 338, Q3 = 380), 269.5 (IQR = 58.5, Q1 = 245.5, Q2 = 263.5, Q3 = 304), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively. Mean times to fixation of the mutant allele were: 280.43 (IQR = 205, Q1 = 153, Q2 = 216, Q3 = 358), 334.03 (IQR = 244.5, Q1 = 170, Q2 = 284, Q3 = 414.5), 286.27 (IQR = 249, Q1 = 146, Q2 = 234, Q3 = 395), 293.69 (IQR = 212, Q1 = 152.5, Q2 = 237, Q3 = 364.5), 321.84 (IQR = 263.5, Q1 = 174, Q2 = 263.5, Q3 = 437.5), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively (Figure 5.11).

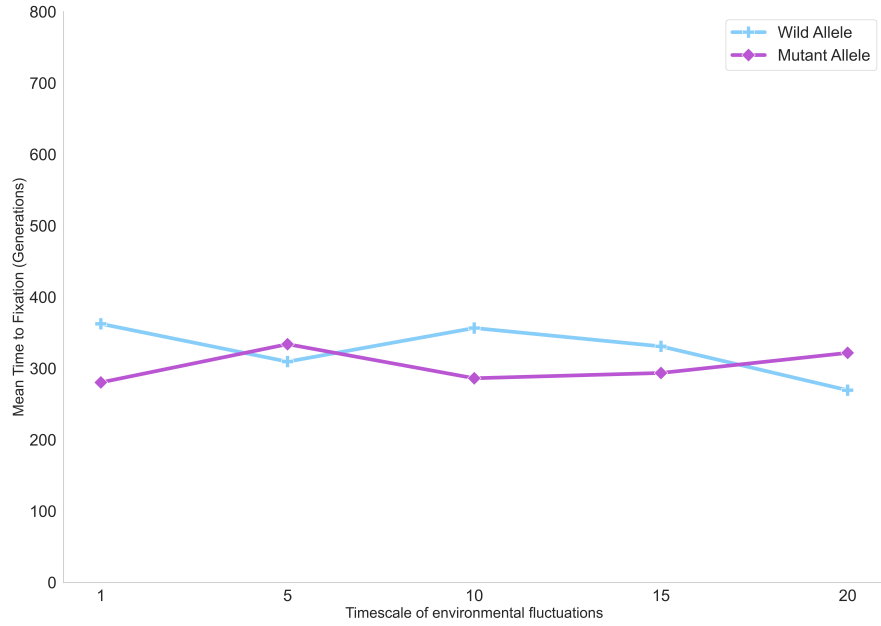


FIGURE 5.11: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on mean times to fixation of the two alleles (light blue line = $+$, violet line = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 10 and *Cost-of-Plasticity-%* = 1.

When *Degree-of-Plasticity-%* = 20, the rel. f of fixation of $+$ was 11%, 9%, 17%, 15%, and 14%, with *Environmental-Cycles* = 1, 5, 10, 15, and 20, respectively (Figure 5.12).

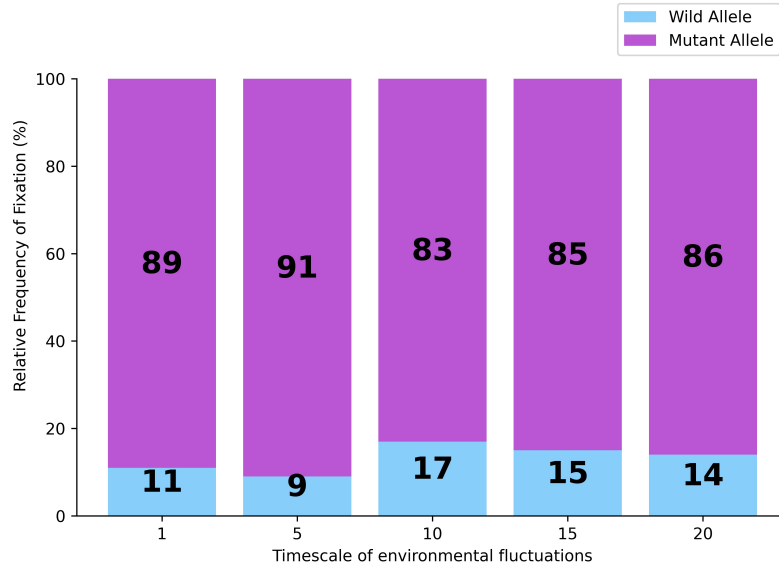


FIGURE 5.12: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on the relative frequencies of fixation of the two alleles (light blue bar = $+$, violet bar = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 20 and *Cost-of-Plasticity-%* = 1.

Mean times to fixation of the wild allele were: 360.82 (IQR = 223.5, Q1 = 236, Q2 = 338, Q3 = 459.5), 230.11 (IQR = 183, Q1 = 140, Q2 = 225, Q3 = 323), 351.18 (IQR = 216, Q1 = 215, Q2 = 240, Q3 = 431), 199.27 (IQR = 147.5, Q1 = 119, Q2 = 158, Q3 = 266.5), 315.36 (IQR = 257.5, Q1 = 147.25, Q2 = 272, Q3 = 404.75), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively. Mean times to fixation of the mutant allele were: 337.38 (IQR = 225, Q1 = 182, Q2 = 272, Q3 = 407), 332.67 (IQR = 302, Q1 = 164, Q2 = 260, Q3 = 466), 344.9 (IQR = 263, Q1 = 181, Q2 = 279, Q3 = 444), 351.93 (IQR = 261, Q1 = 206, Q2 = 271, Q3 = 467), 333.07 (IQR = 250.25, Q1 = 180.25, Q2 = 263, Q3 = 430.5), when *Environmental-Cycles* was 1, 5, 10, 15, and 20, respectively (Figure 5.13).

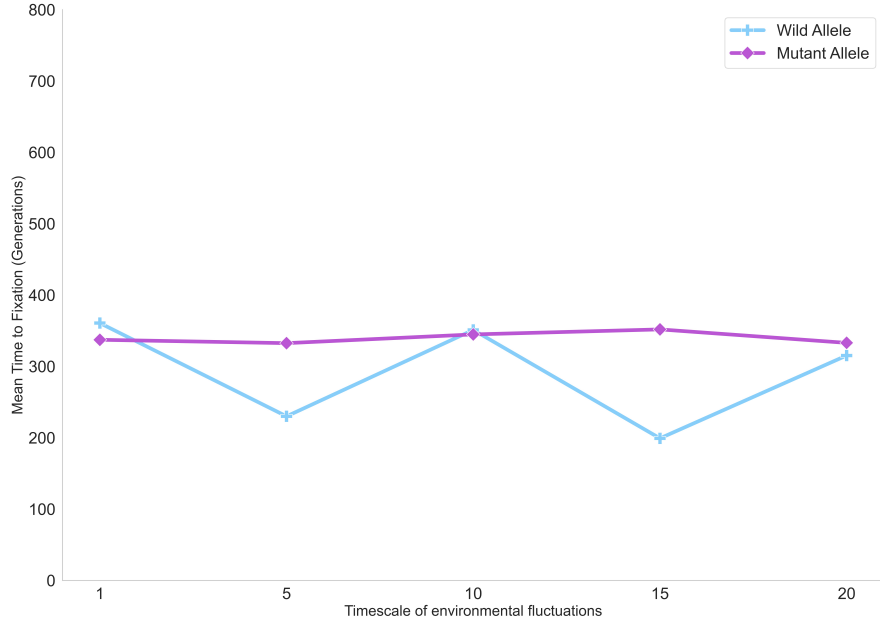


FIGURE 5.13: Effects of different timescales of environmental change in terms of generations (*Environmental-Cycles*) on mean times to fixation of the two alleles (light blue line = $+$, violet line = e). This plot was constructed using data from the SA named “Impact of environmental change and degree of plasticity”, with *Degree-of-Plasticity-%* = 20 and *Cost-of-Plasticity-%* = 1.

Although the reported frequency of fixation of the wild-type allele is low in each set of runs due to the application of the cost of plasticity, these outcomes seem to reflect some of the main tendencies observed with *Cost-of-Plasticity-%* = 0: the higher the *Degree-of-Plasticity-%*, the greater the number of fixations of $+$. This is true at least when *Degree-of-Plasticity-%* is above a threshold value between 10 and 20, since the frequency of fixation of $+$ was comparable in most cases where *Degree-of-Plasticity-%* = 0 and 10, whereas it was consistently higher when *Degree-of-Plasticity-%* = 20, especially when *Environmental-Cycles* were moderate-to-high, that is, when an inversion in dietary conditions happens every 10-20 generations. In the latter case, the frequency of fixation of $+$ was even six times higher (with *Environmental-Cycles* = 10) than the corre-

sponding value obtained with half the degree of plasticity (10%).

To sum up, higher levels of responsiveness to environmental cues (i.e., more pronounced shifts in phenotype ratios in favour of the most beneficial morph for each dietary regime) seem to be positively related to the frequency of fixation of the wild-type allele when the level of plasticity is above a specific tipping point.

While the timescale of environmental change seems to have no relevant effect on mean times to fixation of the two alleles, a *Degree-of-Plasticity-%* of 20 could favour the wild allele, since it seems to decrease the number of generations required to reach its irreversible fixation in the gene pool of the population. More specifically, on average, the $+$ allele reached fixation faster than e when *Environmental-Cycles* = 5, 15, and 20. However, any consideration on mean times until fixation is limited by small sample sizes, especially when *Degree-of-Plasticity-%* = 0 or 10 (e.g., there were just 3 fixations of $+$ when *Environmental-Cycles* = 10).

To what extent does the cost of plasticity favour the non-plastic strain?

The SA named “Tolerance of plastic strains towards the cost of plasticity” is based on the following settings: *Initial-++* = 150, *Initial-ee* = 150, *Carrying-Capacity* = 300, alternate presence of abundant bacteria and *C. elegans* preys (i.e., the environment is fluctuating), *Environmental-Cycles* = 20, *Probability-of-Non-Disjunction-%* = 1, *Degree-of-Plasticity-%* = 20, *Cost-of-Plasticity-%* = 0, 1, 2, and 3, *Cost-of-Starvation-%* = 3, equal benefits in fecundity for both mouth phenotypes = 2 (i.e., *Benefit-of-Faster-Development-%* = *Benefit-of-Predation-%* = 2), and *Efficiency-of-Attack-%* = 8. The high level of plasticity and timescale of environmental change were selected because it turned out from previous runs (see sections above) that these settings can increase the probability of fixation of the wild-type allele, putting the latter in a better position to resist the costs associated with plasticity.

This scenario clearly shows that even moderate costs of plasticity are sufficient to prevent the fixation of the wild allele. Indeed, while the rel. f of fixation of $+$ was 77% when *Cost-of-Plasticity-%* = 0 and 14% when *Cost-of-Plasticity-%* = 1 (see above), the number of fixations of $+$ collapsed to 0 both with *Cost-of-Plasticity-%* =

2 and *Cost-of-Plasticity-%* = 3. Therefore, only low or null costs of plasticity allow the fixation of the plastic strain, otherwise the wild-type allele is doomed to extinction and the non-plastic strains will inevitably proliferate, even if the environmental conditions fluctuate over time.

Moreover, higher costs of plasticity may also decrease the time of coexistence of alternative genotypes under symmetric conditions by favouring the mutant allele. Indeed, while with *Cost-of-Plasticity-%* = 0 the mean time until fixation of the allele *e* was 469.74 (IQR = 436.5, Q1 = 201, Q2 = 365, Q3 = 637.5), this average value steadily decreased when *Cost-of-Plasticity-%* = 1 (333.07, with IQR = 250.25, Q1 = 180.25, Q2 = 263, and Q3 = 430.5), 2 (180.09, with IQR = 116, Q1 = 115.5, Q2 = 151, and Q3 = 231.5), and 3 (130.97, with IQR = 61.25, Q1 = 89, Q2 = 121, and Q3 = 150.25) (Figure 5.14). Thus, there is a clear negative relation between the costs associated with phenotypic plasticity and the mean time to fixation of the mutant allele (it is important to recall that this allele promotes the constitutive expression of the ST morph).

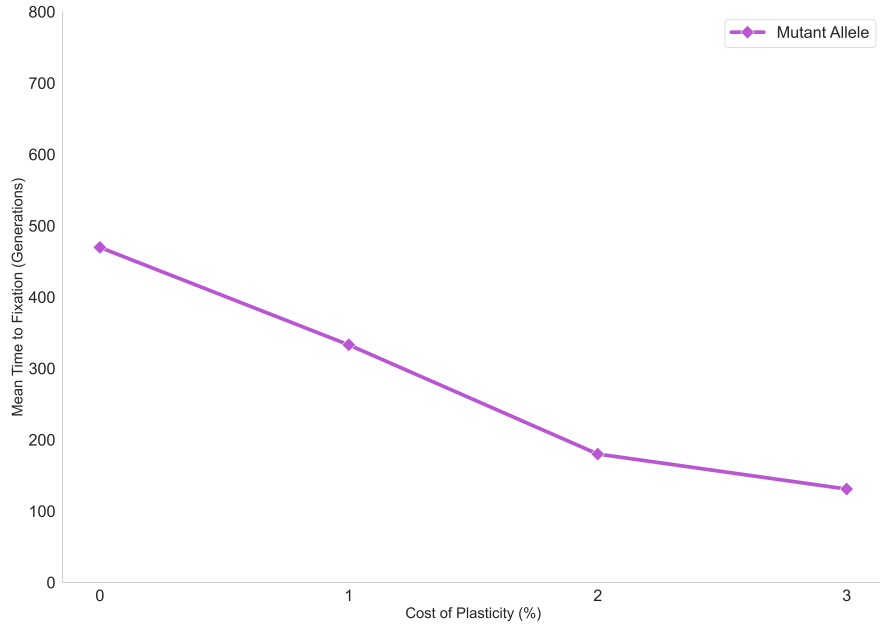


FIGURE 5.14: Effects of variation of *Cost-of-Plasticity-%* on mean times to fixation of the mutant allele. This plot was constructed using data from the SA named “Tolerance of plastic strains towards the cost of plasticity”.

Capacity of the non-plastic strain to invade a plastic population in both stable and oscillating environmental contexts

The evolutionary dynamics of digital populations of *P. pacificus* were also investigated using more realistic initial conditions, that is, through Invasibility Analyses (IAs). Invasibility is the chance or the ability of a population (in this case, a particular strain) to increase its frequency from rarity and become established (Pande et al., 2022; Kalirad and Sommer, 2024a)¹³⁹¹⁴⁰. All IAs presented here involve a single initial organism belonging to a different strain

¹³⁹Pande, J., Tsubery, Y., and Shnerb, N. M. (2022). Quantifying invasibility. *Ecology Letters*, 25(8):1783–1794.

¹⁴⁰Kalirad, A. and Sommer, R. J. (2024a). The role of plasticity and stochasticity in coexistence. *Ecology Letters*, 27(2).

from the rest of the population, e.g., due to mutation (i.e., $++ \rightarrow +e$ or $ee \rightarrow +e$) or gene flow. Note that the genotype/strain $+e$ is still able to express mouth dimorphism and plasticity, but was used as a potential parent of non-plastic worms in scenarios where the prevalent strain was $++$. All following scenarios include 1000 runs.

The IA named “Impact of cost of plasticity on the fixation of non-plastic strains” is based on the following initial settings: *Initial-++* = 299, *Initial-+e* = 1, *Carrying-Capacity* = 300, stable presence of abundant bacteria, absence of *C. elegans* preys (i.e., the environment is not fluctuating), *Probability-of-Non-Disjunction-%* = 1, *Degree-of-Plasticity-%* = 10, *Cost-of-Plasticity-%* = 0, 1, 2, and 3, *Cost-of-Starvation-%* = 3, equal benefits in fecundity for both mouth phenotypes = 2 (i.e., *Benefit-of-Faster-Development-%* = *Benefit-of-Predation-%* = 2), and *Efficiency-of-Attack-%* = 8. This scenario is aimed at identifying the frequency and mean time to fixation of a newly arisen allele in response to variation in the cost associated with plasticity.

When *Cost-of-Plasticity-%* = 0, the absolute f of fixation of e was 15. The frequency was more than doubled (31) when *Cost-of-Plasticity-%* = 1, which suggests that the existence of this cost can be relevant in increasing the frequency of fixation of the mutant allele. Nevertheless, further increases in the cost of plasticity seem to have very moderate or even null effects on the capacity of fixation of e , that is suggested by the outcomes obtained with *Cost-of-Plasticity-%* = 2 (f of fixation of e = 39) and 3 (f of fixation of e = 38) (Figure 5.15).

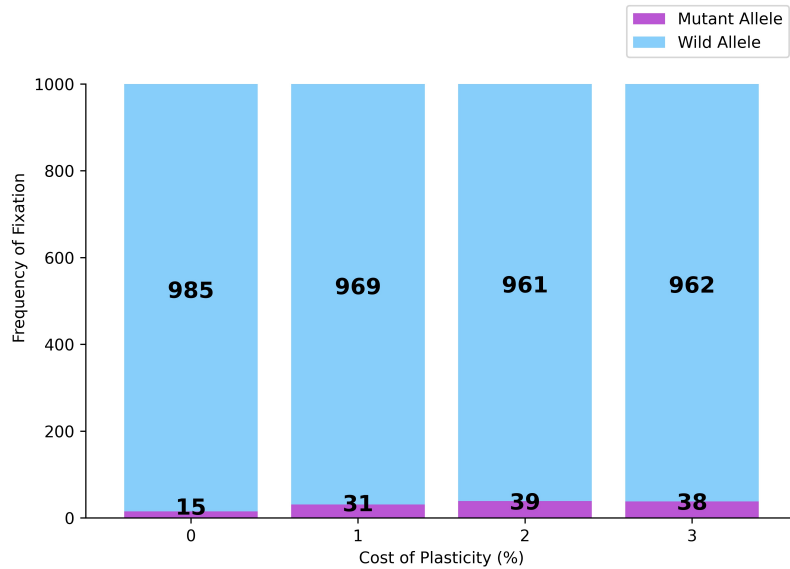


FIGURE 5.15: Effects of variation of *Cost-of-Plasticity-%* on the frequency of fixation of the mutant allele (light blue bar = $+$, violet bar = e). This plot was constructed using data from the IA named “Impact of cost of plasticity on the fixation of non-plastic strains”.

Similarly, this parameter was also relevant for mean times to fixation when passing from a condition with no costs to *Cost-of-Plasticity-%* = 1, whereas any further increase had modest effects. More specifically, mean times to fixation of e were: 351.4 (IQR = 78, Q1 = 282.5, Q2 = 313, Q3 = 360.5), 226.13 (IQR = 86.5, Q1 = 180.5, Q2 = 213, Q3 = 267), 196.85 (IQR = 38.5, Q1 = 170, Q2 = 188, Q3 = 208.5), and 160.39 (IQR = 69.5, Q1 = 122, Q2 = 147.5, Q3 = 191.5), when *Cost-of-Plasticity-%* = 0, 1, 2, and 3, respectively (Figure 5.16).

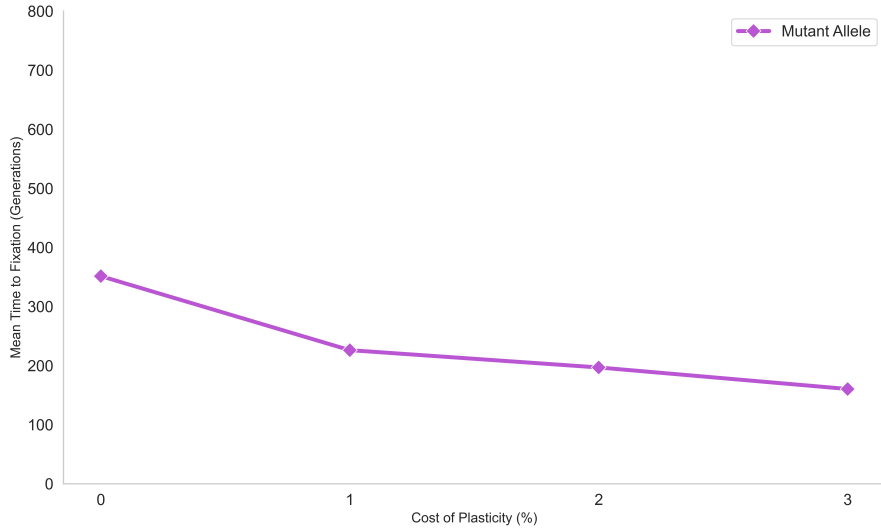


FIGURE 5.16: Effects of variation of *Cost-of-Plasticity-%* on mean times to fixation of the mutant allele. This plot was constructed using data from the IA named “Impact of cost of plasticity on the fixation of non-plastic strains”.

Interestingly, in an additional scenario carried out with the same basic settings of “Impact of cost of plasticity on the fixation of non-plastic strains”, this time with fixed *Cost-of-Plasticity-%* = 1, fluctuating environment (with *Environmental-Cycles* = 10), and *Degree-of-Plasticity-%* = 20, which would be expected to favour the plastic strain, the non-plastic strain still reached fixation in 7/1000 replicas. In particular, the mean time to fixation of e was 538.14 (IQR = 171, Q1 = 423.5, Q2 = 488, Q3 = 594.5), that is a period of coexistence between the two strains higher than those extracted from all the symmetric scenarios (although the sample size is very small here). Therefore, non-plastic strains can invade plastic populations even when the dietary conditions are fluctuating. Moreover, although an absorbing state was reached in very few generations in the vast majority of runs, these initial conditions may increase the time of coexistence between alternative strains in cases where the mutant allele succeeds in becoming fixed.

Effects of variable benefit of predation on the fixation of plastic strains in a slowly changing environment

While all previous experimental scenarios included equal benefits in fecundity for both mouth forms (i.e., ST and EU), the IA “Effects of benefit of predation on the fixation of plastic strains” was designed to assess the evolutionary effects of differences between these two benefits. In fact, it is reasonable to hypothesise that differences in fecundity between ST and EU nematodes may favour or harm each strain in relation to its ability to express the phenotype most attuned to the current environmental conditions with appreciable frequencies. Moreover, since the ST morph is shared by both strains, whereas the EU phenotype is only developed by carriers of at least one copy of the wild-type allele, the global dynamics resulting from bringing imbalances between the two benefits might uncover several unpredictable patterns.

This scenario is based on the following initial settings: *Initial-ee* = 299, *Initial-+e* = 0 or 1, *Initial-++* = 0 or 1, *Carrying-Capacity* = 300, alternate presence of abundant bacteria and *C. elegans* preys (i.e., the environment is oscillating), *Environmental-Cycles* = 10, *Probability-of-Non-Disjunction-%* = 1, *Degree-of-Plasticity-%* = 0 or 20, *Cost-of-Plasticity-%* = 0 or 1, *Cost-of-Starvation-%* = 3, *Benefit-of-Faster-Development-%* = 1, *Benefit-of-Predation-%* = 1, 2, or 3, and *Efficiency-of-Attack-%* = 8. The timescale of environmental change and the level of plasticity were chosen to reflect values shown to be most advantageous for *+* in SAs with *Cost-of-Plasticity-%* = 1 (see above).

When the genotype of the initial invader was *+e*, that is, when it is most likely a mutant, the outcomes were perhaps surprising: in all three cases (i.e., when *Benefit-of-Predation-%* - *Benefit-of-Faster-Development-%* = 0, 1, and 2, respectively), the wild-type allele never reached fixation in any of the 1000 runs carried out for each set. The most advantageous parameter values for plastic strains (i.e., high levels of plasticity and moderate timescale of environmental change, see above) combined with remarkable benefits for the EU phenotype were not enough to overcome even a low value of *Cost-of-Plasticity-%*. This result informs that the presence of a cost associated with the expression of plastic responses is an insurmountable barrier for mutants reverting to a plastic genotype.

When the genotype of the initial invader was *++*, that is, when

it is most likely an immigrant, the outcomes were even more unexpected, since nematodes of this strain are able to develop the advantaged morph (EU) with a 100% probability when bacteria are scarce (*Degree-of-Plasticity-%* = 20). When the difference between the two benefits in fecundity was 0% or 1%, the $+$ allele was still not able to reach fixation in any of the 1000 replicas. A difference of 2% in favour of the EU phenotype was critical to allow a very low number of fixations (3/1000). Thus, there is a very small probability that a wild-type immigrant colonises a non-plastic population if plasticity has an inherent cost.

However, an alternative starting condition involving again a $+e$ nematode, this time with a purely stochastic regulation of the plastic response for which it could be assumed a null cost of plasticity (i.e., *Degree-of-Plasticity-%* = *Cost-of-Plasticity-%* = 0), everything else being equal, revealed that a benefit in fecundity for EU higher than the benefit associated with ST may lead to fixation of the wild-type allele in several replicas (in the performed simulations, in 9 runs out of 1000). Although a single fixation event was also reached with equal benefits in fecundity for both phenotypes, any asymmetry in favour of EU between these parameters, regardless of the specific difference between them, seems to be a relevant requirement to ensure several fixations of $+$ (Figure 5.17).

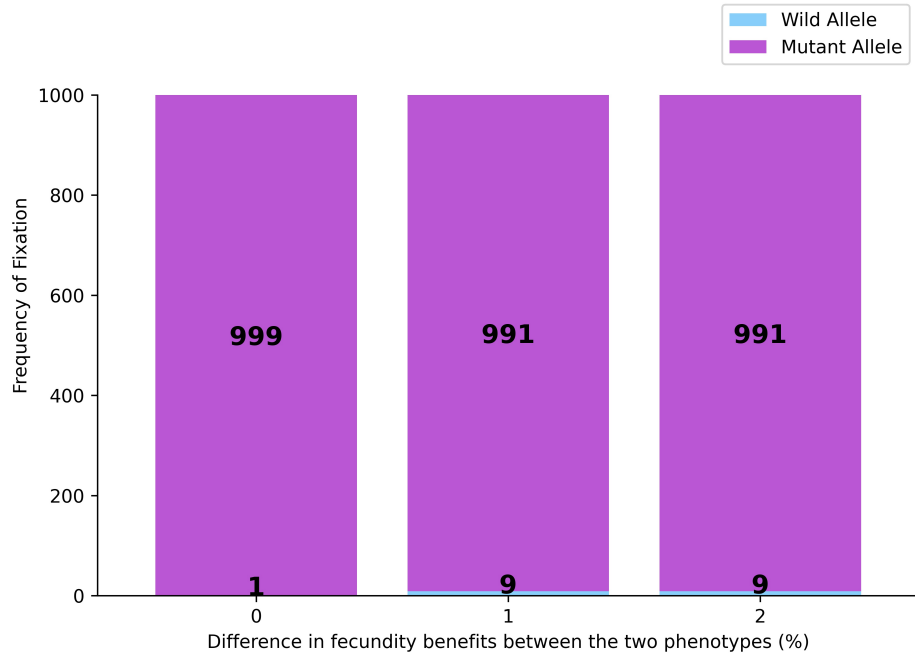


FIGURE 5.17: Effects of variation in differences between *Benefit-of-Predation-%* and *Benefit-of-Faster-Development-%* on the frequency of fixation of the wild-type allele. The initial settings included a single *+e* nematode and *Degree-of-Plasticity-%* = *Cost-of-Plasticity-%* = 0. This plot was constructed using data from the IA named “Impact of differences between the two benefits”.

Furthermore, the outcomes related to mean times to fixation, other than showing an expected pattern (i.e., a negative relation between the number of generations it takes for *+* to become fixed and the difference between benefits), suggest that invasions by mutant/immigrant genotypes with a stochastic determination of mouth-form ratio could enable very long times of coexistence between alternative strains. Indeed, mean times to fixation of *+* were: 753, 676.78 (IQR = 538, Q1 = 441, Q2 = 606, Q3 = 979), and 412.78 (IQR = 134, Q1 = 324, Q2 = 406, Q3 = 458) when *Benefit-of-Predation-%* - *Benefit-of-Faster-Development-%* = 0, 1, and 2, respectively (Figure 5.18).

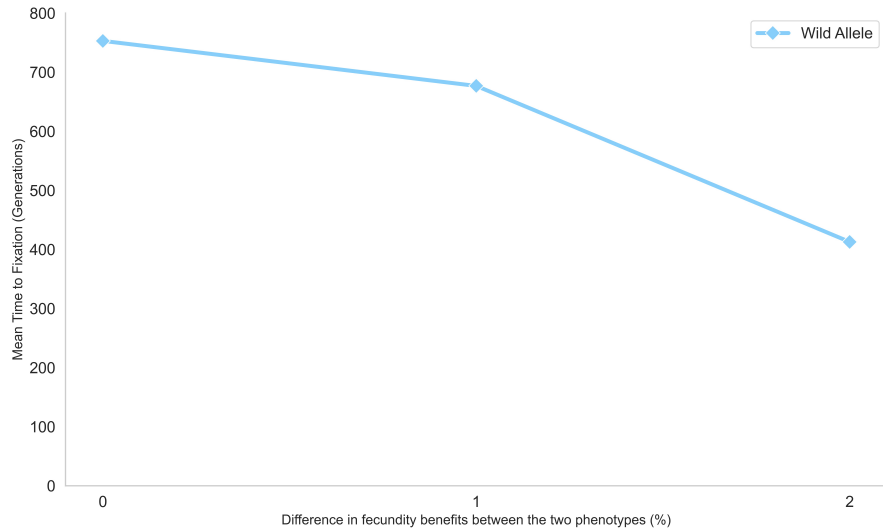


FIGURE 5.18: Effects of variation in differences between *Benefit-of-Predation-%* and *Benefit-of-Faster-Development-%* on mean times to fixation of the wild-type allele. The initial settings included a single *+e* nematode and *Degree-of-Plasticity-%* = *Cost-of-Plasticity-%* = 0. This plot was constructed using data from the IA named “Impact of differences between the two benefits”.

Finally, using the latter settings, I tested whether the highest level of plasticity selectable in this model (i.e., *Degree-of-Plasticity-%* = 20), again in association with no intrinsic cost of plasticity and everything else being equal, could enhance fixation of the wild-type allele. These settings are compatible with a situation where conditional regulation of plasticity is not inherently costly, or where this degree of plasticity is not so high as to entail an inherent cost. The outcomes could suggest a slightly increased advantage for *+* only when the benefit attributed to the EU morph exceeds the benefit attributed to the ST morph in 2 percentual points, as fixation was reached in 13 runs out of 1000. Conversely, fixation was reached only in 1 and 6 runs out of 1000 when the difference between benefits was 0% and 1%, respectively (Figure 5.19). Although these numbers are still low, this pattern could imply that the advantage of plasticity (which was clear in SAs) is only detectable when the exclusive morph of plastic strains (EU) is far more beneficial in terms

of fecundity than the morph shared by both strains (ST) in the proper environments.

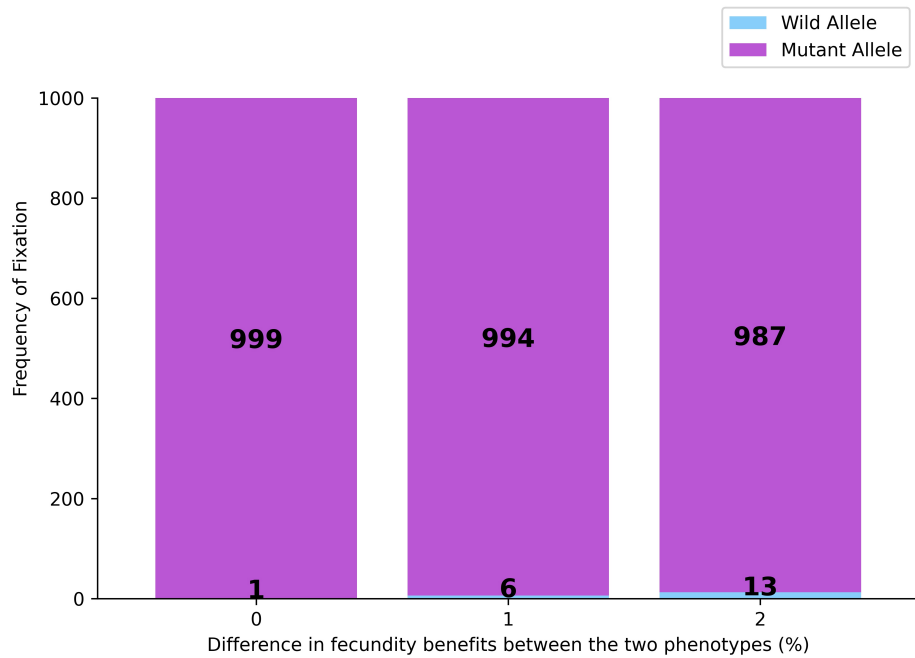


FIGURE 5.19: Effects of variation in differences between *Benefit-of-Predation-%* and *Benefit-of-Faster-Development-%* on the frequency of fixation of the wild-type allele. The initial settings included a single *+e* nematode, *Degree-of-Plasticity-%* = 20, and *Cost-of-Plasticity-%* = 0. This plot was constructed using data from the IA named “Impact of differences between the two benefits”.

Although the sample size is again limited, mean times to fixation of the wild-type allele seem to be negatively related to the degree of plasticity. Indeed, mean times to fixation of *+* were: 318, 467.33 (IQR = 132.25, Q1 = 384.25, Q2 = 487.5, Q3 = 516.5), and 363.85 (IQR = 155, Q1 = 287, Q2 = 347, Q3 = 442) when *Benefit-of-Predation-%* - *Benefit-of-Faster-Development-%* = 0, 1, and 2, respectively (Figure 5.20). This is another indication that, when the cost of plasticity is absent, the combined effect of higher expres-

sion of the optimal morph depending on diet and higher benefits assigned to EU might favour the plastic strain more than the case where plasticity is only stochastically regulated.

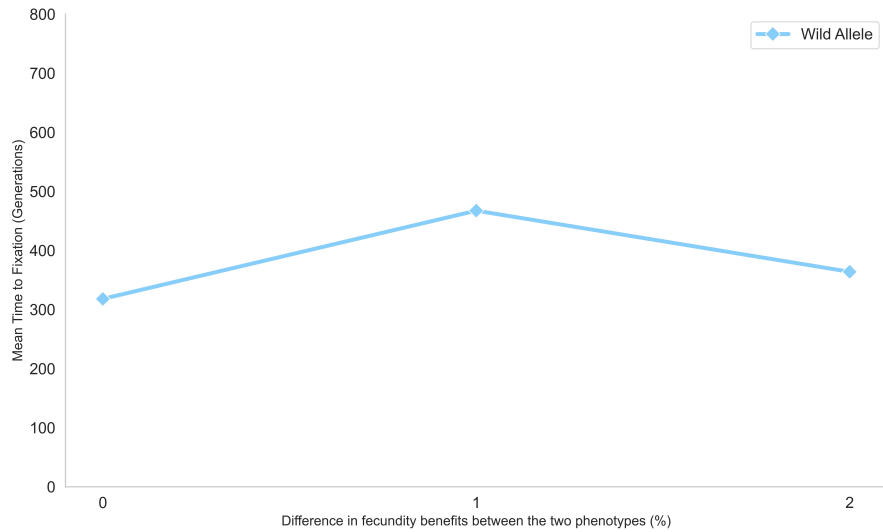


FIGURE 5.20: Effects of variation in differences between *Benefit-of-Predation-%* and *Benefit-of-Faster-Development-%* on mean times to fixation of the wild-type allele. The initial settings included a single *+e* nematode, *Degree-of-Plasticity-%* = 20, and *Cost-of-Plasticity-%* = 0. This plot was constructed using data from the IA named “Impact of differences between the two benefits”.

Altogether, these results highlight the critical role of the intrinsic cost of plasticity in the modelled system. Indeed, a low cost may prevent invasion by a plastic strain even when considering the most favourable conditions for the latter, that is, high degree of plasticity and environmental fluctuations of moderate-to-high duration (see SAs). Thus, the absence of an intrinsic plasticity-related cost might be a necessary condition both for plastic strains to outcompete non-plastic strains and for achieving a more stable coexistence between alternative strains of *P. pacificus*. Moreover, as shown by SAs, higher degrees of plasticity might promote fixation of the plastic genotype more than stochastic regulation of plasticity alone, but

only as long as there are no associated costs.

5.3 Discussion

High degree of plasticity and moderate-to-long environmental cycles could favour plastic strains, but the absence of an intrinsic cost of plasticity is crucial

Other than providing insights on the evolutionary interplay between plastic and non-plastic strains of *P. pacificus* depending on the state of the developmental switch, the outcomes of the model could also give some broader indications on critical parameters and specific conditions that favour each of the two alternative strategies/genotypes in nature.

In the first place, SAs (“Impact of environmental change and degree of plasticity”) suggest that both the timescale of environmental change and the degree of plasticity are relevant variables in the simulated *P. pacificus* system, regardless of whether plasticity is costly or not. If one assumes the absence of an inherent cost of plasticity, simulations clearly show that the combined effect of stochastic and conditional regulation of the dimorphism is highly beneficial for the plastic strain: the “baseline” mouth-form ratio of PS312, that is stochastically determined and intrinsically favourable in fluctuating dietary regimes, is powered by environmental responsiveness, that further increases the fraction of individuals developing the optimal phenotype under each diet. In line with theoretical expectations (Levins, 1974; van Tienderen, 1997; Lind and Johansson, 2009)¹⁴¹¹⁴²¹⁴³, the plastic strategy is always favoured over the non-plastic one in a changing environment under this cost-free condition, whereas the introduction of a low cost assigned to the plastic strain turns out to be critical in tilting the balance of the competition, making the constitutive expression of a single phenotype advantageous.

¹⁴¹Levins, R. A. (1974). Evolution in changing environments. Number 2 in Monographs in population biology. Princeton Univ. Press, Princeton, NJ, 3. printing edition.

¹⁴²van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution*, 51(5):1372–1380.

¹⁴³Lind, M. I. and Johansson, F. (2009). Costs and limits of phenotypic plasticity in island populations of the common frog *rana temporaria* under divergent selection pressures. *Evolution*, 63(6):1508–1518.

However, simulations carried out considering both conditions (i.e., no cost or low cost of plasticity) share a common general trend: the more reactive the plastic strain is to environmental stimuli, the more the wild-type allele will tend to reach fixation, especially when the environment does not change very quickly, that is, when periodic inversions in prevalent food sources happen every 10-20 generations. In addition, a high degree of plasticity might also make the reaching of fixation by the wild-type allele slightly faster, causing a small decrease in the time of coexistence between the two strains.

In the light of these outcomes, the degree of plasticity is certainly among the factors affecting the system in a relevant way. It is important to recall that since the model deals with two discrete alternative phenotypes, I treat this variable as the probability that an individual with a plastic genotype develops the optimal morph in response to dietary change. At the population level, any increase in the degree of plasticity results in a higher percentage of organisms developing the adaptive morph than expected based on the stochastically determined ratio of the strain alone. Considering the discrete nature of mouth form, the reaction norm of each mouth phenotype is flat when *Degree-of-Plasticity-%* = 0 and switch-like with a variable steep when *Degree-of-Plasticity-%* > 0, when plotting the relationship between the probability of developing that phenotype (y-axis) and the quantity of bacterial food (x-axis). Despite the relevance of switch-like reaction norms in nature, research on their impact on the evolution of discrete traits in response to changing environments is still limited (Crowther et al., 2023)¹⁴⁴. *P. pacificus*, by virtue of the abundance of data on its mouth polyphenism, is a perfect candidate for exploring these dynamics.

Since the degree of environmental sensitivity is plausibly controlled by gene regulatory networks (e.g., the pathway including *eud-1* as a key node), natural selection can affect its magnitude (Susoy and Sommer, 2016)¹⁴⁵. Therefore, populations may be composed of individuals or strains with different responsiveness and performances within a fluctuating environment. Indeed, intraspecific variation in

¹⁴⁴Crowther, C., Bonser, S. P., and Schwanz, L. E. (2023). Plasticity and the adaptive evolution of switchlike reaction norms under environmental change. *Evolution Letters*, 8(1):64–75.

¹⁴⁵Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

the level of plasticity is common and has been documented in many species, from chordates to plants Walls (1997); Nussey et al. (2005); Lind and Johansson (2009); Bhat et al. (2015); Grewell et al. (2016)¹⁴⁶¹⁴⁷¹⁴⁸¹⁴⁹¹⁵⁰. In particular, greater temporal variation and reliable environmental cues are among the main conditions promoting higher levels of plasticity (Hendry, 2015)¹⁵¹, as shown by many case studies reported in the literature. For instance, animals living across multiple variable environments, including some invasive species of prawns and springtails, often show a high level of plasticity (Chevin and Hoffmann, 2017)¹⁵². Among vertebrates, increasing selection in favour of individuals carrying highly plastic genotypes was directly observed in a wild Dutch population of great tits over a 32-year period (Nussey et al., 2005)¹⁵³. Similarly, a higher degree of plasticity might be selected in some populations of a cosmopolitan species like *P. pacificus*, as long as they live in sufficiently reliable fluctuating environments, e.g., when nematodes experience roughly periodic changes in the average dietary conditions.

Nevertheless, both theoretical (van Tienderen, 1997)¹⁵⁴ and empirical studies (Lind and Johansson, 2009)¹⁵⁵ hypothesised/found a possible positive relation between the degree of plasticity and the cost of plasticity, suggesting that the latter might be mostly found in highly plastic populations. If this is also applicable to *P. pacificus*,

¹⁴⁶Walls, M. (1997). Phenotypic plasticity of daphnia life history traits: the roles of predation, food level and toxic cyanobacteria. *Freshwater Biology*, 38(2):353–364.

¹⁴⁷Nussey, D. H., Postma, E., Gienapp, P., and Visser, M. E. (2005). Selection on heritable phenotypic plasticity in a wild bird population. *Science*, 310(5746):304–306.

¹⁴⁸Lind, M. I. and Johansson, F. (2009). Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution*, 63(6):1508–1518.

¹⁴⁹Bhat, A., Greulich, M. M., and Martins, E. P. (2015). Behavioral plasticity in response to environmental manipulation among zebrafish (*danio rerio*) populations. *PLOS ONE*, 10(4):e0125097.

¹⁵⁰Grewell, B. J., Castillo, J. M., Skaer Thomason, M. J., and Drenovsky, R. E. (2016). Phenotypic plasticity and population differentiation in response to salinity in the invasive cordgrass *spartina densiflora*. *Biological Invasions*, 18(8):2175–2187.

¹⁵¹Hendry, A. P. (2015). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *Journal of Heredity*, 107(1):25–41.

¹⁵²Chevin, L.-M. and Hoffmann, A. A. (2017). Evolution of phenotypic plasticity in extreme environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1723):20160138.

¹⁵³Nussey, D. H., Postma, E., Gienapp, P., and Visser, M. E. (2005). Selection on heritable phenotypic plasticity in a wild bird population. *Science*, 310(5746):304–306.

¹⁵⁴van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution*, 51(5):1372–1380.

¹⁵⁵Lind, M. I. and Johansson, F. (2009). Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution*, 63(6):1508–1518.

lower levels of phenotypic plasticity may be associated to the absence of the related cost, especially when a plastic strain only shows stochastic regulation of the plastic response (i.e., when *Degree-of-Plasticity*-% = 0). Since simulations proved that the wild-type Californian strain PS312 is favoured over hypothetical *eud-1* mutants when conditional regulation of plasticity is not included and plasticity is not costly, the absence of environmental responsiveness could be a highly beneficial condition for plastic strains.

This finding would corroborate the hypothesis that developmental stochasticity is a valuable bet-hedging strategy for *P. pacificus*, that is, a risk spreading strategy that is observed in contexts characterised by unpredictably changing environments. More technically, bet-hedging leads to a reduction in both mean fitness and fitness variance, which is a beneficial trade-off experienced by bet-hedger lineages across generations (Susoy and Sommer, 2016; Yasui and Garcia-Gonzalez, 2016; Sieriebriennikov and Sommer, 2018)¹⁵⁶¹⁵⁷¹⁵⁸. Among the several reasons why stochastic expression of alternative mouth morphs might be adaptive, there is the fact that the life cycle of *P. pacificus* might be too slow in relation to the rate of environmental change faced during its lifetime, that may prevent the anticipation of future environment (Susoy and Sommer, 2016)¹⁵⁹. Surprisingly, since the dietary regimes considered here do not change at an unpredictable rate and are all longer than a generation of nematodes, bet-hedging could be beneficial even in more stable, periodically oscillating environments. Therefore, this adaptive strategy that plausibly evolved in response to fast, unpredictable environmental change may also be advantageous on much longer timescales and when environmental change is reliable.

This advantageous condition could be reached if a mutation suppresses the sensory apparatus that enables conditional plasticity while preserving the dimorphism, assuming that the latter is realised through molecular mechanisms which do not require environmental responsiveness (e.g., stochastic gene expression), and that these

¹⁵⁶Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹⁵⁷Yasui, Y. and Garcia-Gonzalez, F. (2016). Bet-hedging as a mechanism for the evolution of polyandry, revisited: Polyandry as bethedging. *Evolution*, 70(2):385–397.

¹⁵⁸Sieriebriennikov, B. and Sommer, R. J. (2018). Developmental plasticity and robustness of a nematode mouth-form polyphenism. *Frontiers in Genetics*, 9.

¹⁵⁹Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

mechanisms are not associated with some intrinsic costs. Of course, as indicated by SAs, removing the ability to increase the probability of developing the optimal morph in a given environment would still be a cost of plasticity, because a larger part of the plastic population expresses a suboptimal morph (i.e., cost of phenotype-environment mismatching (Auld et al., 2009)¹⁶⁰). Nevertheless, research on unicellular organisms showed that, under certain conditions, stochastic switching can prevail over sensing, suggesting that this indirect cost may be less impactful than the cost associated with the molecular machinery used to sense the environment (Raj and van Oudenaarden, 2008)¹⁶¹. The hypothesis that a purely stochastic regulation of plasticity could be beneficial for *P. pacificus* would also support previous comparisons between its mouth dimorphism and bacterial bistability (Susoy and Sommer, 2016)¹⁶². However, further speculations on the relationship between degree and costs of plasticity are discussed later, taking into account the outcomes of IAs.

Another important factor to be analysed is the timescale of environmental change. Understanding the interaction between environmental change and the role of plasticity in evolution is of striking relevance in the context of population dynamics and viability, especially when considering the consequences of climate change (Vinton et al., 2022)¹⁶³. Most environments change over time, and periodic fluctuations are widespread and characterised by the most diverse timescales (Abdul-Rahman et al., 2021)¹⁶⁴. Even if fluctuations may be fast and unpredictable in *P. pacificus* (Susoy and Sommer, 2016)¹⁶⁵, some specific contexts may guarantee longer timescales of change and more reliable environments, similarly to the conditions applied here. For instance, one of these ecosystems could be repre-

¹⁶⁰Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681):503–511.

¹⁶¹Raj, A. and van Oudenaarden, A. (2008). Nature, nurture, or chance: Stochastic gene expression and its consequences. *Cell*, 135(2):216–226.

¹⁶²Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

¹⁶³Vinton, A. C., Gascoigne, S. J., Sepil, I., and Salguero-Gomez, R. (2022). Plasticity’s role in adaptive evolution depends on environmental change components. *Trends in Ecology & Evolution*, 37(12):1067–1078.

¹⁶⁴Abdul-Rahman, F., Tranchina, D., and Gresham, D. (2021). Fluctuating environments maintain genetic diversity through neutral fitness effects and balancing selection. *Molecular Biology and Evolution*, 38(10):4362–4375.

¹⁶⁵Susoy, V. and Sommer, R. J. (2016). Stochastic and conditional regulation of nematode mouth-form dimorphisms. *Frontiers in Ecology and Evolution*, 4.

sented by carcasses of beetles co-infested by other nematodes, where mouth-form ratios can undergo major changes within a few weeks, that is, across several generations (Renahan and Sommer, 2022)¹⁶⁶. The existence of more stable and predictable, albeit transient phases would also be compatible with the kind of plasticity shown by wild-type *P. pacificus* nematodes, that is, an irreversible developmental decision. In fact, developmental plasticity is expected to evolve in environments that vary *across* generations, rather than *within* generations, which would instead favour reversible plastic responses (Gabriel and Lynch, 1992; Gabriel, 1999; Kassen, 2002)¹⁶⁷¹⁶⁸¹⁶⁹.

Interestingly, the finding that a moderate-to-long duration of alternative dietary regimes may favour the plastic strain in *P. pacificus* is in line with previous theoretical results, for instance, those obtained with a mathematical model based on the Lotka-Volterra equations and focusing on the competition between one generalist and two specialist genotypes (clones) of an asexually reproducing species (Kasada and Yoshida, 2020)¹⁷⁰. In this context, “generalist” and “specialist” are used as synonyms of “plastic” and “non-plastic”, respectively, although the former terms are primarily linked to the fitness of these genotypes under different environmental conditions and are not exclusively related to the concept of plasticity (van Tienderen, 1997; Fraebel et al., 2020)¹⁷¹¹⁷². The framework underlying this model is comparable with that adopted here and its outcomes suggest that both on moderate and long timescales of environmental fluctuations (in the latter case, only if the cost of plasticity is sufficiently low) the population with plasticity increases in number and, therefore, the generalist genotype may dominate over the specialist one. As argued by the authors, a possible explanation for this

¹⁶⁶Renahan, T. and Sommer, R. J. (2022). Multidimensional competition of nematodes affects plastic traits in a beetle ecosystem. *Frontiers in Cell and Developmental Biology*, 10.

¹⁶⁷Gabriel, W. and Lynch, M. (1992). The selective advantage of reaction norms for environmental tolerance. *Journal of Evolutionary Biology*, 5(1):41–59.

¹⁶⁸Gabriel, W. (1999). *Evolution of Reversible Plastic Responses: Inducible Defenses and Environmental Tolerance*, pages 286–305. Princeton University Press.

¹⁶⁹Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, 15(2):173–190.

¹⁷⁰Kasada, M. and Yoshida, T. (2020). The timescale of environmental fluctuations determines the competitive advantages of phenotypic plasticity and rapid evolution. *Population Ecology*, 62(4):385–394.

¹⁷¹van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution*, 51(5):1372–1380.

¹⁷²Fraebel, D. T., Gowda, K., Mani, M., and Kuehn, S. (2020). Evolution of generalists by phenotypic plasticity. *iScience*, 23(11):101678.

pattern is that the population with plasticity gains more benefits from the fitted environment for a longer interval of time, whereas the specialist genotype that experienced the maladaptive environment requires a longer time to recover in the next environmental cycle. Net of random oscillations in the reported frequencies, this is also the most likely explanation for the overall dynamics generated by *PhePlastiComp*: when the type of food available changes at a slower pace, non-plastic sub-populations face a longer “desert crossing” where they are severely affected by starvation, whereas plastic sub-populations can exploit the benefits of each environment for more generations by developing a variable fraction of the optimal morph.

A further fascinating aspect of research on timescales favouring either generalist or specialist genotypes is the possibility of extending the scope of these findings beyond the frontiers of evolutionary biology in the strict sense, for instance, by approaching topics in the field of immunology. Affinity maturation, that is, the process that enables the immune system to produce high-affinity antibodies to bind any antigen, is a peculiar kind of evolutionary mechanism which takes place inside the body of an organism (Mishra and Mariuzza, 2018)¹⁷³. Also in this case, the resulting products can be generalist (e.g., antibodies effective against a large variety of viral strains) or specialist (e.g., antibodies that only bind very specific viral strains). Providing dynamic environments in terms of switching between different antigens was found to be critical to select generalist antibodies. More specifically, using an intermediate timescale of change, that is, when the environment changes slow enough for significant changes to accumulate but fast enough to prevent the population from reaching a steady state, turned out to be the best protocol to obtain this outcome (Sachdeva et al., 2020)¹⁷⁴. Although the timescales considered in the latter work are not comparable with those implemented in my agent-based model, this study also supports the general idea that rapidly changing environments are not the most suitable for favouring generalist genotypes, whereas longer environmental cycles can perturb specialist strains to a greater ex-

¹⁷³Mishra, A. K. and Mariuzza, R. A. (2018). Insights into the structural basis of antibody affinity maturation from next-generation sequencing. *Frontiers in Immunology*, 9.

¹⁷⁴Sachdeva, V., Husain, K., Sheng, J., Wang, S., and Murugan, A. (2020). Tuning environmental timescales to evolve and maintain generalists. *Proceedings of the National Academy of Sciences*, 117(23):12693–12699.

tent than generalist strains.

To sum up, if the intrinsic cost of plasticity is zero, then higher levels of plasticity are more advantageous for plastic strains, whereas if this cost is present (even if low) conditional regulation of plasticity is no more convenient in the context of a competition with a non-plastic strain. In the latter case, bet-hedging is a better strategy which may allow plastic strains to outcompete non-plastic strains. Moreover, whatever the degree of plasticity, an environment with regular and moderate-to-slow temporal fluctuations enables dimorphic individuals to gain the maximum advantage from their condition.

Even a moderate cost of plasticity prevents the fixation of the plastic strain and causes the collapse of the time of coexistence

A further clear indication emerging from SAs (“Tolerance of plastic strains towards the cost of plasticity”) is that the magnitude of the cost of plasticity is another pivotal factor capable of radically changing the dynamics of the system. Indeed, under the considered conditions, any cost of plasticity that exceeds even very low or null values (i.e., 1% or 0%) is extremely penalising for generalists, both in terms of frequency and mean time to fixation.

Assuming the aforementioned positive relationship between cost and degree of plasticity (van Tienderen, 1997; Lind and Johansson, 2009)^{175,176}, the outcomes generated by the model when the cost of plasticity is moderate-to-high are particularly suitable to describe contexts where the degree of plasticity is also intermediate or high. Based on this finding, a possible biological justification of the absence of highly plastic strains of *P. pacificus* (compared to other diplogastrid species (Susoy et al., 2015)¹⁷⁷, see below) could be the evolution of a bet-hedging strategy in association with reduced activity of the sensory apparatus responsible for the production of the plastic reaction, which could perhaps allow a dramatic decrease in

¹⁷⁵van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution*, 51(5):1372–1380.

¹⁷⁶Lind, M. I. and Johansson, F. (2009). Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution*, 63(6):1508–1518.

¹⁷⁷Susoy, V., Ragsdale, E. J., Kanzaki, N., and Sommer, R. J. (2015). Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. *eLife*, 4.

the intrinsic cost of plasticity.

Speculations aside, the remarkable effect of increasing costs of plasticity on population dynamics is a further confirmation of the well-acknowledged theoretical prediction stating that a low cost of plasticity is a fundamental requirement for the proliferation and maintenance of plasticity itself (Hendry, 2015)¹⁷⁸. More specifically, the *P. pacificus* model system proves that this principle is also applicable in the case of a polyphenism whose regulation is both stochastic and conditional.

Regardless of environmental conditions and cost of plasticity, the non-plastic strain can invade a plastic population

Although it is widely recognised that fixed environments should favour specialist genotypes over generalists (Kassen, 2002; Herron and Doebeli, 2011)¹⁷⁹¹⁸⁰, the benefits of the former strategy in static environments have rarely been documented (Condon et al., 2013)¹⁸¹. Simulations carried out with *PhePlastiComp* provide several insights into this and other related dynamics by monitoring the evolutionary fate of hypothetical *eud-1* mutants/immigrants of *P. pacificus* invading a plastic population. It is important to recall that the specialist strategy is represented here by the non-plastic strain. IAs (“Impact of cost of plasticity on the fixation of non-plastic strains”) strongly suggest that, albeit infrequently, a newly arising allele repressing the plastic response can become established in the population across a broad variety of conditions, uncovering at least two surprising phenomena.

In the first place, unexpectedly, the mutant allele reaches fixation not only when the amount of available microbial food is abundant and stable over time, but even when the environment is periodically oscillating in terms of food sources and non-plastic strains have to face multiple time intervals in which they must pay a cost due to

¹⁷⁸Hendry, A. P. (2015). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *Journal of Heredity*, 107(1):25–41.

¹⁷⁹Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, 15(2):173–190.

¹⁸⁰Herron, M. D. and Doebeli, M. (2011). Adaptive diversification of a plastic trait in a predictably fluctuating environment. *Journal of Theoretical Biology*, 285(1):58–68.

¹⁸¹Condon, C., Cooper, B. S., Yeaman, S., and Angilletta, M. J. (2013). Temporal variation favors the evolution of generalists in experimental populations of *drosophila melanogaster*: Evolution of thermal plasticity. *Evolution*, 68(3):720–728.

bacterial starvation (Seroby et al., 2014)¹⁸², as long as plasticity has at least a minimum cost (i.e., 1%). I speculate that this counterintuitive fact could be explained by considering that, although the non-plastic strain pays a relevant starvation-related cost (i.e., 3%), this cost is also shared with the plastic strain, that must additionally bear the cost of plasticity. Furthermore, differently from the intrinsic cost of plasticity, the cost of starvation is cyclic. This means that while non-plastic strains can recover from diminishing densities during phases when microbial food is abundant (on moderate timescales), the benefits gained by the expression of higher percentages of the optimal morph in the proper environment might not be sufficient for the maintenance of a high net advantage by plastic strains.

Therefore, simulations support not only the benefits of developing a single, optimal phenotype in a static environment (even when plasticity is not inherently costly), but also the possible advantages of unconditional expression of a single phenotype in a fluctuating environment, assuming that plasticity is costly *per se*. In other terms, regardless of environmental variability, the existence of an intrinsic (even if low) cost of plasticity seems to be a sufficient condition to guarantee chances of invasion to strains defective in the expression of the EU morph. Also in this case, this surprising outcome has already been reported in bacteria. In particular, the glycolysis pathway can switch from conditional to constitutive expression when colonies of *E. coli* experience a regularly fluctuating environment over time, even if an inherent cost of plasticity is absent (Herron and Doebeli, 2011)¹⁸³. However, since the vast majority of natural isolates of *P. pacificus* are dimorphic and EU-biased (Ragsdale et al., 2013)¹⁸⁴, it could be hypothesised that invasions by *eud-1* mutants are unlikely due to the possible absence of an intrinsic cost of plasticity (see section below) or to a reduction in fitness experienced by some mutants (e.g., caused by pleiotropy, which is not considered in this model).

The second unexpected finding is that fixations of the non-

¹⁸²Seroby, V., Ragsdale, E. J., and Sommer, R. J. (2014). Adaptive value of a predatory mouth-form in a dimorphic nematode. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791):20141334.

¹⁸³Herron, M. D. and Doebeli, M. (2011). Adaptive diversification of a plastic trait in a predictably fluctuating environment. *Journal of Theoretical Biology*, 285(1):58–68.

¹⁸⁴Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

plastic strain do not increase in number as the cost of plasticity increases beyond a certain threshold. More specifically, the number of fixations does not increase steadily when increasing the cost of plasticity above 1%. Indeed, a small increase in the number of fixations (from a cost of 1% to a cost of 2%) is followed by the achievement of a steady state (from a cost of 2% to a cost of 3%). A plausible explanation for this behaviour of the system is that the same cost is also applied to heterozygotes carrying a single mutated *eud-1* gene, since they are still plastic. As a matter of fact, within the framework underlying this model and in line with observations conducted with mutagenized *P. pacificus* nematodes (Ragsdale et al., 2013)¹⁸⁵, a single mutation cannot completely defuse the molecular machinery responsible for the developmental decision. Conversely, it may be assumed that *ee* mutants produced by heterozygous parents do not have to bear such costs due to deactivation upstream of the gene regulatory network including the developmental switch, that leads to a fixed phenotype across environments. The assumption that the intrinsic cost of plasticity should not be applied to homozygous mutants because of the prominent epistatic position of *eud-1* within the pathway underlying mouth plasticity, although apparently trivial, is of crucial importance. Indeed, it avoids the paradox that occurs when some small downstream changes in the response pathway may not be sufficient to eliminate the costs associated with the plastic response, even if the phenotype is fixed, because the sensory apparatus is still fundamentally intact (Agrawal, 2001; Callahan et al., 2008)¹⁸⁶¹⁸⁷.

A further interesting remark concerns the time of coexistence between alternative strains. While in the case of a stable environment the presence of *Cost-of-Plasticity-%* (but not its magnitude) accelerates the average time until fixation of the mutant allele in a relevant way, it is not the same when the environment is oscillating. In this case, we see a longer, although transient coexistence between the two strains, suggesting that a combination between a low cost

¹⁸⁵Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

¹⁸⁶Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*, 294(5541):321–326.

¹⁸⁷Callahan, H. S., Maughan, H., and Steiner, U. K. (2008). Phenotypic plasticity, costs of phenotypes, and costs of plasticity: Toward an integrative view. *Annals of the New York Academy of Sciences*, 1133(1):44–66.

of plasticity and some specific timescales of periodic change in diet could be the key to finding a possible long-term balance between generalists and specialists in *P. pacificus*. However, in line with previous computational assessments also involving this species (Kalirad and Sommer, 2024a)¹⁸⁸, long-term coexistence between alternative strains is never observed in any of the runs reported here, as they all ended with the fixation of one of the two alleles (see also the next section).

The invasion of a non-plastic population by a plastic strain is highly dependent on the nature of the cost of plasticity

Another surprising finding comes from the IA involving a single $+e$ mutant within a population of non-plastic nematodes (“Impact of differences between the two benefits”). Even using starting conditions which are expected to be highly favourable for the plastic strain based on SAs, that is, moderate timescale of environmental change, high level of plasticity and low cost of plasticity, and introducing higher benefits in fecundity assigned to the EU morph compared to ST, the plastic revertant/immigrant is never able to invade the non-plastic population. Assuming the presence of an inherent cost of plasticity, the wild-type allele can only become established when is present in double copy at the beginning of runs, e.g., carried by a $++$ individual, that is able to develop the highest possible frequency of the optimal phenotypes for each environment already from the first generation. Furthermore, this could only happen if the difference between the benefit in fecundity assigned to EU and that assigned to ST is 2%. However, the heavy disadvantage of the plastic strain emerging from these outcomes seems very much at odds with the overwhelming prevalence of dimorphic/plastic strains found in nature (Ragsdale et al., 2013)¹⁸⁹. Therefore, this initial configuration is probably unrealistic and some of the assumptions need to be reconsidered to allow the model to generate patterns that better match the empirical data. I argue that the variable which is more capable of radically changing the overall behaviour

¹⁸⁸Kalirad, A. and Sommer, R. J. (2024a). The role of plasticity and stochasticity in coexistence. *Ecology Letters*, 27(2).

¹⁸⁹Ragsdale, E. J., Muller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

of the system is the cost of plasticity and speculate that, in natural populations of *P. pacificus*, the inherent cost could be circumvented so that the real cost which individuals have to bear could be entirely due to phenotype-environment mismatching (Auld et al., 2009)¹⁹⁰.

To discuss this hypothesis, I focus on both variants of this IA including no intrinsic costs of plasticity. Indeed, when the *ability* to express plastic responses is not penalised, invasion of the non-plastic population can be successful for plastic genotypes in a few replicas, with similar outcomes observed in both the examined cases (i.e., exclusively stochastic regulation of plasticity and stochastic + conditional regulation). In particular, the $\pm$ allele can become established in a higher number of cases when the EU phenotype is favoured over ST.

Let us first focus on the case where mouth polyphenism is not conditionally regulated in any way, that is, when *Degree-of-Plasticity*-% = 0. This strategy could be selected because, as stated above, stochastic regulation of plasticity may be exempted by costs related to the maintenance of the sensory apparatus that enables conditional regulation, although the cost of expressing a number of sub-optimal phenotypes for a certain environment (Raj and van Oudenaarden, 2008; Auld et al., 2009)¹⁹¹¹⁹² would be slightly increased. A plausible reason why these “exclusive bet-hedgers” would not bear any additional cost other than that related to mismatching is that they would not need any mechanism aimed at dampening the developmental noise underlying phenotypic stochasticity (Donaldson-Matasci et al., 2013; Woods, 2014)¹⁹³¹⁹⁴. Therefore, in case *any* (even minimal) level of conditional regulation of plasticity is costly, also considering the outcomes of SAs, diversifying bet-hedging without dietary responsiveness could be selected even if the environment fluctuates at regular intervals (i.e., when it is not

¹⁹⁰Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681):503–511.

¹⁹¹Raj, A. and van Oudenaarden, A. (2008). Nature, nurture, or chance: Stochastic gene expression and its consequences. *Cell*, 135(2):216–226.

¹⁹²Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681):503–511.

¹⁹³Donaldson-Matasci, M. C., Bergstrom, C. T., and Lachmann, M. (2013). When unreliable cues are good enough. *The American Naturalist*, 182(3):313–327.

¹⁹⁴Woods, H. A. (2014). Mosaic physiology from developmental noise: within-organism physiological diversity as an alternative to phenotypic plasticity and phenotypic flexibility. *Journal of Experimental Biology*, 217(1):35–45.

unpredictable). This evolutionary outcome, however, is only plausible assuming the possibility of disentangling the gene regulatory network including the developmental switch and the mechanism on which stochastic regulation depends, so that developmental sensitivity can be disabled without compromising the molecular dynamics required to implement the bet-hedging strategy.

The second examined case is closer to the configuration which is currently observed in wild isolates of *P. pacificus* (e.g., see Lenuzzi et al. (2021)¹⁹⁵), as it involves both stochastic regulation and a level of conditional regulation (20%, that is the maximum value allowed in the current version of *PhePlastiComp*). Also in this situation, the absence of an inherent cost of plasticity increases the probability of invasion by the wild-type allele, giving the plastic strain a higher level of competitiveness, that is more in line with empirical observations. Indeed, this mixed strategy is typical of this species and is commonly referred to as “coin-flipping plasticity”, that has also been reported in other Ecdysozoans. For instance, in pea aphids wing polyphenism (Grantham et al., 2016)¹⁹⁶, the probability of producing winged offspring depends on the level of crowding experienced by asexual mothers, but both winged and wingless offspring are always produced by individual aphids. It is already acknowledged that this balance between developmental sensitivity and stochasticity may be due to a combination of partial reliability of environmental cues and between-generations environmental variation (Donaldson-Matasci et al., 2013)¹⁹⁷, which could provide some additional information on the ecology of *P. pacificus*.

However, based on the outcomes of the agent-based model I also suggest that, at least under some conditions (e.g., when only the phenotype most frequently expressed across environments has remarkable benefits, see below), this mixed strategy might be selected because it could drastically reduce or even eliminate the intrinsic cost of plasticity. Indeed, if an intrinsic cost of plasticity is

¹⁹⁵Lenuzzi, M., Witte, H., Riebesell, M., Rodelsperger, C., Hong, R. L., and Sommer, R. J. (2021). Influence of environmental temperature on mouth-form plasticity in *pristionchus pacificus* acts through daf-11-dependent cgmmp signaling. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 340(2):214– 224.

¹⁹⁶Grantham, M. E., Antonio, C. J., O’Neil, B. R., Zhan, Y. X., and Brisson, J. A. (2016). A case for a joint strategy of diversified bet hedging and plasticity in the pea aphid wing polyphenism. *Biology Letters*, 12(10):20160654.

¹⁹⁷Donaldson-Matasci, M. C., Bergstrom, C. T., and Lachmann, M. (2013). When unreliable cues are good enough. *The American Naturalist*, 182(3):313–327.

present, but is positively related to the degree of plasticity, then an intermediate genotype with low plasticity could be a beneficial compromise for plastic strains (van Tienderen, 1997)¹⁹⁸, which could be the case when much of the phenotypic diversity is determined stochastically. More specifically, since the majority of diplogastrid nematodes not belonging to the genus *Pristionchus* show a much higher degree of plasticity than the maximum possible value implemented here in response to the two alternative feeding regimes (Susoy et al., 2015)¹⁹⁹, a relatively high degree of plasticity in the model might be low in absolute terms, and therefore might not be associated with an appreciable intrinsic cost of plasticity. This is a plausible scenario also considering some evidence suggesting that the fitness cost depending on the maintenance of additional regulatory machinery may be negligible in most cases (Latta et al., 2012; Murren et al., 2015)²⁰⁰²⁰¹, and that an inherent cost of plasticity is mainly found and expected in highly plastic populations (Lind and Johansson, 2009)²⁰². This consideration could also suggest the plausible dynamics of a competition between generalist genotypes showing different levels of plasticity: the evolutionary fate of the population would depend on a trade-off between degree and inherent cost of plasticity, so a very likely outcome would be the spread of the most plastic genotype among those which do not pay that cost. In this context, the emergence of a non-negligible direct cost associated with plasticity would be an impassable upper limit for plastic strains.

Alternatively, it is important to highlight that assigning a cost to the ability of expressing plastic responses may not accurately reflect the type of cost associated with the plastic strains of *P. pacificus* in nature. For instance, regardless of the level of plasticity, this

¹⁹⁸van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution*, 51(5):1372–1380.

¹⁹⁹Susoy, V., Ragsdale, E. J., Kanzaki, N., and Sommer, R. J. (2015). Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. *eLife*, 4.

²⁰⁰Latta, L. C., Weider, L. J., Colbourne, J. K., and Pfrender, M. E. (2012). The evolution of salinity tolerance in daphnia: a functional genomics approach. *Ecology Letters*, 15(8):794–802.

²⁰¹Murren, C. J., Auld, J. R., Callahan, H., Ghalambor, C. K., Handelsman, C. A., Heskell, M. A., Kingsolver, J. G., Maclean, H. J., Masel, J., Maughan, H., Pfennig, D. W., Relyea, R. A., Seiter, S., Snell-Rood, E., Steiner, U. K., and Schlichting, C. D. (2015). Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity*, 115(4):293–301.

²⁰²Lind, M. I. and Johansson, F. (2009). Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution*, 63(6):1508–1518.

cost might simply be related to the expression of the suboptimal morph in a specific environment by a fraction of the plastic population (Auld et al., 2009)²⁰³. In other words, the production and maintenance of the environmentally-sensitive decision may have no inherent cost. An analogous assumption has already been included in previous models also dealing with the evolution of plasticity, e.g., Tufto (2000)²⁰⁴. If there is no need to add a cost of plasticity other than that deriving from the decreased fecundity of suboptimal phenotypes, then all runs involving an intrinsic cost of plasticity would not apply to the modelled nematode system. This hypothesis could also explain in a simple way why wild isolates of *P. pacificus* are consistently dimorphic and plastic.

It is also very relevant to note that outcomes generated by runs excluding an intrinsic cost of plasticity uncover the crucial role of differences in fecundity between the two mouth morphs, as increasing the fitness of the morph which is only expressed by plastic strains (EU) resulted in higher frequencies of fixation of the wild-type allele than those observed when each phenotype was equally rewarded in the proper environment. A likely explanation is that this higher benefit may greatly enhance the reproductive performance of the plastic strains when nematode preys are present, while during cycles when microbial food is abundant the non-plastic strain is only slightly advantaged by its ability to develop 100% individuals capable of exploiting the benefits applied to ST. Furthermore, there is a single explicit cost in fecundity for both strains (the cost of starvation) and is experienced by both for the same time intervals. The fact that the plastic strain is perhaps more advantaged when the difference between these two benefits is high and the maximum available degree of plasticity is selected (i.e., 20%) makes even more evident the importance of the level of conditional regulation of plasticity in reducing the phenotype-environment mismatching (Auld et al., 2009)²⁰⁵. Thus, the interplay between the degree of plas-

²⁰³Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681):503–511.

²⁰⁴Tufto, J. (2000). The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *The American Naturalist*, 156(2):121–130.

²⁰⁵Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681):503–511.

ticity, the cost of plasticity, and the difference in fitness between phenotypes proves to be crucial and deserves further investigation to understand how a plastic genotype could prevail in a changing environment dominated by specialists.

Finally, when considering a context devoid of intrinsic costs of plasticity and with no (or low) environmental responsiveness, this scenario could also generate runs showing the longest time of coexistence between the plastic and the non-plastic strain. Besides very low sample sizes, invasions of non-plastic populations by a plastic genotype simulated in this IA could involve the most promising starting configuration to observe at least some cases of long-term coexistence. However, to ascertain this, it would be necessary to re-run the experiment with a much larger number of replicas.

5.4 Concluding remarks

Keeping in mind the unavoidable simplifications implicit in the implementation process, agent-based modelling can broaden the scientific scope of model organisms that have already proven to be powerful experimental subjects in a variety of areas, such as *P. pacificus* in the context of phenotypic plasticity. In particular, emerging patterns arising from multiple interacting digital entities can enable the assessment of multiple key variables including the degree of plasticity, the cost of plasticity, and the timescale of environmental change. Collectively, the insights suggested by the simulated scenarios could shed new light on competition and coexistence in *P. pacificus* and other species showing discrete plasticity, especially those characterised by a combination of bet-hedging and environmental responsiveness (e.g., see Grantham et al. (2016)²⁰⁶).

Among the many possible extensions which could be added to the current version of *PhePlastiComp*, it could be of great interest the implementation of random mutations occurring during runs or an environment varying at an unpredictable rate, as well as the inclusion of some spatial components, that are already present in other models also dealing with *P. pacificus* (Dardiry et al., 2023;

²⁰⁶Grantham, M. E., Antonio, C. J., O’Neil, B. R., Zhan, Y. X., and Brisson, J. A. (2016). A case for a joint strategy of diversified bet hedging and plasticity in the pea aphid wing polyphenism. *Biology Letters*, 12(10):20160654.

Kalirad and Sommer, 2024a,b)²⁰⁷²⁰⁸²⁰⁹ and enable a higher level of realism of the simulated scenarios. Indeed, the latter changes could increase the heterogeneity of the environment both in space *and* time, which may have an impact on the cost of plasticity and give further details on which types of environmental changes are more likely to promote the evolution of plasticity (Scheiner, 2013; King and Hadfield, 2019; Dardiry et al., 2023; Kalirad and Sommer, 2024b)²¹⁰²¹¹²¹²²¹³. Moreover, further fascinating outcomes could be obtained by investigating the same scenarios with other baseline values of mouth-form ratios, using empirical data from strains different from PS312. In this regard, an interesting attempt would be to test whether the combined effects of alternative stochastically-regulated ratios and conditional regulation of plasticity could enable invasion by the wild-type allele when plasticity is intrinsically costly. Of course, the same approach could be used to simulate the population dynamics of other members of the family of the Diplogastridae, some of which show relevant characteristics similar to those of *P. pacificus*, e.g., *Allodiplogaster sudhausi*, that displays three alternative mouth morphs depending on different diets and whose plastic response also involves a developmental switch gene (Wighard et al., 2024)²¹⁴.

²⁰⁷Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

²⁰⁸Kalirad, A. and Sommer, R. J. (2024a). The role of plasticity and stochasticity in coexistence. *Ecology Letters*, 27(2).

²⁰⁹Kalirad, A. and Sommer, R. J. (2024b). Spatial and temporal heterogeneity alter the cost of plasticity in *Pristionchus pacificus*. *PLOS Computational Biology*, 20(1):e1011823.

²¹⁰Scheiner, S. M. (2013). The genetics of phenotypic plasticity.xii. temporal and spatial heterogeneity. *Ecology and Evolution*, 3(13):4596–4609.

²¹¹King, J. G. and Hadfield, J. D. (2019). The evolution of phenotypic plasticity when environments fluctuate in time and space. *Evolution Letters*, 3(1):15–27.

²¹²Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

²¹³Kalirad, A. and Sommer, R. J. (2024b). Spatial and temporal heterogeneity alter the cost of plasticity in *Pristionchus pacificus*. *PLOS Computational Biology*, 20(1):e1011823.

²¹⁴Wighard, S., Witte, H., and Sommer, R. J. (2024). Conserved switch genes that arose via whole-genome duplication regulate a cannibalistic nematode morph. *Science Advances*, 10(15).

Chapter 6

Appendix A: Code of the NetLogo model *PhePlastiComp*

```
breed [ nematodes nematode ]
breed [ bacteria bacterium ]

globals [ temperature stored-cost-of-starvation-\% ]

nematodes-own [ species genotype phenotype sex partner
  developmental-stage developmental-progression time-
  to-oviposition generation parent-1 parent-2
  nematodes-killed ]

to setup

  ca

  ask patches [ set pcolor 89 ]

  set temperature 20

  set stored-cost-of-starvation-\% cost-of-starvation-\%

  ifelse abundant-bacteria?
  [
    create-bacteria 800 [
      set shape "circle"
      set color 46
```

```

        set size 0.45
        setxy random-xcor random-ycor
    ]
]
[
    create-bacteria 200 [
        set shape "circle"
        set color 46
        set size 0.45
        setxy random-xcor random-ycor
    ]
]

ifelse c.elegans?
[
    create-nematodes 300 [
        set shape "nematode"
        set color grey
        set size 0.45
        setxy random-xcor random-ycor
        set species "c.elegans"
    ]
]
[ create-nematodes 0 ]

create-nematodes initial-++ [
    set shape "circle"
    set color grey
    set size 0.5
    setxy random-xcor random-ycor
    set species "p.pacificus"
    set genotype "++"
    set phenotype 0
    set sex "hermaphrodite"
    set partner nobody
    set developmental-stage "none"
    set developmental-progression 0
    set generation 0
    set parent-1 who

```

```

    set parent-2 nobody
    set nematodes-killed 0
]

create-nematodes initial-+e [
    set shape "circle"
    set color grey
    set size 0.5
    setxy random-xcor random-ycor
    set species "p.pacificus"
    set genotype "+e"
    set phenotype 0
    set sex "hermaphrodite"
    set partner nobody
    set developmental-stage "none"
    set developmental-progression 0
    set generation 0
    set parent-1 who
    set parent-2 nobody
    set nematodes-killed 0
]

create-nematodes initial-ee [
    set shape "circle"
    set color grey
    set size 0.5
    setxy random-xcor random-ycor
    set species "p.pacificus"
    set genotype "ee"
    set phenotype 0
    set sex "hermaphrodite"
    set partner nobody
    set developmental-stage "new-egg"
    set developmental-progression 0
    set generation 0
    set parent-1 who
    set parent-2 nobody
    set nematodes-killed 0
]

```

```

create-nematodes initial-+0 [
  set shape "circle"
  set color grey
  set size 0.5
  setxy random-xcor random-ycor
  set species "p.pacificus"
  set genotype "+0"
  set phenotype 0
  set sex "male"
  set partner nobody
  set developmental-stage "new-egg"
  set developmental-progression 0
  set generation 0
  set parent-1 who
  set parent-2 nobody
  set nematodes-killed 0
]

create-nematodes initial-e0 [
  set shape "circle"
  set color grey
  set size 0.5
  setxy random-xcor random-ycor
  set species "p.pacificus"
  set genotype "e0"
  set phenotype 0
  set sex "male"
  set partner nobody
  set developmental-stage "new-egg"
  set developmental-progression 0
  set generation 0
  set parent-1 who
  set parent-2 nobody
  set nematodes-killed 0
]

reset-ticks

```

```

end

to go

  set temperature 20

  ifelse abundant-bacteria?
  [
    if count bacteria > 800 [
      ask n-of ( count bacteria - 800 ) bacteria [ die ]
    ]
    set cost-of-starvation-\% 0
  ]
  [
    if count bacteria > 200 [
      ask n-of ( count bacteria - 200 ) bacteria [ die ]
    ]
    set cost-of-starvation-\% stored-cost-of-starvation
      -\%
  ]

  ifelse c.elegans?
  [
    ifelse any? nematodes with [ species = "c.elegans" ]
    [
      if count nematodes with [ species = "c.elegans" ]
        > 300 [
        ask n-of ( count nematodes with [ species = "c.
          elegans" ] - 300 ) nematodes with [ species =
            "c.elegans" ] [ die ]
        ]
    ]
    [
      create-nematodes 300 [
        set shape "nematode"
        set color grey
        set size 0.45
        setxy random-xcor random-ycor
        set species "c.elegans"
      ]
    ]
  ]

```

```

    ]
  ]
]
[ ask nematodes with [ species = "c.elegans" ] [ die ]
  ]

ask bacteria [
  fd 0.02
  rt random 90
  lt random 90
  if ticks mod 10 = 0 [
    hatch 1
  ]
]

ask nematodes with [ species = "c.elegans" ] [
  move-worms
  if ticks mod 10 = 0 [
    hatch 1 [ set size 0.45 ]
  ]
]

if fluctuating-environment? [
  if ( ticks != 0 ) and ( ticks mod ( 820 *
    environmental-cycles) = 0 ) [
    ifelse abundant-bacteria? = TRUE
    [ set abundant-bacteria? FALSE ]
    [ set abundant-bacteria? TRUE ]
    ifelse c.elegans? = TRUE
    [ set c.elegans? FALSE ]
    [ set c.elegans? TRUE ]
  ]
]

if count nematodes with [ species = "p.pacificus" ]
  with [ developmental-stage = "none" ] > carrying-
  capacity [
  ask n-of ( count nematodes with [ species = "p.
    pacificus" ] with [ developmental-stage = "none"

```

```

    ] - carrying-capacity ) nematodes with [ species
    = "p.pacificus" ] with [ developmental-stage = "
    none" ] [ die ]
]

ask nematodes with [ species = "p.pacificus" ] with [
    developmental-stage = "none" ] with [
    developmental-progression = 1 ] [
    set developmental-stage "new-egg"
    set parent-1 who
]

ask nematodes with [ species = "p.pacificus" ] [
    set developmental-progression developmental-
    progression + 1
    if developmental-progression = 150 [
        set developmental-stage "J1"
        set size 0.75
    ]
    ifelse developmental-progression < 240
    [ move-eggs ]
    [ move-worms ]
    if ( developmental-stage = "J1" ) and (
        developmental-progression = 200 ) [
        set developmental-stage "J2"
        set size 1
    ]
    if developmental-stage = "J2" [
        if developmental-progression = 240 [
            set shape "nematode"
            set size 4
        ]
    ]
    if developmental-progression = 400 [
        set developmental-stage "J3"
        set size 5
    ]
    if developmental-progression = 480 [
        set developmental-stage "J4"

```

```

    set size 6
]
if developmental-progression = 700 [
    set developmental-stage "adult"
    if genotype = "++" [
        ifelse random 100 < probability-of-developing-EU
            -++
        [
            set phenotype 1
            set shape "EU"
            set color blue
        ]
        [
            set phenotype 2
            set shape "ST"
            set color red
        ]
    ]
]
if genotype = "+e" [
    ifelse random 100 < probability-of-developing-EU
        -+e
    [
        set phenotype 1
        set shape "EU"
        set color blue
    ]
    [
        set phenotype 2
        set shape "ST"
        set color red
    ]
]
if genotype = "ee" [
    set phenotype 2
    set shape "ST"
    set color red
]
if genotype = "+0" [

```



```

        ifelse random 100 < probability-of-developing-EU
            +0
        [
            set phenotype 1
            set shape "EU"
            set color blue
        ]
        [
            set phenotype 2
            set shape "ST"
            set color red
        ]
    ]
    if genotype = "e0" [
        set phenotype 2
        set shape "ST"
        set color red
    ]
    set time-to-oviposition 120
    set size 7
    if sex = "male" [
        if any? nematodes with [ sex = "hermaphrodite" ]
            with [ partner = nobody ] [
                set partner one-of nematodes with [ sex = "
                    hermaphrodite" ] with [ partner = nobody ]
                ask partner [
                    set partner myself
                    set parent-2 [ who ] of partner
                ]
            ]
    ]
]
if ( developmental-progression > 700 ) and (
    developmental-progression <= 820 ) [
    set time-to-oviposition time-to-oviposition - 1 ;
    time-to-oviposition is updated at each tick
    if ( sex = "hermaphrodite" ) and ( time-to-
        oviposition = 0 ) [ reproduce ]

```

```

if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "++" ) and
    ( partner = nobody ) [
    ask n-of ( cost-of-plasticity-\% + cost-of-
        starvation-\% ) other nematodes-here with [
        developmental-stage = "none" ] with [ parent
        -1 = [ parent-1 ] of myself ] [ die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "+e" ) and
    ( partner = nobody ) [
    ask n-of ( cost-of-plasticity-\% + cost-of-
        starvation-\% ) other nematodes-here with [
        developmental-stage = "none" ] with [ parent
        -1 = [ parent-1 ] of myself ] [ die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "ee" ) and
    ( partner = nobody ) [
    ask n-of cost-of-starvation-\% other nematodes-
        here with [ developmental-stage = "none" ]
        with [ parent-1 = [ parent-1 ] of myself ] [
        die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "++" ) and
    ( partner != nobody ) and ( [ genotype ] of
    partner = "+0" ) [
    ask n-of ( 2 * cost-of-plasticity-\% + 2 * cost-
        of-starvation-\% ) other nematodes-here with
        [ developmental-stage = "none" ] with [
        parent-1 = [ parent-1 ] of myself ] with [
        parent-2 = [ parent-2 ] of myself ] [ die ]
    die
]

```

```

if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "+e" ) and
    ( partner != nobody ) and ( [ genotype ] of
    partner = "+0" ) [
    ask n-of ( 2 * cost-of-plasticity-\% + 2 * cost-
        of-starvation-\% ) other nematodes-here with
        [ developmental-stage = "none" ] with [
        parent-1 = [ parent-1 ] of myself ] with [
        parent-2 = [ parent-2 ] of myself ] [ die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "ee" ) and
    ( partner != nobody ) and ( [ genotype ] of
    partner = "+0" ) [
    ask n-of ( cost-of-plasticity-\% + 2 * cost-of-
        starvation-\% ) other nematodes-here with [
        developmental-stage = "none" ] with [ parent
        -1 = [ parent-1 ] of myself ] with [ parent-2
        = [ parent-2 ] of myself ] [ die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "++" ) and
    ( partner != nobody ) and ( [ genotype ] of
    partner = "e0" ) [
    ask n-of ( cost-of-plasticity-\% + 2 * cost-of-
        starvation-\% ) other nematodes-here with [
        developmental-stage = "none" ] with [ parent
        -1 = [ parent-1 ] of myself ] with [ parent-2
        = [ parent-2 ] of myself ] [ die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "+e" ) and
    ( partner != nobody ) and ( [ genotype ] of
    partner = "e0" ) [
    ask n-of ( cost-of-plasticity-\% + 2 * cost-of-
        starvation-\% ) other nematodes-here with [

```

```

        developmental-stage = "none" ] with [ parent
        -1 = [ parent-1 ] of myself ] with [ parent-2
        = [ parent-2 ] of myself ] [ die ]
    die
]
if ( sex = "hermaphrodite" ) and ( developmental-
    progression = 820 ) and ( genotype = "ee" ) and
    ( partner != nobody ) and ( [ genotype ] of
    partner = "e0" ) [
    ask n-of ( 2 * cost-of-starvation-\% ) other
        nematodes-here with [ developmental-stage = "
        none" ] with [ parent-1 = [ parent-1 ] of
        myself ] with [ parent-2 = [ parent-2 ] of
        myself ] [ die ]
    die
]
]
if ( sex = "male" ) and ( partner = nobody ) and (
    developmental-progression >= 820 ) [ die ]
if developmental-progression > 240 [
    if ( phenotype = 1 ) and ( nematodes-killed < 5 )
    [
        if any? nematodes in-radius 1 with [ species = "
            c.elegans" ] [
            if random 100 < efficiency-of-attack-\% [
                ask one-of nematodes in-radius 1 with [
                    species = "c.elegans" ] [ die ]
                set nematodes-killed nematodes-killed + 1
            ]
        ]
    ]
]
]
]
tick
end
to move-eggs

```

```

    fd 0.1
    rt random 30
    lt random 30

end

to move-worms

    fd 0.25
    rt random 15
    lt random 15

end

to reproduce

    ifelse partner = nobody
    [
        if genotype = "++" [
            ifelse ( phenotype = 2 ) and ( abundant-bacteria?
                = TRUE )
            [
                hatch 100 + benefit-of-faster-development-\% [
                    set shape "circle"
                    set color grey
                    set size 0.5
                    set genotype "++"
                    set phenotype 0
                    ifelse random 100 < probability-of-non-
                        disjunction-\%
                    [
                        set sex "male"
                        set genotype "+0"
                    ]
                    [ set sex "hermaphrodite" ]
                    set developmental-stage "none"
                    set developmental-progression 0
                    set generation generation + 1
                ]
            ]
        ]
    ]

```

```

        set nematodes-killed 0
    ]
]
[
    hatch 100 + benefit-of-predation-\% * nematodes-
        killed [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "++"
            set phenotype 0
            ifelse random 100 < probability-of-non-
                disjunction-\%
            [
                set sex "male"
                set genotype "+0"
            ]
            [ set sex "hermaphrodite" ]
            set developmental-stage "none"
            set developmental-progression 0
            set generation generation + 1
            set nematodes-killed 0
        ]
    ]
]
if genotype = "+e" [
    ifelse ( phenotype = 2 ) and ( abundant-bacteria?
        = TRUE )
    [
        let chance-of-offspring-increase-1 random 4
        ifelse chance-of-offspring-increase-1 = 0
        [
            hatch 25 + benefit-of-faster-development-\% [
                set shape "circle"
                set color grey
                set size 0.5
                set genotype "++"
                set phenotype 0
            ]
        ]
    ]
]

```

```

        ifelse random 100 < probability-of-non-
            disjunction-\%
        [
            set sex "male"
            set genotype "+0"
        ]
        [ set sex "hermaphrodite" ]
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "++"
        set phenotype 0
        ifelse random 100 < probability-of-non-
            disjunction-\%
        [
            set sex "male"
            set genotype "+0"
        ]
        [ set sex "hermaphrodite" ]
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse ( chance-of-offspring-increase-1 = 1 ) or
    ( chance-of-offspring-increase-1 = 2 )
[
    hatch 50 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
    ]
]

```

```

set size 0.5
set genotype "+e"
set phenotype 0
ifelse random 100 < probability-of-non-
  disjunction-\%
[
  set sex "male"
  ifelse random 2 = 0
  [ set genotype "+0" ]
  [ set genotype "e0" ]
]
[ set sex "hermaphrodite" ]
set developmental-stage "none"
set developmental-progression 0
set generation generation + 1
set nematodes-killed 0
]
[
hatch 50 [
  set shape "circle"
  set color grey
  set size 0.5
  set genotype "+e"
  set phenotype 0
  ifelse random 100 < probability-of-non-
    disjunction-\%
  [
    set sex "male"
    ifelse random 2 = 0
    [ set genotype "+0" ]
    [ set genotype "e0" ]
  ]
  [ set sex "hermaphrodite" ]
  set developmental-stage "none"
  set developmental-progression 0
  set generation generation + 1
  set nematodes-killed 0
]
]

```



```

]
ifelse chance-of-offspring-increase-1 = 3
[
  hatch 25 + benefit-of-faster-development-\% [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "ee"
    set phenotype 0
    ifelse random 100 < probability-of-non-
      disjunction-\%
    [
      set sex "male"
      set genotype "e0"
    ]
    [ set sex "hermaphrodite" ]
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "ee"
    set phenotype 0
    ifelse random 100 < probability-of-non-
      disjunction-\%
    [
      set sex "male"
      set genotype "e0"
    ]
    [ set sex "hermaphrodite" ]
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
  ]
]

```

```

        set nematodes-killed 0
    ]
]
[
let chance-of-offspring-increase-2 random 4
ifelse chance-of-offspring-increase-2 = 0
[
    hatch 25 + benefit-of-predation-\% * nematodes
        -killed [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "++"
            set phenotype 0
            ifelse random 100 < probability-of-non-
                disjunction-\%
            [
                set sex "male"
                set genotype "+0"
            ]
            [ set sex "hermaphrodite" ]
            set developmental-stage "none"
            set developmental-progression 0
            set generation generation + 1
            set nematodes-killed 0
        ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "++"
        set phenotype 0
        ifelse random 100 < probability-of-non-
            disjunction-\%
        [
            set sex "male"

```

```

        set genotype "+0"
    ]
    [ set sex "hermaphrodite" ]
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
]
]
ifelse ( chance-of-offspring-increase-2 = 1 ) or
( chance-of-offspring-increase-2 = 2 )
[
    hatch 50 + benefit-of-predation-\% * nematodes
    -killed [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        ifelse random 100 < probability-of-non-
            disjunction-\%
        [
            set sex "male"
            ifelse random 2 = 0
            [ set genotype "+0" ]
            [ set genotype "e0" ]
        ]
        [ set sex "hermaphrodite" ]
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5

```

```

set genotype "+e"
set phenotype 0
ifelse random 100 < probability-of-non-
  disjunction-\%
[
  set sex "male"
  ifelse random 2 = 0
  [ set genotype "+0" ]
  [ set genotype "e0" ]
]
[ set sex "hermaphrodite" ]
set developmental-stage "none"
set developmental-progression 0
set generation generation + 1
set nematodes-killed 0
]
]
ifelse chance-of-offspring-increase-2 = 3
[
  hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "ee"
      set phenotype 0
      ifelse random 100 < probability-of-non-
        disjunction-\%
      [
        set sex "male"
        set genotype "e0"
      ]
      [ set sex "hermaphrodite" ]
      set developmental-stage "none"
      set developmental-progression 0
      set generation generation + 1
      set nematodes-killed 0
    ]
  ]
]
]

```

```

[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "ee"
    set phenotype 0
    ifelse random 100 < probability-of-non-
      disjunction-\%
    [
      set sex "male"
      set genotype "e0"
    ]
    [ set sex "hermaphrodite" ]
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
]
]
if genotype = "ee" [
  ifelse abundant-bacteria? = TRUE
  [
    hatch 100 + benefit-of-faster-development-\% [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "ee"
      set phenotype 0
      ifelse random 100 < probability-of-non-
        disjunction-\%
      [
        set sex "male"
        set genotype "e0"
      ]
      [ set sex "hermaphrodite" ]
      set developmental-stage "none"
    ]
  ]
]

```

```

        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 100 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "ee"
        set phenotype 0
        ifelse random 100 < probability-of-non-
            disjunction-\%
        [
            set sex "male"
            set genotype "e0"
        ]
        [ set sex "hermaphrodite" ]
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]
[
    if ( genotype = "++" ) and ( [ genotype ] of partner
        = "+0" ) [
        ifelse ( phenotype = 2 ) and ( [ phenotype ] of
            partner = 2 ) and ( abundant-bacteria? = TRUE )
        [
            let chance-of-offspring-increase-3 random 2
            ifelse chance-of-offspring-increase-3 = 0
            [
                hatch 50 + benefit-of-faster-development-\% [
                    set shape "circle"
                    set color grey

```

```

        set size 0.5
        set genotype "++"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "++"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-3 = 1
[
    hatch 50 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]

```

```

[
  hatch 50 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "+0"
    set phenotype 0
    set sex "male"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
[
  let chance-of-offspring-increase-4 random 2
  ifelse chance-of-offspring-increase-4 = 0
  [
    hatch 50 + benefit-of-predation-\% * nematodes
      -killed [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "++"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
      ]
  ]
  [
    hatch 50 [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "++"
    ]
  ]
]

```



```

        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-4 = 1
[
    hatch 50 + benefit-of-predation-\% * nematodes
        -killed [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "+0"
            set phenotype 0
            set sex "male"
            set developmental-stage "none"
            set developmental-progression 0
            set generation generation + 1
            set nematodes-killed 0
        ]
    ]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]

```

```

]
if ( genotype = "+e" ) and ( [ genotype ] of partner
  = "+0" ) [
  ifelse ( phenotype = 2 ) and ( [ phenotype ] of
    partner = 2 ) and ( abundant-bacteria? = TRUE )
  [
    let chance-of-offspring-increase-5 random 4
    ifelse chance-of-offspring-increase-5 = 0
    [
      hatch 25 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "++"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
      ]
    ]
  ]
  [
    hatch 25 [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "++"
      set phenotype 0
      set sex "hermaphrodite"
      set developmental-stage "none"
      set developmental-progression 0
      set generation generation + 1
      set nematodes-killed 0
    ]
  ]
]
ifelse chance-of-offspring-increase-5 = 1
[
  hatch 25 + benefit-of-faster-development-\% [

```

```

        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-5 = 2
[
    hatch 25 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]

```

```

]
]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "+e"
    set phenotype 0
    set sex "hermaphrodite"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
ifelse chance-of-offspring-increase-5 = 3
[
  hatch 25 + benefit-of-faster-development-\% [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "e0"
    set phenotype 0
    set sex "male"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "e0"
    set phenotype 0
    set sex "male"
  ]
]

```

```

        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
let chance-of-offspring-increase-6 random 4
ifelse chance-of-offspring-increase-6 = 0
[
    hatch 25 + benefit-of-predation-\% * nematodes
        -killed [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "++"
            set phenotype 0
            set sex "hermaphrodite"
            set developmental-stage "none"
            set developmental-progression 0
            set generation generation + 1
            set nematodes-killed 0
        ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "++"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]

```

```

ifelse chance-of-offspring-increase-6 = 1
[
  hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "+0"
      set phenotype 0
      set sex "male"
      set developmental-stage "none"
      set developmental-progression 0
      set generation generation + 1
      set nematodes-killed 0
    ]
]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "+0"
    set phenotype 0
    set sex "male"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
ifelse chance-of-offspring-increase-6 = 2
[
  hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "+e"
      set phenotype 0
    ]
]

```

```

        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-6 = 3
[
    hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [

```

```

        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]
]
if ( genotype = "ee" ) and ( [ genotype ] of partner
    = "+0" ) [
    ifelse ( phenotype = 2 ) and ( [ phenotype ] of
        partner = 2 ) and ( abundant-bacteria? = TRUE )
    [
        let chance-of-offspring-increase-7 random 2
        ifelse chance-of-offspring-increase-7 = 0
        [
            hatch 50 + benefit-of-faster-development-\% [
                set shape "circle"
                set color grey
                set size 0.5
                set genotype "+e"
                set phenotype 0
                set sex "hermaphrodite"
                set developmental-stage "none"
                set developmental-progression 0
                set generation generation + 1
                set nematodes-killed 0
            ]
        ]
    ]
    [
        hatch 50 [
            set shape "circle"
            set color grey

```



```

        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-7 = 1
[
    hatch 50 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]

```

```

]
[
  let chance-of-offspring-increase-8 random 2
  ifelse chance-of-offspring-increase-8 = 0
  [
    hatch 50 + benefit-of-predation-\% * nematodes
      -killed [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
      ]
  ]
[
  hatch 50 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "+e"
    set phenotype 0
    set sex "hermaphrodite"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
ifelse chance-of-offspring-increase-8 = 1
[
  hatch 50 + benefit-of-predation-\% * nematodes
    -killed [
      set shape "circle"
      set color grey
    ]

```

```

        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]
if ( genotype = "++" ) and ( [ genotype ] of partner
    = "e0" ) [
    ifelse ( phenotype = 2 ) and ( abundant-bacteria?
        = TRUE )
    [
        let chance-of-offspring-increase-9 random 2
        ifelse chance-of-offspring-increase-9 = 0
        [
            hatch 50 + benefit-of-faster-development-\% [
                set shape "circle"
                set color grey
                set size 0.5
                set genotype "+e"
            ]
        ]
    ]
]

```

```

        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-9 = 1
[
    hatch 50 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [

```

```

        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    let chance-of-offspring-increase-10 random 2
    ifelse chance-of-offspring-increase-10 = 0
    [
        hatch 50 + benefit-of-predation-\% * nematodes
        -killed [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "+e"
            set phenotype 0
            set sex "hermaphrodite"
            set developmental-stage "none"
            set developmental-progression 0
            set generation generation + 1
            set nematodes-killed 0
        ]
    ]
    [
        hatch 50 [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "+e"
            set phenotype 0
            set sex "hermaphrodite"

```

```

        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-10 = 1
[
    hatch 50 + benefit-of-predation-\% * nematodes
    -killed [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]

```

```

if ( genotype = "+e" ) and ( [ genotype ] of partner
  = "e0" ) [
  ifelse ( phenotype = 2 ) and ( abundant-bacteria?
    = TRUE )
  [
    let chance-of-offspring-increase-11 random 4
    ifelse chance-of-offspring-increase-11 = 0
    [
      hatch 25 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
      ]
    ]
  ]
  [
    hatch 25 [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "+e"
      set phenotype 0
      set sex "hermaphrodite"
      set developmental-stage "none"
      set developmental-progression 0
      set generation generation + 1
      set nematodes-killed 0
    ]
  ]
  ifelse chance-of-offspring-increase-11 = 1
  [
    hatch 25 + benefit-of-faster-development-\% [
      set shape "circle"

```

```

        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-11 = 2
[
    hatch 25 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "ee"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]

```



```

]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "ee"
    set phenotype 0
    set sex "hermaphrodite"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
ifelse chance-of-offspring-increase-11 = 3
[
  hatch 25 + benefit-of-faster-development-\% [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "e0"
    set phenotype 0
    set sex "male"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "e0"
    set phenotype 0
    set sex "male"
    set developmental-stage "none"

```

```

        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
let chance-of-offspring-increase-12 random 4
ifelse chance-of-offspring-increase-12 = 0
[
    hatch 25 + benefit-of-predation-\% * nematodes
        -killed [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "+e"
            set phenotype 0
            set sex "hermaphrodite"
            set developmental-stage "none"
            set developmental-progression 0
            set generation generation + 1
            set nematodes-killed 0
        ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "+e"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-12 = 1

```

```

[
  hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "+0"
      set phenotype 0
      set sex "male"
      set developmental-stage "none"
      set developmental-progression 0
      set generation generation + 1
      set nematodes-killed 0
    ]
]
[
  hatch 25 [
    set shape "circle"
    set color grey
    set size 0.5
    set genotype "+0"
    set phenotype 0
    set sex "male"
    set developmental-stage "none"
    set developmental-progression 0
    set generation generation + 1
    set nematodes-killed 0
  ]
]
ifelse chance-of-offspring-increase-12 = 2
[
  hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
      set shape "circle"
      set color grey
      set size 0.5
      set genotype "ee"
      set phenotype 0
      set sex "hermaphrodite"
    ]
]

```

```

        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "ee"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-12 = 3
[
    hatch 25 + benefit-of-predation-\% * nematodes
    -killed [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 25 [
        set shape "circle"

```

```

        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]
]
if ( genotype = "ee" ) and ( [ genotype ] of partner
    = "e0" ) [
    ifelse abundant-bacteria? = TRUE
    [
        let chance-of-offspring-increase-13 random 2
        ifelse chance-of-offspring-increase-13 = 0
        [
            hatch 50 + benefit-of-faster-development-\% [
                set shape "circle"
                set color grey
                set size 0.5
                set genotype "ee"
                set phenotype 0
                set sex "hermaphrodite"
                set developmental-stage "none"
                set developmental-progression 0
                set generation generation + 1
                set nematodes-killed 0
            ]
        ]
    ]
    [
        hatch 50 [
            set shape "circle"
            set color grey
            set size 0.5
            set genotype "ee"

```

```

        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
ifelse chance-of-offspring-increase-13 = 1
[
    hatch 50 + benefit-of-faster-development-\% [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
[

```

```

    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "ee"
        set phenotype 0
        set sex "hermaphrodite"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
    hatch 50 [
        set shape "circle"
        set color grey
        set size 0.5
        set genotype "e0"
        set phenotype 0
        set sex "male"
        set developmental-stage "none"
        set developmental-progression 0
        set generation generation + 1
        set nematodes-killed 0
    ]
]
]
]
end

to-report probability-of-developing-EU-++

    ifelse count bacteria >= 500
    [ report 80 ]
    [ report 80 + degree-of-plasticity-\% ]

end

to-report probability-of-developing-EU-+e

```

```

    ifelse count bacteria >= 500
    [ report 25 ]
    [ report 25 + degree-of-plasticity-\% ]
end

to-report probability-of-developing-EU-+0

    ifelse count bacteria >= 500
    [ report 25 ]
    [ report 25 + degree-of-plasticity-\% ]
end

to-report generations

    if [ developmental-progression ] of one-of nematodes
    with [ species = "p.pacificus" ] >= 0 [ report [
    generation ] of one-of nematodes with [ species =
    "p.pacificus" ] ]

end

to-report population-size

    if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report count nematodes with [ species =
    "p.pacificus" ] ]

end

to-report hermaphrodites

    if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report count nematodes with [ species =
    "p.pacificus" ] with [ sex = "hermaphrodite" ] ]

```



```

end

to-report males

  if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report count nematodes with [ species =
      "p.pacificus" ] with [ sex = "male" ] ]

end

to-report frequency-of-++

  if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report ( count nematodes with [ species
      = "p.pacificus" ] with [ genotype = "++" ] / count
      nematodes with [ species = "p.pacificus" ] ) ]

end

to-report frequency-of-+e

  if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report ( count nematodes with [ species
      = "p.pacificus" ] with [ genotype = "+e" ] / count
      nematodes with [ species = "p.pacificus" ] ) ]

end

to-report frequency-of-ee

  if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report ( count nematodes with [ species
      = "p.pacificus" ] with [ genotype = "ee" ] / count
      nematodes with [ species = "p.pacificus" ] ) ]

```

```

end

to-report frequency-of-+0

  if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report ( count nematodes with [ species
    = "p.pacificus" ] with [ genotype = "+0" ] / count
    nematodes with [ species = "p.pacificus" ] ) ]

end

to-report frequency-of-e0

  if ( ticks = 0 ) or ( [ developmental-progression ] of
    one-of nematodes with [ species = "p.pacificus" ]
    ) >= 1 [ report ( count nematodes with [ species
    = "p.pacificus" ] with [ genotype = "e0" ] / count
    nematodes with [ species = "p.pacificus" ] ) ]

end

to-report frequency-of-++

  report ( ( count nematodes with [ species = "p.
    pacificus" ] with [ genotype = "++" ] * 2 ) + (
    count nematodes with [ species = "p.pacificus" ]
    with [ genotype = "+e" ] ) + ( count nematodes
    with [ species = "p.pacificus" ] with [ genotype =
    "+0" ] ) ) / ( count nematodes with [ species = "
    p.pacificus" ] * 2 )

end

to-report frequency-of-e

  report ( ( count nematodes with [ species = "p.
    pacificus" ] with [ genotype = "ee" ] * 2 ) + (

```

```

        count nematodes with [ species = "p.pacificus" ]
        with [ genotype = "+e" ] ) + ( count nematodes
        with [ species = "p.pacificus" ] with [ genotype =
        "e0" ] ) ) / ( count nematodes with [ species = "
        p.pacificus" ] * 2 )

end

to-report ++

    report count nematodes with [ species = "p.pacificus"
        ] with [ genotype = "++" ]

end

to-report +e

    report count nematodes with [ species = "p.pacificus"
        ] with [ genotype = "+e" ]

end

to-report ee

    report count nematodes with [ species = "p.pacificus"
        ] with [ genotype = "ee" ]

end

```

Chapter 7

Appendix B: Interview with Professor Alessandro Minelli

Short presentation

Alessandro Minelli (born 20 December 1948) is a former professor of Zoology at the University of Padua, a specialist in the field of zoological systematics, and a prominent figure in evolutionary developmental biology, whose conceptual foundations he has contributed to establish in the last decades. He is a member of the National Academy of Sciences and the Veneto Institute of Sciences Letters and Arts, and an honorary member of the Royal Entomological Society (London). Furthermore, he was president of the International Commission on Zoological Nomenclature and vice-president of the European Society for Evolutionary Biology, as well as a member of the editorial board of several journals dealing with evolution, development, and zoology. His most relevant publications include the seminal book *Forms of becoming: the evolutionary biology of development* (Minelli, 2009b)¹.

Interview

Q1: Dear Sandro, it is a great opportunity to be able to dialogue with you about evolution, so thank you again for your availability and kindness. I would like to start with something very subjective, that is, what are the most recent discoveries or studies that have impressed you the most in the context of evolutionary developmental biology, and what do you think might be the most promising future

¹Minelli, A. (2009b). *Forms of becoming*. Princeton University Press, Princeton.

research directions in this area?

A1: Dear Riccardo, many thanks for inviting me to this dialogue.

To answer your first question: the most important innovation in this area of the life sciences was the emergence of evo-devo as an autonomous discipline. Evo-devo is not a simple sum, or juxtaposition, of developmental biology and evolutionary biology. Concepts like *modularity* and *evolvability* are specific to it and give rise to new research agendas. According to many colleagues, we should add another topic, *evolutionary innovations*, regarding which, however, my position is close to that of François Jacob (Jacob, 1977)²: evolution does not produce innovations starting from scratch, but operates on what already exists.

Evo-devo is not the sum of evo and devo, but in these first decades of its existence it has already led to important rethinks regarding both disciplines. In the field of evolution, there is a widespread opinion (which I also accept with reservations; (Minelli, 2009a)³) that the advent of evo-devo has unmasked very important aspects of evolution that require a transition from the traditional Neo-Darwinian paradigm to an Extended Evolutionary Synthesis (Pigliucci, 2010a; Laubichler, 2009)⁴⁵. More radical has been the impact of evo-devo on developmental biology, of which in my opinion it has undermined many assumptions that have long been uncritically accepted, such as the very notion of development as the process that starting from an egg, or a seed, leads to the adult

²Jacob, F. (1977). Evolution and tinkering. *Science*, 196(4295):1161– 1166.

³Minelli, A. (2009a). Evolutionary developmental biology does not offer a significant challenge to the neo-darwinian paradigm.

⁴Pigliucci, M. (2010a). *Evolution, the Extended Synthesis*. The MIT Press Ser. MIT Press, Cambridge.

⁵Laubichler, M. D. (2009). Evolutionary developmental biology offers a significant challenge to the neo-darwinian paradigm.

organism. A targeted effort to remove this *adultocentrism* from developmental biology has long been one of my favorite battles Minelli (2003, 2009b, 2011, 2014a, 2015, 2019, 2021a,b); Minelli and Valero-Gracia (2022)⁶⁷⁸⁹¹⁰¹¹¹²¹³¹⁴. At last, this conceptual revision seems to spread, at least among the philosophers of biology (for example, see (Silvestros, 2023)¹⁵.

In an article written for the launch of the journal *Frontiers in Evolutionary Developmental Biology*, I enumerated and discussed (Minelli, 2015)¹⁶ a dozen major challenges that evo-devo must confront and I am very pleased to note that your theoretical and experimental work falls fully within one of the two very great challenges, as I called them in that article, namely the transition from polyphenism to polymorphism through the internalization of releasing inputs.

Q2: I know you also dealt with phenotypic plasticity (e.g., in (Fusco and Minelli, 2010)¹⁷). In my perspective, adaptive phenotypic plasticity is one of the most fascinating topics in current evolutionary research. Although genes are commonly seen as the embodiment of biological determinism, they may instead be the “activators” of an increased responsiveness to environmental cues, which could even seem paradoxical to those who are not into these issues. In other words, natural selection can promote this environmentally-induced flexibility in developmental processes by increasing the frequency of

⁶Minelli, A. (2003). *The Development of Animal Form: Ontogeny, Morphology, and Evolution*. Cambridge University Press.

⁷Minelli, A. (2009b). *Forms of becoming*. Princeton University Press, Princeton. Includes bibliographical references (pages 215-220) and index.

⁸Minelli, A. (2011). Animal development, an open-ended segment of life. *Biological Theory*, 6(1):4–15.

⁹Minelli, A. (2014a). Developmental disparity, pages 227–245. Oxford University Press.

¹⁰Minelli, A. (2015). Grand challenges in evolutionary developmental biology. *Frontiers in Ecology and Evolution*, 2.

¹¹Minelli, A. (2019). 8. A Principle of Developmental Inertia, pages 116–134. University of California Press.

¹²Minelli, A. (2021a). On the nature of organs and organ systems – a chapter in the history and philosophy of biology. *Frontiers in Ecology and Evolution*, 9.

¹³Minelli, A. (2021b). *Understanding Development*. Cambridge University Press.

¹⁴Minelli, A. and Valero-Gracia, A. (2022). Spatially and temporally distributed complexity—a refreshed framework for the study of grn evolution. *Cells*, 11(11):1790.

¹⁵Silvestros, P. (2023). An operational definition of biological development. *Biology & Philosophy*, 38(5).

¹⁶Minelli, A. (2015). Grand challenges in evolutionary developmental biology. *Frontiers in Ecology and Evolution*, 2.

¹⁷Fusco, G. and Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1540):547–556.

certain genotypes within a population. Conversely, as exemplified by processes such as genetic assimilation or the Baldwin effect (Crispo, 2007)¹⁸, in other cases the tendency is quite the opposite, that is, fixed phenotypes may be favoured over plastic ones. In my PhD thesis, I use a computational model to simulate this evolutionary interplay between fixed and plastic phenotypes. To your knowledge, do we have any case studies using an experimental evolution approach which were carried out in the field and clearly showed some events of replacement of fixed phenotypes with plastic ones (and/or vice versa)? More generally, do you think that the experimental evolution approach will be able to play a critical role in the context of the evolution of reaction norms and polyphenism in the near future?

A2: I have not been following the literature on these topics systematically for some years, so my answer cannot be exhaustive. However, in principle one would expect to find examples of fixation as genetic polymorphism of traits previously manifested as polyphenism, rather than the other way around. Indeed, over the last fifteen years, the idea has become increasingly popular that new phenotypes occur primarily as a result of the response of an unchanged genome to environmental conditions or selective regimes that have not been confronted before, and that genetic fixation is a consequence of prolonged selection in favor of the new phenotype. In short, *gene as followers*, rather than *genes as leaders* –as proposed by Mary Jane West Eberhard (West-Eberhard, 1989, 2020)^{19,20}, and brilliantly discussed in an article by Schwander and Leimar (Schwander and Leimar, 2011)²¹ under this very title. But the divide between polyphenism and polymorphism is sometimes extremely tenuous. In this regard, enlightening is the case of the pea aphid *Acyrtosiphon pisum* (Brisson, 2010)²², on the borderline

¹⁸Crispo, E. (2007). The baldwin effect and genetic assimilation: Revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution*, 61(11):2469–2479.

¹⁹West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 20(1):249– 278.

²⁰West-Eberhard, M. J. (2020). *Developmental plasticity and evolution*. Oxford scholarship online. Oxford University Press, New York. Previously issued in print: 2003. - Includes bibliographical references. - Description based on print version record and publisher information.

²¹Schwander, T. and Leimar, O. (2011). Genes as leaders and followers in evolution. *Trends in Ecology & Evolution*, 26(3):143–151.

²²Brisson, J. A. (2010). Aphid wing dimorphisms: linking environmental and genetic control of trait variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1540):605– 616.

between natural history and experiment. Pea aphids of either sex can be either winged or wingless, but in the females this is an environmentally controlled polyphenism, whereas in the male the difference is controlled by a single-gene polymorphism (Braendle et al., 2005a)²³. However, the protein encoded by the gene (*aphicarus*) responsible for wing development in the male aphid is also involved in the female’s developmental response to the environmental stimulus (Braendle et al., 2005b)²⁴.

To some extent, this “plasticity-first” hypothesis remains controversial, primarily because comprehensive tests in natural populations are generally lacking. Levis and Pfennig (Levis and Pfennig, 2016)²⁵ identified four key criteria to test it in natural systems. I copy the following list from their article: 1. The focal trait will be environmentally induced in ancestral-proxy lineages. 2. Cryptic genetic variation will be uncovered when ancestral-proxy lineages experience the derived environment. 3. The focal trait will exhibit evidence of having undergone an evolutionary change in its regulation, form, or both in derived lineages. 4. The focal trait will exhibit evidence of having undergone adaptive refinement in derived lineages. All four of these criteria seem to be fulfilled in a few particularly well-studied cases of adaptation to new environments, such as the repeatedly evolved freshwater populations of a little fish of marine origin, the three-spine stickleback *Gasterosteus aculeatus* (Robinson and Parsons, 2002)²⁶.

Q3: Many scholars have criticised, sometimes harshly, the science popularization masterpiece *The selfish gene* (Dawkins, 2009)²⁷ by Richard Dawkins for its simplifications of biological reality, its genetic determinism and, more generally, the gene-centric view of its

²³Braendle, C., Caillaud, M. C., and Stern, D. L. (2005a). Genetic mapping of *aphicarus* – a sex-linked locus controlling a wing polymorphism in the pea aphid (*acyrthosiphon pisum*). *Heredity*, 94(4):435–442.

²⁴Braendle, C., Friebe, I., Caillaud, M. C., and Stern, D. L. (2005b). Genetic variation for an aphid wing polyphenism is genetically linked to a naturally occurring wing polymorphism. *Proceedings of the Royal Society B: Biological Sciences*, 272(1563):657–664.

²⁵Levis, N. A. and Pfennig, D. W. (2016). Evaluating ‘plasticity-first’ evolution in nature: Key criteria and empirical approaches. *Trends in Ecology & Evolution*, 31(7):563–574.

²⁶Robinson, B. W. and Parsons, K. J. (2002). Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 59(11):1819–1833.

²⁷Dawkins, R. (2009). *The selfish gene*. Oxford Univ. Press, Oxford, 2. ed., 30th anniversary ed. edition. Previous ed. : 1989.

author (Ågren, 2021, pp.80-115)²⁸. In your opinion, should we consider Dawkins’ perspective wrong, or would it be more correct to consider it incomplete, i.e., neglecting important aspects such as the complex interplay between genes and the environment during development and between genes with different hierarchical positions and other regions of the genome?

A3: I answer with the words of Carl Schlichting (Schlichting, 2021)²⁹: “gene expression is always context-dependent (Nijhout, 1990)³⁰. Genes require an environmental cue to be expressed whether that cue is an external environment or the product of some other gene, and phenotypes can vary as a function of differences in those cues [...]. So, while one can readily assign a fitness to a DNA sequence in a computer model, in the real world, it is that sequence’s expressed phenotype that has a fitness. [...] we neglect the fact that patterns of gene expression leading to “normal” development have evolved in the context of specific ranges and frequencies of various environmental parameters (Pei et al., 2020)³¹. When organisms are placed in environmental contexts beyond the scope of their evolutionary memory, their development may no longer be predictable.”

Richard Dawkins’ vision, which identifies in the gene - the selfish gene - the only autonomous level at which natural selection can act, suffers, in my opinion, from many flaws, extensively present in the biology of the last half-century (but fortunately countered, in an increasingly convinced manner, in recent times): among these, in addition to a reductionism transformed from epistemological choice to ontological principle, I emphasise the lack of interest in the phenotype and, therefore, in development. It is disappointing to realize how, for decades, the teaching of Conrad Hal Waddington (Waddington, 2014)³² was neglected, who so well understood how natural selection does not directly affect gene variation, but the phenotypes that express it through development.

²⁸Ågren, J. A. (2021). *The gene’s-eye view of evolution*. Oxford University Press, Oxford, first edition edition.

²⁹Schlichting, C. D. (2021). *Plasticity and Evolutionary Theory: Where We Are and Where We Should Be Going*, pages 367–394. CRC Press.

³⁰Nijhout, H. F. (1990). Problems and paradigms: Metaphors and the role of genes in development. *BioEssays*, 12(9):441–446.

³¹Pei, Y., Forstmeier, W., and Kempenaers, B. (2020). Offspring performance is well buffered against stress experienced by ancestors. *Evolution*, 74(7):1525–1539.

³²Waddington, C. H. (2014). *The Strategy of the Genes*. Routledge Library Editions: 20th Century Science Ser. Taylor & Francis Group, Florence.

In the second half of the last century, there was a shift from a widespread disinterest in biological evolution (and, in many places, from an outright denial of it) to a biology that spearheaded Dobzhansky's (Dobzhansky, 1973)³³ motto *Nothing in biology makes sense except in the light of evolution*. Certainly, an evolutionary vision of all forms and processes in the living world is possible, and often necessary, but there are many important areas of biology that have progressed, and continue successfully to progress, without an evolutionary reading of their objects of study. I particularly emphasise, once again, development. While I welcome the advent of evo-devo with great enthusiasm, I cannot ignore the legitimacy of a developmental biology that pursues a research agenda in which evolution is not necessarily mentioned.

Q4: In this regard, do you think that genes (at least for what concerns loci enabling plastic responses) may share some characteristics with polysemous words, whose meaning changes in relation to their context of utterance and linguistic surroundings? If the main points of this analogy hold, we could perhaps say that what is passed from one generation to the next is a nucleus of potential “biological meanings”, which often needs to be conjugated with both the environment and the genomic surroundings in order to generate actual forms. As I argue in my thesis, an analogy emphasising the “polysemy of genes” could highlight these latter aspects more effectively than the “selfish gene” metaphor, at least from a communicative point of view.

A4: I agree with you: not only about the welcome overcoming of the atomistic vision of Dawkins' selfish gene, but also about the usefulness of an extended parallelism between the world of genes and the world of words. I too indulged, in an essay published ten years ago (Minelli, 2014b)³⁴, in a reading of genes as words. I'll pick for you and freely translate some passages from that text: “The use of the letters A, T, C and G to indicate the nitrogenous bases in the DNA molecule, has long been extended to describe suggested analogies between the genetic and the linguistic material. Nucleotides

³³Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *The American Biology Teacher*, 35(3):125–129.

³⁴Minelli, A. (2014b). *Geni come parole - uno, nessuno e centomila*, pages 107–117. Roma-Messina: Corisco Edizioni.

are like the letters of the alphabet, which can be joined together in sequences of different lengths to form a multitude of words. The molecular (DNA or RNA) alphabet is made up of only four letters, but the number of words (i.e., different molecules) that can be formed with them is immense, because these words are very long, up to hundreds of letters. In addition to DNA and RNA, this analogy could also be applied to proteins, where, however, the alphabet is much richer and, with its 20 different amino acids, singularly close in richness to the alphabet of many languages. The linguistic analogy showed its solidity from many points of view. Just as, in a given language, the words made up of N letters are only a subset of all the combinations of N letters that the alphabet would potentially allow to be formed, so also in the world of molecules the strings of nucleotides or amino acids present in nature they are only a subset of the molecules of equal length that could potentially be composed. Expressions built on this analogy with written language have increasingly taken hold in molecular genetics. For example, given that DNA and RNA molecules are very similar in structure, it seemed reasonable to speak of *transcription*, to indicate the synthesis of a messenger RNA molecule as a copy of a DNA segment. To indicate instead the passage from mRNA to proteins, molecules built on the basis of different alphabets, we preferred to borrow the term *translation*, again from the world of languages.”

Q5: In my thesis, alongside a more theoretical/philosophical perspective, I also adopt a computational approach by creating an agent-based model to simulate the competition and coexistence between alternative strains (plastic vs non-plastic) of the nematode worm *Pristionchus pacificus*. In short, agent-based modelling is a technique used to simulate the evolution of natural systems with a bottom-up approach, that is: i) writing a computer program which describes the algorithmic behaviour of several digital individuals (the agents, e.g., cells, viruses, animals), and ii) letting the agents generate the global dynamics of the system spontaneously, from their reciprocal interactions. So, for instance, agent-based modelling allows to simulate a virtual environment where digital worms develop, move, eat, reproduce, and die, since all the necessary “ingredients” to simulate this biological system, including whole life cycles, gene segregation ratios, and so on, may be synthesized in

several code lines. One of the main benefits of this technique is its ability to speed up in a remarkable way otherwise slower phenomena, so we can observe the evolution of a population over thousands of generations in a few seconds or minutes. Is there any phenomenon or evolutionary process you would be curious to explore using computer simulations based on multiple, interacting animals?

A5: Yes, of course. Particularly intriguing are the phenomena of mimicry, as well as the most sophisticated instances of *coadaptation between flowers and pollinators*.

Together with Marta Marchini, at the time a graduate student of mine, and Daniele Sommaggio, an expert of hoverflies, I studied one of the most paradigmatic examples of Batesian mimicry, involving wasps as poisonous models and hoverflies (Syrphidae) as their mimics (Marchini et al., 2016)³⁵. Our question was not about the adaptive significance of the similarity in the black-and-yellow livery of the two groups of insects, or the likely limited stringency of the same, but about a plausible explanation for the inception of their coevolution. Our hypothesis was, that color patterns that can eventually be selected for their likeness between poisonous and harmless insects are perhaps more likely to evolve than different, although potentially similar, patterns. Specifically, we looked for a possible positive bias in the occurrence of yellow color in homologous cuticle areas of the thorax and abdomen of wasps and syrphids, and the like for the black ones. As a proxy for evolvability of these color patterns we analysed the co-occurrence of multiple color pattern within species (either as serial homologues or as expression of intraspecific variation) in 203 species of syrphids and 127 species of wasps. Results supported our hypothesis of positively biased evolvability. Experimental evolution studies would be much useful to go ahead with this kind of investigations.

More intriguing are the coadaptations between flowers and pollinators. A first level of intrigue are morphological correspondences like those between the shape of the flowers of many species of the mint family (Labiatae, e.g., *Salvia*) and the body shape and behavior on the flowers of bees, the usual pollinators of many plants in this family. More intriguing, still on the level of morphology,

³⁵Marchini, M., Sommaggio, D., and Minelli, A. (2016). Playing with black and yellow: The evolvability of a batesian mimicry. *Evolutionary Biology*, 44(1):100–112.

are the similarities between the shape of flowers of the orchid genus *Ophrys* and their usual pollinators, the males of a number of wasp- or bee-like hymenopterans. But the top of puzzling coadaptation is found on the level of chemistry. The perfume through which these orchids attract their pollinators is a mixture of very numerous compounds. As probably expected, this perfume has some similarity to the pheromone produced by the female of the pollinators, thus explaining why the visit of their males to the orchids are often described as a kind of pseudocopulation. Wasp female pheromone and orchid perfume are both very complex mixtures. It might be speculated that the specificity of their attracting power on the male wasps is given by the combined effect of numerous, similarly dosed compounds, but this does not seem to be actually the way. At least, the most accurate study thus far performed on an orchid-wasp system disprove this naïve hypothesis. In fact, what the perfume and pheromone have in common, as attractant, is likely to be a single chemical, a rare one, and present in very modest amounts both in the orchid perfume and in the insect's pheromone. This has been shown by Florian P. Schiestl and co-workers (Schiestl et al., 2003)³⁶ in the case of an Australian orchid (*Chiloglottis trapeziformis*) and its pollinator, the male of the wasp *Neozeleboria ayptoides*. They exposed males of the same species to molecules present in both natural mixtures (orchid perfume and female pheromone) and found that only one of the many molecules isolated and tested triggers a reaction when it is perceived by the male insect with its antennae.

Q6: One last question: as you already know from our previous interactions, in my thesis I wanted to integrate a philosophical and a scientific approach to investigate phenotypic plasticity. From your perspective, are there ways of doing philosophy that can be considered contiguous with scientific research in the strict sense, or are these two activities always separated from each other, e.g., for their methods and aims (although, of course, they can cooperate)?

A6: Over the years, I extended my interests in the life sciences to the history and, later, also to the philosophy of biology. Once more, developmental biology has been the core of my intellectual

³⁶Schiestl, F. P., Peakall, R., Mant, J. G., Ibarra, F., Schulz, C., Franke, S., and Francke, W. (2003). The chemistry of sexual deception in an orchid-wasp pollination system. *Science*, 302(5644):437–438.

wanderings also in the relationship between biology and the philosophy of biology. I still remember the sense of bewilderment I felt, just over twenty years ago, when writing the preface to my book *The Development of Animal Form*. As is often the case (and I think this is a good thing), I was about to write the preface after I had completed all the book's chapters. I did not intend to write a summary of the work, I tried instead to make sense of it, to place my message in the context of a cultural project that at the time already had a name (evo-devo), but still ill-defined contours and contents that were strongly skewed in favor of a genetic-molecular dimension far removed from my approach to evo-devo. It was then that I realised that, in the face of the very rich theoretical and conceptual literature on the problems of evolution, there was very little of the like for development. I even found it difficult to distil from the literature an acceptable definition of development that could be applied to all the phenomena that, in my opinion, legitimately fall within it. Together with a young French philosopher of biology, Thomas Pradeu, I invited twenty scholars (half of them biologists, the other half philosophers) to contribute to a collective volume entitled *Towards a Theory of Development* (Minelli, 2014c)³⁷. It was an excellent opportunity to take a step forward, but we were still far from reaching a general agreement on the most basic point, the definition of development. We returned to this issue later, with an article signed by nine authors (Pradeu et al., 2016)³⁸, but even today I still think there is work to be done on this point.

Lately, my adventurous forays into philosophy have led me to suggest two epistemological experiments. On the one hand, to reverse Isabelle Stengers' (Andler and Stengers, 1987)³⁹ model, according to which polysemic terms such as species or gene can be described as *nomadic concepts* that take on different meanings depending on the disciplines with which they become linked. In my opinion, it is worth trying a different, dual reading of Stengers', recognising instead these *concepts as anchors* to which disciplines in constant evolution are temporarily associated (Minelli, 2020b)⁴⁰.

³⁷Minelli, A. (2014c). *Towards a Theory of Development*. Oxford University Press, Incorporated, Oxford.

³⁸Pradeu, T., Laplane, L., Prévot, K., Hoquet, T., Reynaud, V., Fusco, G., Minelli, A., Orgogozo, V., and Vervoort, M. (2016). Defining "Development", pages 171–183. Elsevier.

³⁹Andler, D. and Stengers, I., editors (1987). *D'une science 'à l'autre. Science ouverte*. Ed. du Seuil, Paris.

⁴⁰Minelli, A. (2020a). Biology and its disciplinary partitions - intellectual and academic

The divides between scientific disciplines (as far as I am concerned, the biological disciplines) are sometimes defended too strenuously (not only for cultural reasons, but also for academic ones), whereas it would be fruitful to rethink their boundaries from time to time (Minelli, 2020a)⁴¹.

Finally, I recently came across an illuminating observation by Love and Brigandt (Love and Brigandt, 2017, p.320)⁴², who have proposed “to shift attention in discussions of individuality from metaphysics to epistemology,” arguing that “some of the controversies involve epistemological differences rather than metaphysical disagreement.” A similar shift from a metaphysical position to an epistemological position could be a winning strategy in other areas as well, and I recently tried to explore this avenue in a revisiting of the long-standing problem of homology (Minelli and Valero-Gracia, 2022)⁴³.

constraints. *Scienza & Filosofia*, (24):105–126.

⁴¹Minelli, A. (2020b). Disciplinary fields in the life sciences: Evolving divides and anchor concepts. *Philosophies*, 5(4):34.

⁴²Love, A. C. and Brigandt, I. (2017). Philosophical dimensions of individuality. In Lidgard, S. and Nyhart, L. K., editors, *Biological Individuality: Integrating Scientific, Philosophical, and Historical Perspectives*, pages 318–348. University of Chicago Press.

⁴³Minelli, A. and Valero-Gracia, A. (2022). Spatially and temporally distributed complexity—a refreshed framework for the study of grn evolution. *Cells*, 11(11):1790.

Chapter 8

Appendix C: Interview with Professor Ralf J. Sommer

Short presentation

Ralf J. Sommer (born 23 September 1963) is a leading scientist in the field of evolutionary developmental biology. The hallmark of his research activity is the integration of genetic, developmental, and ecological data to investigate the mechanisms underlying evolutionary novelty. In recent years, his main interest has been the role of phenotypic plasticity in evolution, specifically in the nematode worm *Pristionchus pacificus*, which he established as a new model organism in evolutionary biology using the island of La Réunion as a case study. He has been a Scientific Member of the Max Planck Society and Director of the Department Integrative Evolutionary Biology at the Max Planck Institute (MPI) for Developmental Biology in Tübingen, Germany since 1999. He has also been Honorary Professor at the University of Tübingen since 2002, and Editor-in-Chief of the Journal of Nematology since 2021.

Interview

Q1: Dear Professor Sommer, it is a real pleasure talking to you about evolution and phenotypic plasticity! Thank you for this valuable opportunity. As a first, introductory question, I would like to ask you something about your scientific background. When and why did you become interested in evolution and, more specifically, in the relationship between evolution and developmental processes? In this context, why are you convinced that phenotypic plasticity

may be regarded as a critical component to reach a deeper understanding of life? I know you have already discussed this issue (e.g., in (Sommer, 2020)¹, but I would be curious to hear from you if and how much research in the very last few years may have updated this perspective.

A1: My interest in biology and evolution dates back to my school days, when I was an active butterfly collector. From this time stems my interest in the patterns and processes that result in diversity, mainly morphological diversity. At the end of my studies, developmental genetics had started to obtain detailed molecular mechanistic insight into embryonic segmentation in *Drosophila* and later vulva development in *C. elegans*. This understanding allowed the corresponding developmental processes to be studied in other insects and nematodes, the birth of evolutionary developmental biology or “evo-devo”. I was one of the first graduate students to work on the evolution of insect segmentation and later on as a postdoc, started comparative on nematode postembryonic development.

While we were at the time convinced in the field that “evo-devo” could explain how diversity is generated it became clear to me that the “merger of development and evolution” on its own would be insufficient. Given the overall conservation of developmental control genes, something more must be important. Taking the environment “on board” was for me the logical extension and still missing aspect of evolution, something that also dated back to my experience as a butterfly collector. In this context, phenotypic (developmental) plasticity is of upmost importance and several monographs from the late 1990ies and early 2000s reinforced this suggestion. I saw developmental plasticity as the unique phenomenon to bring all aspects of biology that are important for generating diversity together: development, evolution and the environment. At the same time, plasticity was totally understudied, but my model system *Pristionchus* had a potentially highly rewarding example of this. And it worked out for us. Although, as always in science, it was not clear at the beginning that things would work out that way.

The most important novel research directions in the last few years are on long-term environmental induction experiments follow-

¹Sommer, R. J. (2020). Phenotypic plasticity: From theory and genetics to current and future challenges. *Genetics*, 215(1):1–13.

ing the experimental evolution idea from Lenski. While I proposed this in my 2020 review, we have only started to get involved in such studies later the same year. This is highly rewarding and has changed our current research and the way we think about plasticity. In reality, “things” happen much faster in evolution than we thought. This is really very exciting times for my lab.

Q2: I think one of the most surprising things about plasticity is its immense spread in nature, from the environmentally-induced morphology of unicellular organisms like protists (Mulot et al., 2017)² to the growth of human muscle tissue (Hoppeler and Fluck, 2002)³. This is even more remarkable when considering the counterbalancing effect of the costs of production and maintenance of plasticity, which you contributed to show and measure in one of your recent papers (Dardiry et al., 2023)⁴. Do you think that many traits from all domains of life showing some degree of plasticity might have been poorly investigated by evolutionary biologists due to an old “prejudice” that sees plasticity as a confounding factor in evolution?

A2: Yes, most traits will have some elements of plasticity. Biologists were long not interested in this, in particular because bringing organisms into the laboratory was done to keep traits as fixed as possible so that mechanistic analysis would be feasible. Judgement on plasticity is an interesting question: Botanists have been very open and build the importance of plasticity into their thinking early on. Zoologists in contrast, ignored and neglected the phenomenon, likely as you say because it was seen as a confounding factor. For me that was hard to believe because as a butterfly collector one knows that every butterfly with two generations per year shows at least some degree of wing polyphenism. Therefore, entomologist should have known better for a long time. Also, some influential early review articles considered plasticity unimportant for evolution and the subsequent work and literature pointing towards its importance had

²Mulot, M., Marcisz, K., Grandgirard, L., Lara, E., Kosakyan, A., Robroek, B. J. M., Lamentowicz, M., Payne, R. J., and Mitchell, E. A. D. (2017). Genetic determinism vs. phenotypic plasticity in protist morphology. *Journal of Eukaryotic Microbiology*, 64(6):729–739.

³Hoppeler, H. and Fluck, M. (2002). Normal mammalian skeletal muscle and its phenotypic plasticity. *Journal of Experimental Biology*, 205(15):2143–2152.

⁴Dardiry, M., Piskobulu, V., Kalirad, A., and Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1):48–57.

to work against these early contributions.

Q3: By reading a lot of works by you and your research team, I matured the idea that *Pristionchus pacificus* could indeed have a place in the Olympus of model organisms, together with *Escherichia coli* bacteria, *Drosophila melanogaster* fruit flies, and other nematode species like *P. pacificus*' famous "cousin", *Caenorhabditis elegans*. Other than a huge amount of data about its development, genetics, and behaviour, *P. pacificus* is also perfect for experimental aims due to its short life cycle and prevalence of self-fertilization as a mode of reproduction. I feel that this species still has so much more to offer us in terms of understanding of evolutionary mechanisms. What discoveries do you think these worms may still bring in the future, and how far do you imagine all these discoveries will go beyond the "boundaries" of the single species and tell us something very general about evolution? In other words, what is the degree of generalisation of present and plausible future findings?

A3: We are quite far already with regard to our understanding of the gene regulatory network controlling mouth-form plasticity. But with regard to its behavioural and ecological consequences, much more is still to be discovered. There are a multitude of organismal interactions at the intra- and interspecies level that still have to be investigated and for sure, the predatory vs. non-predatory state of *Pristionchus* will heavily affect these interactions. Therefore, the next decade will result in many novel striking features around mouth-form plasticity.

The question about generalizations is an important one for every branch of biology. I believe for *P. pacificus* mouth-form plasticity it is similar to other heavily investigated traits. Some principles that we learn about are conserved and thus, we can identify and learn about general principles from our model system, whereas for others we cannot. Therefore, it is always important to add to your "model system approach" with a strong focus on one study system, some comparative investigations. They will rapidly tell you at what level patterns and their underlying processes vary and over which evolutionary time scale variation occurs. We have just this month published a paper on a related nematode *Allodiplogaster sudhausi* that tells us in a striking way about several aspects of mouth-form

plasticity that are rapidly changing with strong ecological adaptations, whereas other aspects are surprisingly conserved (Wighard et al., 2024)⁵.

Q4: One of your research lines which impressed me most includes the studies on the crucial role of the *eud-1* gene in the maintenance/-suppression of mouth dimorphism in *P. pacificus* (e.g., (Ragsdale et al., 2013)⁶. In this case, the plastic response is highly dependent on the state of a single, epistatic gene, so the “barrier” between a plastic and a non-plastic strain is, to some extent, “weak”, because even a single point mutation can deactivate (or reactivate) plasticity. This also reminds me of another interesting study where the authors found that a SNP was responsible for the reduction of the sensitivity to environmental stimuli promoting egg-laying behaviour in *C. elegans* (Vigne et al., 2021)⁷. In contrast, there are also cases where plasticity does not depend on a single key gene, e.g., the temperature-dependent height of *Sorghum bicolor* plants, that is regulated simultaneously by three loci (Mu et al., 2021)⁸. Considering these aspects, would it be reasonable to assume the presence of single developmental switches as a predictor of competition (or coexistence) between generalist and specialist strains within the same population or between different populations of the same species? In the case of *P. pacificus*, do you think that the interplay between plastic and fixed genotypes relying on mutations affecting *eud-1* (with the exclusive effect of suppressing the dimorphism) might be observed/widespread in nature?

A4: This is an interesting question. We have so far, no evidence that species or strains that are monomorphic are due to mutations in *eud-1*. But this might be a sampling bias. There is however,

⁵Wighard, S., Witte, H., and Sommer, R. J. (2024). Conserved switch genes that arose via whole-genome duplication regulate a cannibalistic nematode morph. *Science Advances*, 10(15).

⁶Ragsdale, E. J., Müller, M. R., Rodelsperger, C., and Sommer, R. J. (2013). A developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*, 155(4):922–933.

⁷Vigne, P., Gimond, C., Ferrari, C., Vielle, A., Hallin, J., Pino-Querido, A., El Mouridi, S., Mignerot, L., Frøkjær-Jensen, C., Boulin, T., Teotonio, H., and Braendle, C. (2021). A single nucleotide change underlies the genetic assimilation of a plastic trait. *Science Advances*, 7(6).

⁸Mu, Q., Guo, T., Li, X., and Yu, J. (2021). Phenotypic plasticity in plant height shaped by interaction between genetic loci and diurnal temperature range. *New Phytologist*, 233(4):1768–1779.

another aspect to this: if you, for example, look at the other major example of plasticity in nematodes, dauer development, you see that it is very easy to generate dauer-defective mutants in the lab by genetic means. However, in the wild such forms are never found suggesting that such mutants are maladapted. But this being said, in this years' sampling trip to the Indian ocean we isolated for the first time a *P. pacificus* strain that is completely non-predatory. We are currently testing exactly your question if this phenotype is due to a mutation in *eud-1*.

Q5: I would also like to go into more detail regarding experimental evolution. I think it is fantastic that biologists are now able to study evolutionary processes while they are happening. An astonishing example of this approach is Richard Lenski's *E. coli* long term evolution experiment (Lenski, 2017)⁹. *P. pacificus* is of course suitable as well for this treatment for the reasons listed above, and all its peculiarities also enable a deeper investigation of the effects of the feedback loops between evolution and development. In addition to laboratory and field research, you also had some experience with evolutionary agent-based (or individual-based) modelling (Kalirad and Sommer, 2024a)¹⁰, that is, implementing programmed organisms and letting them develop, interact, and evolve, usually within a simulation environment. What do you think about the future perspectives of this approach in the field of evolutionary developmental biology when it comes to provide some significant, complementary contributions to your work with real animals? Can the increased speed, replicability, and control allowed by computer simulations effectively balance the costs of simplification?

A5: Indeed, as indicated above, we have heavily investigated in experimental evolution and much of our current research is a logical consequence of using these approaches. The theoretical studies we do are important for at least two reasons. First, they can help "us" understand aspects of our system that cannot be studied in the lab or the environment, because timescales of hundreds of generations, in particular in a context of slow-growing beetles, are simply unfea-

⁹Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, 11(10):2181–2194.

¹⁰Kalirad, A. and Sommer, R. J. (2024a). The role of plasticity and stochasticity in coexistence. *Ecology Letters*, 27(2).

sible. Second, we hope that our studies of combining simulations and ABM modelling with empirical life history data, will be seen as a major advantage that can be successfully transferred to other systems. Again, such comparisons would reveal to which extent the nematode predation system can be generalised. I would not be surprised if there would be very similar patterns in vertebrates and mammals.

Q6: One last question (if I may ask): which aspects of *P. pacificus* evolution and, more generally, of the evolution of phenotypic plasticity would you be most curious to explore through such models?

A6: Long-term stability of plasticity and co-existence are of course obvious questions that can be addressed through modelling. But in general, all three historical aspects of plasticity that lab scientists and also field workers cannot easily address in their lifetime – origin of plasticity, long-term stability and canalisation – can be modelled, if proper empirical data are available. This is where I see the biggest value of theory in the coming years.

Glossary

adaptation A process of genetic change in a population whereby, as a result of natural selection, the average state of a character becomes improved with reference to a specific function, or whereby a population is thought to have become better suited to some feature of its environment. Also, an adaptation: a feature that has become prevalent in a population because of a selective advantage conveyed by that feature in the improvement in some function (Futuyma, 2017)¹¹. 42

amino acid An organic molecule possessing both acidic carboxylic acid ($-\text{COOH}$) and basic amino ($-\text{NH}_2$) groups attached to the same tetrahedral carbon atom. Amino acids are the principal building blocks of proteins and enzymes. 15

cell membrane A two-layered structure of material surrounding living cells. Most cell membranes have proteins, such as receptors and enzymes, embedded in them. The membrane holds the cell together, controls which substances go in or out, and maintains homeostasis. 23

chromosome The self-replicating gene-carrying member found in the cell nucleus and composed of a DNA molecule and proteins (chromatin). 15

DNA A high-molecularmass linear polymer, composed of nucleotides containing 2-deoxyribose and linked between positions 3' and 5' by phosphodiester groups. DNA contains the genetic information of organisms. The doublestranded form consists of a double helix of two complementary chains that run in opposite

¹¹Futuyma, D. J. (2017). *Evolution*. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts, fourth edition edition.

directions and are held together by hydrogen bonds between pairs of the complementary nucleotides. 6

environmental homeostasis The ability of an organism to automatically maintain a constant internal condition regardless of the external environment. 36

enzyme macromolecule that functions as a biocatalyst by increasing the reaction rate, frequently enzyme containing or requiring one or more metal ions. In general, an enzyme catalyzes only one reaction type (reaction specificity) and operates on only a narrow range of substrates (substrate specificity). 15

epistasis An effect of the interaction between two or more gene loci on the phenotype or fitness whereby their joint effect differs from the sum of the loci taken separately (Futuyma, 2017)¹².. 41

fitness The measure of an individual's relative genetic contribution to the gene pool of the next generation; the longer an individual survives and the more it reproduces, the higher the fitness and the higher the chance that a hereditary characteristic will be reproduced. 33

gene Structurally, a basic unit of hereditary material; an ordered sequence of nucleotide bases that encodes one polypeptide chain (via mRNA). 6

gene expression A term describing the process of translating information in DNA into an organism's traits. A process by which a gene's code affects the cell in which it is found by synthesizing a protein or RNA product that exerts its effects on the phenotype of the organism. Expressed genes are transcribed into mRNA and translated into protein or transcribed into mRNA but not translated into protein. 15

genetic code The language of genetics. The instructions in a gene that tell the cell how to make a specific protein. The code defines the series of nucleotides in DNA, read as triplets called

¹²Futuyma, D. J. (2017). *Evolution*. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts, fourth edition.

codons, that specifies the sequence of amino acids in a protein. The set comprises 64 nucleotide triplets (codons) that specify the 20 amino acids and termination codons (UAA, UAG, UGA). The code is made up of adenine (A), thymine (T), guanine (G), and cytosine (C), the nucleotide bases of DNA. Each gene's code combines them in various ways to spell out three-letter triplets (codons) that specify which amino acid is needed at each step in making a protein. 23

genome The complete assemblage of chromosomes and extrachromosomal genes of a cell, organelle, organism, or virus; the complete DNA portion of an organism; the complete set of genes shared by members of any reproductive body such as a population or species. 14

genotype The complete genetic makeup of an organism, which may not show in physical appearance; the pair of alleles at a particular locus. 30

glycolysis The anaerobic pathway or enzymatic conversion (using 11 different enzymes) in the cell's cytoplasm of glucose to simpler compounds. Glucose, a six-carbon sugar, is converted into two molecules of pyruvic acid of three carbons each, with two molecules of NADH and two ATPs as by-products. It is the most universal and basic energy harvesting system; it transforms glucose into lactic acid in muscles and other tissues for energy production when there is not enough oxygen available. In aerobic respiration, the two pyruvic acids are further used in the Krebs cycle. 55

mutation A heritable change in the nucleotide sequence of genomic DNA (or RNA in RNA viruses), or in the number of genes or chromosomes in a cell, that can occur spontaneously or be brought about by chemical mutagens or by radiation (induced mutation). 7

natural selection Survival and reproduction of a genotype that leaves more progenies who possess attributes of fitness, survivability, and adaptation to their environment over that of another genotype. Those better adapted to their environment are likely to increase in frequency over a number of generations

over those that are less adapted, and this difference is not due to chance. 7

nucleotides Nucleosides with one or more phosphate groups esterified mainly to the 3- or the 5- position of the sugar moiety. Nucleotides found in cells are adenylic acid, guanylic acid, uridylic acid, cytidylic acid, deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid, and thymidylic acid. A nucleotide is a nucleoside in which the primary hydroxy group of either 2-deoxy-D-ribose or of D-ribose is esterified by orthophosphoric acid. 25

operon A functional unit consisting of a promoter, an operator, and a number of structural genes, found mainly in prokaryotes. An example is the operon NIF. The structural genes commonly code for several functionally related enzymes, and although they are transcribed as one (polycistronic) mRNA, each has its separate translation initiation site. In the typical operon, the operator region acts as a controlling element in switching on or off the synthesis of mRNA. 15

phenotype The outward observable features, functions, or behaviors of an organism, based on the coding of the genotype. 30

phenotypic plasticity A phenotype's capacity to respond differentially to varying environmental conditions. 32

photoperiod The length of day and night in a 24-hour period. 18

polypeptide A polymer chain of amino acids linked by covalent peptide bonds. One or more polypeptides form proteins. Each polypeptide has two terminal ends; one, called the amino terminal or N-terminal, has a free amino group, while the other end is called the carboxyl terminal or C-terminal with a free carboxyl group. 14

polyphenism The capacity of a species or genotype to develop two or more forms, with the specific form depending on specific environmental conditions or cues, such as temperature or day length (Futuyma, 2017)¹³. 32

¹³Futuyma, D. J. (2017). *Evolution*. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts, fourth edition.

promoter The DNA region, usually upstream from the coding sequence of a gene or operon, that binds and directs RNA polymerase to the correct transcriptional start site and thus permits transcription at a specific initiation site. 35

protein A molecule composed of many amino acids and with a complex structure. For example, immunoglobulin, casein. 14

reaction norm An expression or variation in pattern among phenotypes produced by a single genotype caused by a variation in environmental conditions. While phenotypes are an expression of genes in an organism that give it certain traits and characters (anatomical structure, physiology, and behavior) that can be measured, sometimes the traits are actually norms of reaction, i.e., traits expressed in different ways in different environments. For example, human body weight or height is different in identical environments or on different diets. Take a set of genes that produce a particular trait together, list all the possible environmental conditions that they can survive in, and the norm of reaction is the total variation in that trait in all of the survivable environmental conditions. 32

ribosome A subcellular unit composed of specific rRNA and proteins that is responsible for the translation of mRNA into protein synthesis. 23

RNA An RNA molecule that transfers the coding information for protein synthesis from the chromosomes to the ribosomes. Fragments of ribonucleic acid serve as templates for protein synthesis by carrying genetic information from a strand of DNA to ribosomes for translation into a protein. The information from a particular gene or group of genes is transferred from a strand of DNA by constructing a complementary strand of RNA through transcription. Transfer RNA (tRNA), composed of three nucleotide segments attached to specific amino acids, correctly match with a template strand of mRNA, lining up the correct order of amino acids and bonding them, via translation in the ribosome with rRNA (ribosomal RNA), to form a protein. 14

sexual selection Sexual selection based on secondary sex characteristics and on the success of certain individuals over others in relation to the propagation of the species and the enhancement of sexual dimorphism. 45

speciation In biology, it refers to the origination of a new species. 47

splicing The process of removing introns, the noncoding regions of eukaryotic genes that are transcribed into mRNA. 44

trait Any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization (Violle et al., 2007)¹⁴. 13

transcription The process by which the genetic information encoded in a linear sequence of nucleotides in one strand of DNA is copied into an exactly complementary sequence of RNA. 15

translation The unidirectional process that takes place on the ribosomes whereby the genetic information present in an mRNA is converted into a corresponding sequence of amino acids in a protein. 15

¹⁴Violle, C., Navas, M., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5):882–892.

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