



The evolution of mechanism underlying the allocation of resources and consequences on the shape of trade-offs in multicellular organisms

Salomé Bourg

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**The evolution of mechanism underlying the allocation
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The evolution of mechanism underlying the allocation
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“Et ainsi, parfois, la science nous permet de découvrir l’art. Une aventure scientifique, l’épigraphie, le déchiffrement des écritures et langues antiques inconnues nous a donné accès à la splendeur des oeuvres littéraires et des cultures perdues d’une partie des plus anciennes civilisations qui nous ont précédées. Dans toute l’histoire de l’histoire, il n’y a peut être pas d’aventure plus extraordinaire que celle qui en un siècle a ainsi mené les savants d’une première étincelle à laquelle personne n’avait pris garde à tout un flamboiement de découvertes et de déchiffrements tous plus ardu, ingénieux et inimaginables les uns que les autres et de la sorte, à la remise au jour des normes et capitales portions de notre passé jusque là à jamais égarées dans le temps. En vérité, nos contemporains, si ils étaient un peu moins obnubilés par l’utile et le rentable, le terre à terre et les chiffres, si ils avaient un peu plus le sens de la véritable grandeur de l’Homme, laquelle sur le plan intellectuel est dans le savoir, le véritable savoir qui est d’abord de pur connaissance et sans la moindre recherche première d’une utilisation pratique. En vérité, nos contemporains, devraient célébrer cette glorieuse épopée à l’égale des plus mémorables de l’histoire de nos progrès.”

Jean Bottéro - Mésopotamie. L’écriture, la raison et les dieux.

À ma soeur et mes parents

Résumé

Pour se développer, survivre ou se reproduire, les organismes ont besoin d'énergie, généralement acquise par l'alimentation. Or cette ressource alimentaire est présente en quantité fluctuante et limitée dans l'environnement, obligeant les êtres vivants à faire des compromis et à répartir leur énergie entre différentes fonctions. Ces compromis évolutifs sont visibles à l'échelle d'une population sous la forme d'une relation négative entre traits appelée **trade-off**. Les trade-offs ont longtemps été considérés comme étant uniquement la résultante d'une allocation différentielle des ressources et comme étant immuables. Ainsi, allouer davantage d'énergie à un trait, la survie par exemple, réduit nécessairement la part pouvant être redistribuée aux autres traits tel que la fécondité ou la croissance. Or l'allocation différentielle des ressources est un processus régulé par un mécanisme endocrinien, lui-même codé génétiquement et donc capable d'évoluer. L'objectif de cette thèse a été de comprendre, de manière théorique, comment l'évolution du mécanisme endocrinien impacte la forme des trade-offs et comment la forme des trade-offs elle-même évolue.

Pour ce faire, j'ai développé des modèles évolutifs où l'allocation des ressources est régie par un système endocrinien. Ce système peut évoluer, sous l'effet de mutations impactant l'expression et la conformation des hormones et des récepteurs qui composent ce système endocrinien. J'ai ainsi pu montrer que les relations négatives entre traits peuvent évoluer et que leur forme dépend fortement d'un paramètre rarement considéré : le coût du stockage. Dans un second temps, j'ai étudié l'impact de la variabilité temporelle dans l'abondance d'une ressource alimentaire sur l'allocation différentielle et les mécanismes endocriniens sous-jacents.

Mon projet de thèse comprend un troisième volet, complémentaire à la partie théorique, qui s'attache à tester certaines prédictions empiriquement. J'ai mené une expérience de sélection artificielle dans laquelle je contrôlais la topologie d'un paysage de fitness, permettant de sélectionner des combinaisons de trait n'appartenant pas à la relation phénotypique habituellement observée. Cette expérience, conduite chez *Drosophila melanogaster* durant 10 générations, a montré que l'évolution peut effectivement se produire dans ce contexte, remettant partiellement en cause notre compréhension des mécanismes sous-tendant l'expression des traits phénotypiques.

Mots clés : trade-offs, mécanisme endocrinien, pléiotropie, traits d'histoire de vie, génétique quantitative, allocation différentielle des ressources, espace des génotypes

Abstract

In order to grow, survive or reproduce, all organisms need energy, usually acquired through diet. However, this food resource is present both in fluctuating and limited quantities in the environment, forcing living beings to compromise and thus to divide their energy between their different functions. These evolutionary compromises, visible at the scale of a population in the form of a negative relationship between traits, are called trade-off. Trade-offs have long been considered as the result of a differential resource allocation and as immutable. Therefore, allocating more energy to a trait such as survival necessarily reduces the amount that can be redistributed to other traits, such as fecundity or growth. It is noteworthy that the differential allocation of resources is a process regulated by an endocrine mechanism, itself genetically coded and thereby able to evolve. The aim of my PhD thesis was to understand, theoretically, (i) how the evolution of the endocrine mechanism impacts the shape of trade-offs and (ii) how the shape of trade-offs itself evolves.

To do so, I first developed evolutionary models where the allocation of resources is governed by an endocrine system. This system can evolve under the effect of mutations that impact both the expression and the conformation of hormones and receptors constituting this endocrine system. Thanks to this model, I show that the negative relationships between traits can evolve and that their shape strongly depends on a parameter rarely considered: the cost of storage. In a second step, I studied the impact of temporal variability in food abundance on the endocrine mechanisms responsible for the differential allocation of resources.

Lastly, my thesis project includes a component complementary to the theoretical part, which attempts to empirically test certain of the expressed predictions. I conducted an artificial selection experiment in which I controlled the topology of a fitness landscape, thus allowing to select combinations of traits not belonging to the phenotypic relationship usually observed. This experiment, implemented in *Drosophila melanogaster* for 10 generations, has shown that evolution can indeed occur in this context, thereby partially challenging our understanding of the mechanisms underlying the expression of phenotypic traits.

Keywords: trade-offs, endocrine mechanism, pleiotropy, life history traits, quantitative genetics, differential allocation of resources, genotype space

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sont autant d'atouts (trop souvent considérés comme des défauts si ils sont féminins) dans lesquels tu m'as fait infuser. Ce féminisme soigneusement codé dans l'ADN des Maroc depuis au moins 5 générations, je ne te remercierai jamais assez pour cet héritage. Merci maman d'être là dans les meilleurs moments comme dans les moins drôles, merci pour ton soutien et ton amour inconditionnels. Merci pour ta présence et les kilos de linge que tu as lavé. Merci d'être une si bonne oreille et d'avoir toujours 3 longueurs d'avance sur tout le monde. Tu m'inspires au quotidien. Ce n'est pas un hasard non plus si aujourd'hui je suis docteure, que j'ai monté une association et que j'espère continuer à faire mille choses en même temps. Merci d'être ma maman, je t'aime.

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Part I

INTRODUCTION - English version

General introduction

During geological times, the earth has changed in appearance many times. It has gone from an hostile and cold rock bombarded with hazardous UV-radiation to a rich habitat teeming with life. An astonishing number of species has succeeded each other at the rhythm of mass extinctions. Notwithstanding the anthropocene (a new geological era characterized by the major influence of mankind on the environment), the first five extinctions are the results of important environmental variations (Hallam, 1994; Veron, 2008). Most living species from this temporal period were not able to survive under these particular conditions and went to extinction. Each time, after an extreme decrease of species diversity, an explosion of new species emerged from the remaining ones. These crises followed by successive increases in biodiversity illustrate what occurs at shorter timescales. Indeed, evolution acts continuously. In this introduction, I will begin by detailing concepts necessary for the understanding of evolutionary processes. Then I will introduce the questions that interest me particularly.

Each living organism carries molecules of DNA (or RNA for some viruses); as its genetic heritage or genome. To facilitate the study of these organisms (but also because until recently we did not master sequencing techniques) we summarize this information as its genotype – i.e., the allelic composition of all genes of an individual. This allelic diversity present in each individual is however not entirely visible to the naked eye. Indeed, the phenotype constitutes the set of observable traits and is the result of the interaction between the genotype and the environment (e.g., developmental processes, behavior, availability in food resource). The relationship between genotype and phenotype is complex : some genetic variation affects phenotype variation while other variation does not. An important aspect of current research in evolutionary biology is focused on deciphering the link between genotype and phenotype and on establishing a map of these relationships called the Genotype-Phenotype map¹. During the transmission of genetic information

¹I present a description of this work in the second part of this introduction.

from parents to offspring, successive DNA replications take place. If these replications were perfect, then the replicated DNA would be identical to that of the parents. Despite many mechanisms to ensure a uniform copy, germline cells accumulate rare errors. These random modifications of the genome are named mutations, and represent an important mechanism in the emergence of new genetic variation. A part of mutations have no or little impact on the phenotype (Eyre-Walker & Keightley, 2007) and are called neutral (even if there is debate around this qualification). Other mutations can have an important impact and a majority of them are deleterious and represent a disadvantage for the organism. But sometimes, a mutation can improve a phenotype (e.g., by allowing it to exploit a new resource). Thus, by having this advantage (access to more resources), it might be able to reproduce and/or to survive better. Hence, its offspring will carry this heritable character, which will most likely spread in the population. This principle represents the core idea of natural selection enunciated by Charles Darwin in ‘On the origin of species’ (1859). From random modifications of inherited information, new variants, if they perform better, can establish in a population and replace old ones. In a context of poor species diversity, many niches are not inhabited. Mutants can colonize the available ones. Hence, generations after generations, specific diversity can rise again (of course, diversity can also emerge in other situations such as in a strong competitive environment). The description of this diversity and the study of its emergence and maintenance constitutes a central question for evolutionary biologists. For decades, they have tried to decipher interactions between species and their evolution. To understand these, evolutionary biologists focused on one central parameter: fitness. Fitness can be described as the ability of a genotype to transmit its genetic information to future generations. For this transmission to occur, it is necessary that the focal individual survive until reproduction. Hence fitness is often considered as a combination of survival and reproduction of the individual.

Evolutionary biologists have attempted to describe and then to understand the variations in reproduction and survival among organisms. In order to maximize gene propagation through generations, a simplistic solution might be to gradually increase the capacity to produce offspring. A positive relationship between clutch/litter size (which is often used as a proxy of reproduction) and time could be expected. The study of variations in clutch/litter size has particularly occupied evolutionary biologists during 1800s and 1900s. This question has been especially studied in birds. In 1947, Lack published ‘The significance of Clutch-size’, a detailed review of observed variations in clutch size in European birds where the majority of bird species described are indeterminate egg

layers, meaning that they can replace any egg that is lost and do not lay a set number of eggs. Indeed, experiments consisting in repetitively removing eggs from the nest have been conducted. Then, experimenters observed a new laying until bird reaches the 'typical' number in the population studied (Leopold, 1987; Phillips, 1887). This was a very puzzling observation for researchers in the 1900s: why a bird capable of laying more than twice the number of eggs it produces would restrain itself to a specific lower quantity (Rensch, 1938; Moreau, 1944). One of the assumptions was to involve a demographic reason (Moreau, 1944). A bird population in a given environment has an optimal density, allowing it to exploit resources optimally and to maintain a stable population size. Two demographic processes can act simultaneously on the population size: birth and death. Global mortality is the result of intrinsic mortality (*i.e.*, mostly aging) and extrinsic mortality (*e.g.*, predation, pathogens, bad environmental conditions). Hence, global mortality eliminates a part of the population. The population can only be stable if the number of individuals removed by death is replaced by an equal number of births. Thus, to allow this balance, and following the reasoning of Moreau et al., the clutch size would correspond to the number of dead birds divided by the number of sexually mature couples, without taking into account the juvenile survival. However, this reasoning is inconsistent with the process of natural selection (Lack, 1947). Indeed, this could work if natural selection were acting at a population (or a group) scale but yet it acts on individuals. If an individual produces more offspring and if this trait is genetically encoded, then this character will spread within the population even if it is deleterious for the whole population. From this verbal demonstration, Lack established a new theory (and specifies that most bird species have a constant clutch size no matter the environmental conditions). He proposed this limitation as a positive act (*i.e.*, the cessation of laying) contrary to an incurred operation (*i.e.*, the inability to produce more eggs). In particular, he argued that producing too many eggs could be disadvantageous for parents. Hence, the clutch size would reflect the maximum number of chicks that parents can raise without risking the loss of one or several offspring, and would be season- and region-dependent. Natural selection action under this hypothesis is much clearer : birds with large brood would be favored (genes coding for a vast clutch size should rapidly increase in the population) but birds with a too large brood would be selected against (in order to avoid losing the whole clutch). In '*The significance of the clutch size*', Lack does not propose an explanatory mechanism for this phenomenon but has various intuitions.

First, clutch size can not increase indefinitely because parents would not be able to

feed chicks properly. This particular argument is pivotal. He introduced the core idea that a trait (here, fecundity) can not vary without consequences on other traits (here, the quality and survival of offspring). This balance effect is now called a trade-off (a term that Lack did not use in 1947) and constitutes the main topic of my PhD. Variations in one trait should not be considered independently of variations in others (Houle *et al.*, 1996) because genetic or physiological relationships (like trade-offs) are link variation across traits. Trade-offs are defined by Stearns in 1989 as "the cost paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another". One of the simplest mechanisms for explaining trade-offs is the differential allocation of resources. This mechanism is the one intuitively seen by Lack. The main idea is that every organisms has access to a finite amount of energy and has to allocate this energy in a manner that maximizes both survival and reproduction. The partitioning of energy between two competing traits reflects a compromise.

Second, the clutch/litter size can depend on the physical condition of the mother (Plard *et al.*, 2015). We easily understand that a mother in good condition can raise more offsprings because she can offer better parental care or produce better gametes. Hence, body mass is often linked with the quality of an individual and its ability to acquire a lot of resources. Acquisition also represents an essential parameter in the study of trade-offs.

After the work of Lack, many studies investigated in trade-offs. Some have focused on the detection of trade-offs in various species (**mammals**: Derting & McClure (1989); Rutkowska *et al.* (2011); Oksanen *et al.* (2002); Charnov & Ernest (2006); **birds**: Smith *et al.* (1989); Ketterson & Nolan Jr (1992); Ghilambor & Martin (2001); Brzęk & Konarzewski (2007); Soler *et al.* (2003); **insects**: Cotter *et al.* (2008); Flatt & Kawecki (2007); Desouhant *et al.* (2005); Czesak & Fox (2003); Khokhlova *et al.* (2014); **fishes**: Jonsson & Jonsson (1999); Fleming & Gross (1990); Gunderson (1997); Abrahams (1993); **reptiles and amphibians**: Uller & Olsson (2005); Massot *et al.* (2011); Rivalan *et al.* (2005); Camargo *et al.* (2008); Sinervo & Licht (1991)). Others have sought to identify negative relationships between traits that had not been considered before (Gustafsson *et al.*, 1995; Siikamäki *et al.*, 1994). This variety will be addressed in the section Trade-offs : debate and underlying mechanisms. In a second step, evolutionary biologists sought to understand more precisely how trade-offs emerged and to verify the underlying hypotheses (Roff, 1990; Flatt *et al.*, 2005; Zera & Zhao, 2006; Flatt & Heyland, 2011). My thesis work is a continuation of these studies. I focused on understanding the evolution

of trade-offs in multicellular organisms. This question has been rarely considered for a variety of reasons. First, a fairly old literature considered trade-offs as resulting from physico-chemical constraints only, and therefore immutable (Tuomi *et al.*, 1983). In addition, the term trade-off is defined in a very broad way that generates a blur around what this concept represents (discussed in the Part 2). Finally, trade-offs may have emerged at various scales and some authors have attempted to categorize them. However, I think it is necessary to study them in an integrative way (by considering the underlying mechanisms) and I detail this opinion in the third part of this introduction. In the next part (I), I will describe important evolutionary processes and the different scales interacting during these processes. In the second part, I will detail mechanisms responsible for the emergence of trade-offs with theoretical explanations and empirical examples. Finally, in the last part, I will present the few studies where the evolution of trade-offs is envisaged as well as a theoretical framework to study this question.

The Genotype-Phenotype map: description and evolutionary consequences

To predict evolution, one must know which individuals are favored in a given environment. However, the emergence of new characteristics and their selection do not work at the same level. Hence, evolution is the results of 2 processes: **mutation** (*i.e.*, formation of new genotypes – at the genetic level) and **selection** (*i.e.*, phenotypes selected depending on the encountered environment and the selective pressure – at the phenotypic level) (McCandlish & Stoltzfus, 2014). One perspective of evolutionary study has been to look at the link between genotype and fitness. In the 1930s Sewall Wright realized the multidimensional nature of such a work of predicting adaptation. Indeed, it seems very ambitious to imagine a space containing all the possible allelic combinations for each gene and the associated fitness value. To overcome this complexity, Wright suggested an abstract continuous representation of fitness variations in the form of a landscape. The first illustration in two dimensions (figure 1.1) is a topography such as that found on hiking maps. Lines of variations (dotted lines) of altitude are represented and let us imagine a surface like mountains. In his graphical model, these variations of altitude symbolize fitness variations. Peaks are associated with high values of fitness and brutal changes of altitude like a steep mountain. Conversely, valleys represent areas associated with low fitness values. In the representation of Wright’s adaptive landscape in 1932, the x and y axes are not labeled and are not clearly defined, probably because it is impossible to elaborate a sufficiently precise unit to describe such a multidimensional object summarized in two dimensions (Dietrich & Skipper Jr, 2012). Although the axes have a symbolical representation, positions within the graph do have a rough biological meaning. Indeed, each dot would correspond to a genotype and euclidean distance between two dots would be proportional to the number of mutations separating these two genotypes. Hence, via mutation and fixation processes, genotypes can move in the landscape and thanks to selection these moves are ‘guided’ towards fitness peaks.

Variants associated with low fitness values and represented in fitness valleys are elim-

inated by selection. After this major contribution, several authors re-used (and sometimes modified) this illustrative tool (Dobzhansky *et al.*, 1937; Simpson, 1944; Lewontin & Kojima, 1960; Lewontin & White, 1960) and even today, this metaphor is used as a conceptual backdrop (Tenaillon *et al.*, 1999; Martin & Lenormand, 2006; Mills & Watson, 2006; Manna *et al.*, 2011; Ferretti *et al.*, 2016; Bajić *et al.*, 2018; Fragata *et al.*, 2018). The fitness landscape has thus largely contributed to a better understanding of the mechanisms of evolution. Nevertheless, fitness landscape studies omit an important part of the biological reality: phenotypes. Indeed, in some cases it may be relevant to be interested in fitness changes associated with mutations but in fluctuating environments for instance, the same mutation may have different effects on fitness in relation to phenotype performance in the environment considered. Thus, other approaches are interested in the link that exists between genotype and phenotype and try to establish the map of these relations, the so-called Genotype- Phenotype map (GP-map, hereafter). The following section will focus on this topic: I will begin by describing what is the GP-map (the elements that structure it) but also the evolutionary consequences associated.

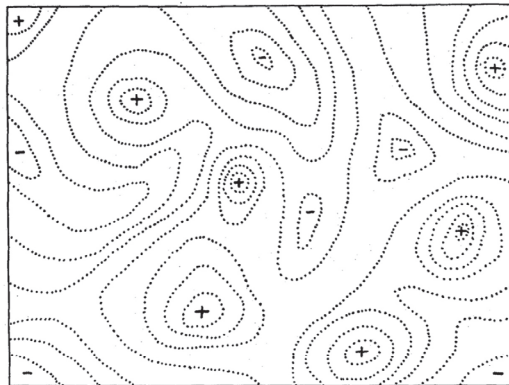


Figure 1.1: Adaptive landscape diagram from Wright (1932). First representation of this tool, where dotted lines represent variation in altitude (*i.e.*, fitness). Axis are not clearly described in the seminal paper of Wright but should correspond to summary units symbolizing a multidimensional object.

1.1 Essential notions structuring GP-map studies

Schlosser & Wagner (2004) define the Genotype-Phenotype map as "*the relationship between genetic variation and phenotypic variation ; that is ; it specifies which genetic differences give rise to which phenotypic differences*". The main objective is to build a map containing the effect of each possible mutation on each trait. First, I will focus on mutations and the relationship between genotype and a trait. Then, I will present the

processes involved on a multitrait scale and I will list different methods to build parts of the GP-map.

1.1.1 Genotype spaces in relation to phenotype

The production of new genotypes in a population at generation $t + 1$ depends on genotypes present in the population at generation t . For example, a genotype far removed from those present in the population has no chance of being generated at the next generation. Conversely, a genotype close to one of those present in the population has a high probability of being part of the next generation. Hence, the array of genotypes at a particular time constrains the genotypes that can be produced in the next generation by mutation. A very useful tool to consider which variations are likely to emerge, is the concept of genotype space. The genotype space is a representative tool used to symbolise genotype networks (figure 1.2). It is composed of nodes and connections between them. Each node is a genotype and each connection is a mutation or a recombination event (Smith, 1970; Draghi *et al.*, 2010; Rajon & Masel, 2013). The genotype space includes all possible genotypes as well as all combinations of connections between them. Hence, the whole genotype space of a population represents an enormous complex network and multi-dimensional object. The size of such an object can be tricky to define but studying the evolution of a sub-unit of this object (like a transcription- factor binding site) represents an achievable challenge. Jörg *et al.* (2008) determined the size of a neutral space (*i.e.*, a subset of the genotype space containing all genotypes coding for a similar phenotype, Masel & Trotter (2010)). They estimated the number of genotypes expressing a single phenotype around 1021 (Jörg *et al.*, 2008), which, if extrapolated to an entire organism, represents a staggering number of genotypes.

At any point in time, a population occupies a subset of the whole genotype space. Evolution occurs through movements in this space, due to mutations and recombination events, and thus, population can reach new regions of the genotype space. In direct contact with the cloud of points formed by the population, there are other genotypes accessible with a unique event. This closer sub-unit of genotypes constitutes the mutational neighborhood of the population (see figure 1.2).

Depending on different parameters (such as genetic variation, recombination, mutation rate, defined in the next section), a population has a larger or smaller mutational neighborhood (Draghi *et al.*, 2010). Rapid access (*i.e.*, few mutation or recombination events)

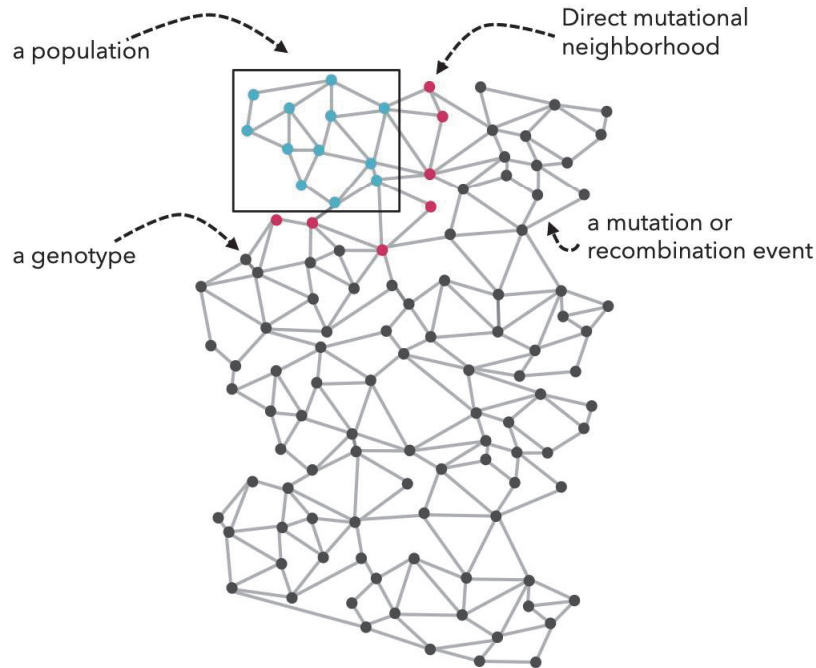


Figure 1.2: A genotype space representation. Each node corresponds to a genotype and connections between them are mutations or recombination events. Blue dots represent a population occupying a subset of the whole genotype space.

to new genotypes partly determines the **evolvability** of a population. Evolvability can be defined "*as the capacity of a population to produce heritable phenotypic variation of a kind that is not unconditionally deleterious*" (Masel & Bergman, 2003; Masel, 2006; Masel & Trotter, 2010). Hence, in a population with high evolvability, evolution can proceed rapidly and adaptation is favored (Wichman *et al.*, 1999). However, responding quickly at the phenotypic level to mutational events may, in theory, represents a disadvantage. In a stable environment, an individual producing offspring with mutations would probably not exhibit high fitness (the majority of mutations being deleterious; Houle *et al.* (1994)). Moreover, an evolutionary mechanism exists to resist to these disturbances, termed **robustness**. Schlosser & Wagner (2004) describe a biological system as robust if it continues to work in the face of perturbations. Perturbations can be of different natures : genetic changes, noise or environmental changes (Schlosser & Wagner, 2004).

Robustness and evolvability therefore seem to be two opposite situations: one limiting the impact of mutations on phenotypes while the other maximizing them. Paradoxically, these mechanisms act together: robustness can favor evolvability (Wagner *et al.*, 2007). To understand this paradox, it is necessary to consider separately mutational robustness and environmental robustness. An organism can be robust by expressing the same phenotype

despite mutations – *i.e.*, mutational robustness (Montville *et al.*, 2005) – but it can also be robust if the phenotype remains stable in the face of environmental variations – *i.e.*, environmental robustness (De Visser *et al.*, 2003)–. In our case, we are interested in the mutational robustness.

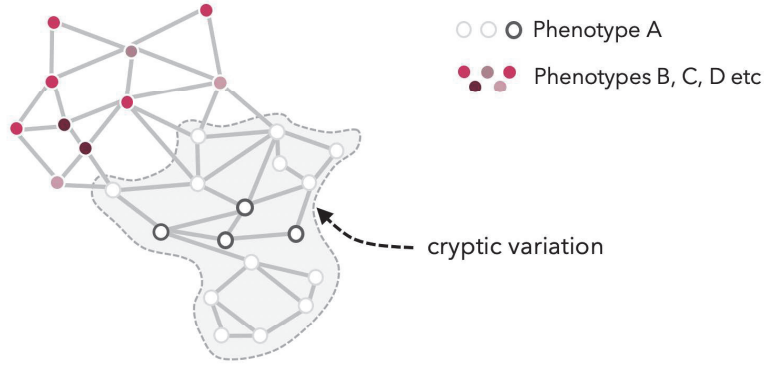


Figure 1.3: Subset of a genotype space hiding genetic variation. White nodes correspond to genotypes expressing the same phenotype. White nodes surrounding by black are genotypes forming a population. Pink nodes are genotypes producing new phenotypes, which could represent a selective advantage (or not). Grey lines are mutations between genotypes. The population can move in a neutral space without modifying the phenotype. Hence, it reveals the robustness of genotypes to mutation, until population reaches pink genotypes, which leads to new phenotypes. The robustness was hiding genetic variation that is called in this particular case "cryptic variation". All of these mutations and genotype spaces are hypothetical.

Neutral spaces mentioned previously are linked with mutational robustness because by definition, a population under mutation effects moves in a neutral space in the case of mutational robustness. These moves do not have consequences on expressed phenotypes but allow the population to reach new sub-genotype spaces with future potential impact on phenotype (and thus innovations). In this context, genetic variation is present, although hidden (*i.e.*, called cryptic variation), and allows neutral evolution to act. An example of cryptic variation is illustrated in figure 1.3, where a (very small) population of 4 different genotypes producing the same phenotype can move in a neutral space without causing change in the phenotype expressed (– white dots in the figure 1.3 –, the population is robust to mutation) but over mutations the population moves and can come closer to genotypes leading to different phenotypes (– pink dots in the figure 1.3 –, the population is evolvable). However, the relationship between evolvability and robustness can reveal to be more complex, as shown in Draghi *et al.* (2010). We could expect a monotonic relationship between evolvability and robustness but it appears that an intermediary of robustness is optimal to maximize evolvability. Indeed, in a population highly robust, almost no mutations have consequences on the phenotype and adaptation is very slow.

Otherwise, in a population with weak robustness, most mutations are deleterious and the population does not contain any "adaptable" individuals which also leads to slower adaptation (Draghi *et al.*, 2010).

Another process can act at the genotypic level and affect a trait: **epistasis**. Epistasis is described as the situation in which the actions of alleles at one locus mask the allelic effects of another locus, in the same way that a completely dominant allele masks the effects of a recessive allele at the same locus (Carlborg & Haley, 2004). A classical example of such situation is found in the pig (illustrated in figure 1.4) between loci *KIT* and *MC1R*. If an individual is heterozygous or homozygous for the dominant allele at the *KIT* locus then allelic variations on the *MC1R* loci will not cause any changes in color: all pigs will be pink/white. However, if the genotype at the *KIT* locus is homozygous recessive, then 2 other phenotypes can emerge: the pigs can be brown or black depending on the alleles of the *MC1R* locus.

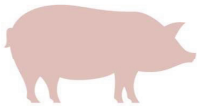
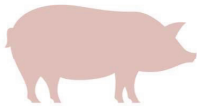
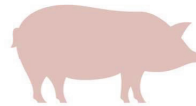
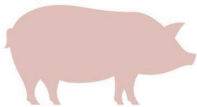
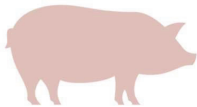
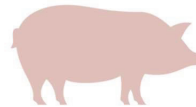
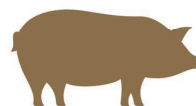
Locus <i>KIT</i>	II			
	li			
	ii			
		EE	Ee	ee
		Locus <i>MC1R</i>		

Figure 1.4: Empirical example of epistasis in pig colour. The gene *KIT* hides the effect of the gene *MC1R* on the expression of pig colour. When the dominant allele I of *KIT* is expressed, the pig is white/pink no matter the allele on *MC1R* loci. On the contrary, when the pig is homozygous recessive (ii), the effect of *MC1R* is revealed and the pig can be black (EE, Ee) or brown (ee). Hence, the gene *KIT* is epistatic to the *MC1R* locus. This example is extracted from Carlborg & Haley (2004).

1.1.2 Multitrait processes involved in the GP-map: pleiotropy and modularity

Some genes have simple effects on the phenotype : they regulate only one trait. On the contrary, other genes can control several traits. For instance the gene *Pax6*, which is found in *Drosophila melanogaster* and other invertebrates, produces a transcription factor essential for cell proliferation, migration, and adhesion. Hence, the development of many organs like the eyes, central nervous system and endocrine glands depends upon it (Simpson & Price, 2002). Another example is the albino locus in *Mus musculus*. Mutant mice do not produce *tyrosinase* (*i.e.*, enzyme necessary for the biosynthesis of melanin) and thus express a white phenotype. This mutation also impacts the production of brain catecholamines (*i.e.*, organic compounds playing the role of neurotransmitter) and have behavioral consequences. Mutant mice are less active, slower to escape from water and worse at white-black discrimination than pigmented counterparts (Fuller, 1967).

These genes affecting multiple traits are called pleiotropic (Zhang & Wagner, 2013). Different empirical methods exist to detect pleiotropic genes, such as simple genetic crosses monitoring a few traits, QTL analysis¹ (*i.e.*, a method using random markers to identify portions of the genome affecting a focal trait) or genome-wide association studies (*i.e.*, used preliminary to QTL studies) (Schlosser & Wagner, 2004; Paaby & Rockman, 2013). Pleiotropy is a widespread phenomenon and is particularly well-studied in evolutionary biology both theoretically (Williams, 1957; Cheverud, 1996) and empirically (Dudley *et al.*, 2005; Ohya *et al.*, 2005; Wagner *et al.*, 2008; Albert *et al.*, 2008; Wagner & Zhang, 2011). The concept of pleiotropy has a very broad definition and it is sometimes used to talk about different things. It depends mainly on what is considered as a "trait", which has to be specified (Paaby & Rockman, 2013). The generality of the pleiotropy concept has been questioned, in particular, after the development of the universal theory of pleiotropy. Universal pleiotropy is defined as the fact that each gene can impact (via mutation) each trait of an organism directly or indirectly (Stearns, 2010; Orr, 2000; Fisher, 1930b). The

¹Quantitative trait loci (QTL) analysis : individual genomes of F2 offspring (classically obtained by inter-crossing an homogeneous F1 originated from crossing two inbred lines), are partially sequenced (length of genome portions considered varies across studies) and measures on their phenotypes are collected. Then, correlations between molecular markers and phenotype data are realized. If an important correlation is detected, then a QTL presence is assessed on the specific section of the genome. Thus, it is possible to identify the genetic architecture on a trait, which means the number of genome regions involved in the expression of the trait. In order to increase the efficiency of a QTL analysis, a complementary technique can be used before and it is called GWAS (Genome wide association studies) (Korte & Farlow, 2013).

hypothesis of universal pleiotropy implies a very difficult adaptation, it would necessitate finding the perfect mutation allowing to modify the whole phenotype in the good way to live in the environment encountered. As Stearns (2010) says, the debate around universal pleiotropy should not revolve around a binary answer: yes, pleiotropy is universal versus no it is not. But studies should rather identify in which context and to what degree the pleiotropy is universal. Indeed, more recent studies showed that pleiotropic genes rarely act on a plethora of traits (Wagner & Zhang, 2011) and pleiotropic genes only represent a small proportion of the totality of genes (Wang *et al.*, 2010).

A direct consequence of pleiotropy on the genetic architecture of traits is called **modularity**. This property means that a set of traits is coordinated by a set of genes forming a module (as illustrated in figure 1.5). Modules are detected as subset of genes with more frequent interactions with a group of traits. Thus, modules can have different levels of independence: some genes can regulate traits not integrated in the module, and some traits of the module can be controlled by genes out of it. The differentiation between modules is due to a maximal integration between genes and traits inside a module but a maximal difference between traits and genes involved in other modules (implying that interactions inter-module should stay minimal). The modular characteristic of the GP-map is essential because it plays a role in evolution. Indeed, traits regulated by genes inside a same module do not evolve independently. They react to selection as a unit (Magwene, 2001). On the contrary, independence maintained between modules implies an independent evolution between traits that comprise the module (Schlosser & Wagner, 2004). As demonstrated by Fisher (1930b), small mutations constitute the genetic basis for adaptation (Orr, 2005) and a restrained pleiotropy combined with a strong modularity should evolve because it increases the probability to find optimal mutations maximizing fitness. Hence, if we follow the metaphor of Fisher about the microscope (where the goal is to perfectly tune it with different setting wheels), universal pleiotropy would mean turning all the wheels randomly at each try which makes it almost impossible to find the perfect setting. While if setting wheels (pleiotropic genes) are grouped in small sets (modules) related with a particular microscope function (traits) such as the eyepiece, objectives or the diaphragm then after a few tests it is possible to find an optimal setting.

The modular nature of the GP-map contradicts the theory of pleiotropy stated decades ago. Under the universal pleiotropy hypothesis, how to explain module detection ? Different authors argue that modularity takes precedence over universal pleiotropy (Wagner *et al.*, 2008; Su *et al.*, 2010) while others attempt to demonstrate the plausibility of this

hypothesis empirically (Knight *et al.*, 2006). Nevertheless, modular pleiotropy allows organisms to respond to mutation more quickly and in a less targeted way. Thus, despite the constraints that can be generated by such structuring, it can potentially promote adaption more quickly. In any case, to study these questions it is necessary to consider the effects of mutations to correctly understand the moves they provoke in the genotype space. For these reasons, my theoretical thesis work has been based on developments that make it possible to realistically take into account moves in the genotype space as well as the consequences generated on the phenotype.

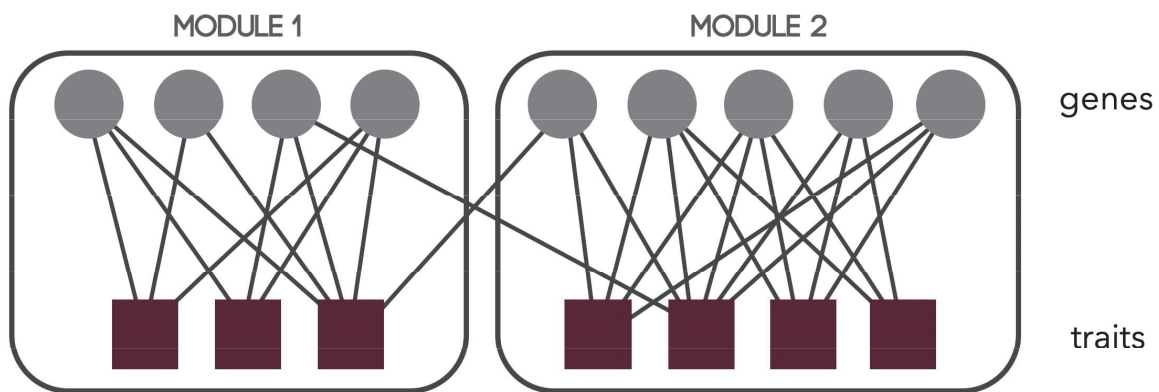


Figure 1.5: The modularity of the GP-map. A grey circle is a gene node and a purple square is a trait node. If a circle and a square are linked with a line, then the gene is implied in the expression of the trait. A set of genes can be involved in the expression of a set of trait. This particular combination is called modular pleiotropy and is represented with 2 separated modules (1 and 2). This illustration is reproduced from (Wagner & Zhang, 2011).

1.1.3 Examples of GP-map constructions

One must take into account that the genotype space explicitly in order to identify major processes that drive evolution but not to identify how genetic variation becomes phenotypic variance. Precise quantification of gene effects on traits at the whole genome scale is, at least, very complex. Indeed, the GP-map is complicated to decipher in living organisms but empirical studies on this question reveal interesting insights on possible arrangements of the GP-map.

The GP-map can be studied at different scales: from protein structure to morphological traits. Some computational studies have focused on a very fine scale in studying the consequences of RNA sequence mutations on the structure of the protein produced. In particular, (Stadler & Stadler, 2006) showed that genetic variation does not lead systematically to variation in expressed protein structure. Indeed, there is always more nucleic

sequences than protein shapes, which is an intuitive result since a part of the mutations is known to be neutral. Bloom *et al.* (2005) demonstrated that with the increase in the number of substitutions, the probability that a protein preserves its structure decreases exponentially. These theoretical results confirm empirical results on the *subtilisin* protease (Shafikhani *et al.*, 1997). Bloom *et al.* (2005) also showed that proteins can have higher mutational robustness if their thermodynamic stability is increased (this stability may be dependent on temperature, pressure or pH).

Studies using QTL analysis have made it possible to determine the genetic architecture of morphological traits. Schlosser & Wagner (2004) compared various QTL studies on morphological traits in mice and noted that traits physiologically related tend to be regulated by the same groups of gene, while they remain disconnected from genes impacting other traits. These results confirm the modular nature of the GP-map (at least for these traits in mice). Nevertheless, authors point out that modularity is not absolute since some genes detected can also impact the entire development of the morphological structure.

On an even broader scale, studies comparing wild type and mutant phenotypes have shown the potent pleiotropy of certain genes such as the impact of homeobox genes on development in *D. melanogaster* (Gehring & Hiromi, 1986). An inter-species study demonstrated that pleiotropy is found in a wide range of organisms and acts similarly in these species (i.e., one gene affecting few traits whereas a very few number of genes control a lot of traits) (Wang *et al.*, 2010). They also proved that the modularity detected in these species is highly higher than found by chance.

1.2 Reality is even more complex

Evolution is a process acting at two different scales: mutation (genotypic level) and selection (fitness level). However, between these scales is the phenotypic level (represented in figure 1.6). Hence, the link between genotype and phenotype constitutes an important part of the global process of evolution. The combinend use of the GP-map and adaptive landscape studies (which appear to be complementary) enhances our general understanding of evolution. Nevertheless, despite these degrees of complexity, several major factors have been completely ignored: the interaction with the environment (biotic or abiotic) or intermediary scales (like physiological level). As described by quantitative genetics, the phenotype is the result of the effect of the genotype, the environment and their interaction

(Lynch *et al.*, 1998). The environmental effect is not taken into account in classical GP-map studies while it is known to have consequences on the expression of the phenotype (Gillespie & Turelli, 1989). For instance, a change in temperature or photoperiod induces significant modifications of phenotypes in many species (**mammals**: Reilly *et al.* (2006); Zimova *et al.* (2014); **amphibians**: Denver (1997); **birds**: McKechnie *et al.* (2006); **insects**: Whitman (2009); **fish**: Johnston (1993)). The ability for a genotype to express different phenotypes in different environments is called plasticity (West-Eberhard, 1989). Sometimes it implies a change in feeding behavior in presence of parasites, or a change in the reproductive behavior in a context of high predation pressure (Chivers *et al.*, 1999). These modifications have consequences on fitness and play an important role in evolution (Relyea, 2002). Recent studies show that some species are able to modify their direct environment by producing useful metabolites. Hence, an eco-evolutionary feedback can even arise and influence the genotype in return.

‘Internal’ environment can also affect the phenotypes of organisms. All eucaryotes live in symbiosis with each other: a lichen is an association between a unicellular algae and a fungus, *Wolbachia* is an intracellular bacterium of many insects, humans live with over 1000 bacteria in their digestive system like *Escherichia coli*, etc. Symbiosis can influence host behavior with a possible impact on its reproduction (Johanowicz & Hoy, 1998; Engelstädter & Hurst, 2009), such that the genotype of the host depends on its own genotype as well as genotypes of its symbionts. These relationships could be considered as extended phenotype (*i.e.*, effects of genes at a higher level than the population level) (Dawkins *et al.*, 1982).

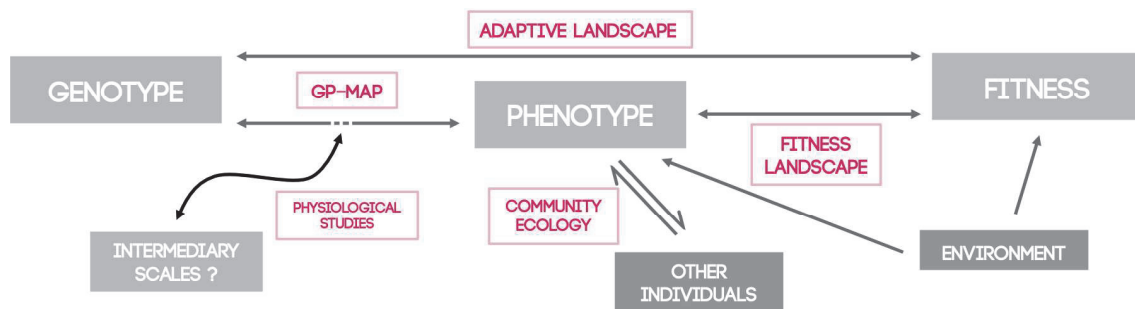


Figure 1.6: Schema of relationship between units (grey boxes) and their associated type of studies (pink boxes). The study of evolution requires an understanding of the interactions between these different levels. The levels represented here as well as the type of associated studies are obviously not exhaustive. The main objective is to illustrate schematically the complexity and diversity of levels that ideally should be taken into account to better understand the evolution of phenotypic traits relationships.

Fields of study (such as quantitative genetics, life history theory, demography and so on) are built around a specific theme and from their own hypothesis. Indeed, other concepts from other fields are rarely integrated into the development of theories. However, nowadays we know these different units and the interactions that bind them, so it seems essential to multiply transverse studies and to create theoretical bridges between disciplines in order to more effectively achieve a global understanding of evolutionary mechanisms.

Trade-offs : debate and underlying mechanisms

2.1 Universality and diversity of measurements

For Stearns (1989), trade offs represent the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another. In this definition, it is assumed that an underlying mechanism causes an internal and individual compromise. The expression of this internal compromise is made visible when, in a population, there is inter-individual variance in the expression of compromise (*i.e.*, individuals do not have the same allocation strategy). In this situation, a negative relationship between traits can then be observed and the detection of these relationships is the preferred tool for highlighting individual internal compromise. However through misnomer, the term trade-off is used to designate two phenomena: the internal compromise but also the negative relationship that emerges from this compromise at the population level (Roff & Fairbairn, 2007). In the following pages, when I refer to the negative relationship between traits I will use the word trade-off, however I will use the term compromise to talk about the individual scale.

Even if trade-offs have mostly been detected in the context of the cost of reproduction (Gadgil & Bossert, 1970; Bell, 1980; Schaffer, 1974), the definition given offers a larger range of possible traits or levels. Hence, trade-offs are observed at different scales like represented in figure 2.1. These different levels of measurement are described below. Moreover, the existence of trade-offs can often be anticipated from first principles. These two characteristics (the broad definition and different levels where they are detected) make trade-offs universal in theory but not, as we will see, in practice.

A first level where trade-offs can be observed is between species (inter-specific scale). Inter-species studies are particular in that they need to focus only on "global" traits shared by numerous species. Mean phenotypic traits values for each species are used, and a least-square regression is fitted to expose the trade-off. In these studies, they do not focus

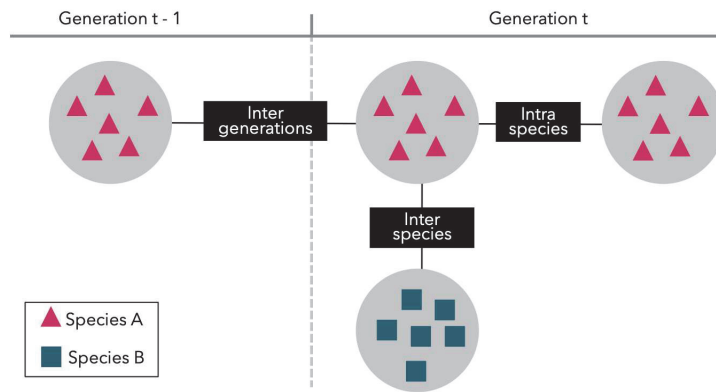


Figure 2.1: An illustrative representation of the diversity of observation levels. Each grey circle represents a population. These populations are made of individuals (triangles for species A and squares for species B). Trade-offs can be observed between: generations (inter-generations), species (inter-species), population of a same species (intra-species).

on intra-specific variance. These methods have revealed general patterns of life-history strategies, such as the slow-fast continuum (*i.e.*, an hypothesis explaining differences in life span across mammals related with demographic rate) (Gaillard *et al.*, 1989; Dobson & Oli, 2007; Jones *et al.*, 2008; Ricklefs, 2010; Salguero-Gómez *et al.*, 2016). Indeed, a negative relationship between longevity and fecundity is found in many species (Read & Harvey, 1989) (represented figure 2.2) even after body size correction¹ (Reznick, 1985; Jeschke & Kokko, 2009). In these studies, it was assumed that variation in acquisition intra-species is negligible compared to inter-species variations. The explanatory factor often proposed is that extrinsic mortality rate is a major shaper of life-history strategies. Indeed, if an individual faces a huge risk of dying quickly (as a mouse), natural selection should favor those which are able to rapidly reproduce responding to the adage: "*living fast and dying young*" (Promislow & Harvey, 1990). On the other hand, in the case of a weak extrinsic mortality (for example in the case of a large vertebrate such as a bear), organisms invest mainly in their survival and tend to produce fewer offspring at each breeding season (on a longer reproductive period). They produce more developed juveniles and can concentrate their parental care on it and thus maximize offspring survival.

Trade-offs are also observed among individuals in the same species (intra-specific scale). These trade-offs are observed when comparing differences in phenotypic traits between individuals of a same population (and sometimes between populations). As presented in Table 2.4 (not exhaustively), this level of detection is widespread. To return to the example of clutch size, a trade-off that is often studied in an intra-specific context is

¹Body weight is also considered as a principal determinant of life history traits. Obviously, being big or small have consequences on life of individuals (Lebreton, 1981) but other factors play an important role in life history strategies (Gaillard *et al.*, 1989) like described here.

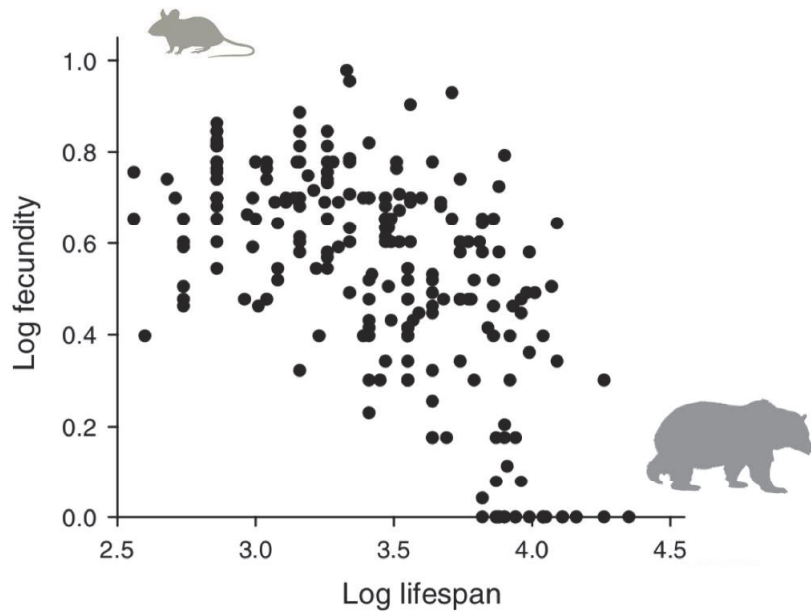


Figure 2.2: The trade-off between fecundity (number of offspring per litter) and maximum lifespan (in days) in mammals native to Europe or North America. This figure is extracted from Jeschke & Kokko (2009) where marsupials and bats are excluded remaining 475 mammal species.

that between quality and quantity of the offspring (Smith *et al.*, 1989; Sinervo & Licht, 1991; Merilä & Wiggins, 1995; Oksanen *et al.*, 2002; Czesak & Fox, 2003; Mappes *et al.*, 2004; Sanchez-Lafuente, 2004; Gibbs *et al.*, 2005; Uller & Olsson, 2005; Charnov & Ernest, 2006; Camargo *et al.*, 2008; Gillespie *et al.*, 2008; Walker *et al.*, 2008; Khokhlova *et al.*, 2014). A typical explanation suggests that individuals have a finite amount of energy to allocate between two traits. They can not produce an unlimited number of offspring and provide sufficient parental care (*i.e.*, parental investment in time and energy to their offspring) to them. Then, a continuum of combinations between these traits is possible. They can give birth to a single offspring and provide extensive parental care to it (purple dot on the left extreme of the trade-off figure 2.3). Or, they can produce a lot of offspring limiting parental care to them and therefore taking the risk of losing a significant proportion (blue dot on the right extreme figure 2.3). The study of intra-specific trade-offs is used to determine which traits are negatively related in a given species. This can later allow one to compare the shape of trade-offs between different species and can highlight different constraints emerging from phylogeny or environment.

A novel observation level of trade-off has been investigated recently. The idea of inter-generational trade-off emerges to answer to *a priori* unexplained observations. A case in study is *Caenorhabditis elegans*, in which some mutant individuals are able to escape from the established trade-off between fecundity and longevity (Dillin *et al.*, 2002;

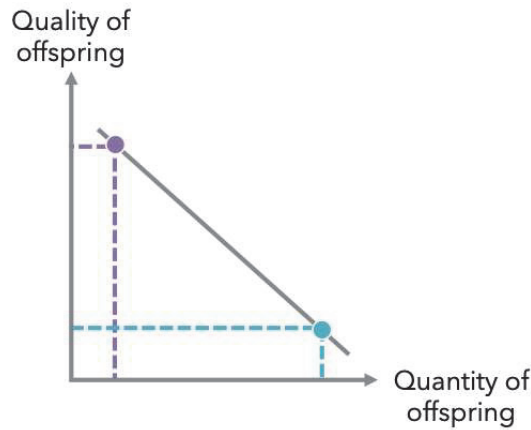


Figure 2.3: An illustrative representation of a linear trade-off between quantity and quality of offspring. Two individual's positions are represented with different colors of circle: in purple, an individual with an extreme strategy (*i.e.*, a large amount of its energy is allocated to the quality of offspring at the expense of the quantity), in blue an opposite extreme strategy (*i.e.*, an inverted investment with the production of a large litter of probably "bad" quality offspring).

Kenyon, 2005) (similar results have been found in *Drosophila* INDY mutant by Rogina *et al.* (2000)). Such mutants live longer than their congeners and seem not to pay any cost on other traits like reproduction. Naturally, we can ask ourselves why this genotype is not fixed in population as it obviously increases fitness. A possible explanation for these observations is that offspring of individuals expressing "better" trait values are probably paying a cost on their own traits (quality) suggesting an inter- generational trade-off. However, this apparent question may be a consequence of the choice for the trait considered. It could be possible to find costs if realized fecundity was measured (Murua *et al.*, 2003). Nevertheless, these studies seek to find trade-offs beyond the life of individuals (by focusing on the impact of individual performances on the next generations) when trade-offs are not "detected" during the life of individuals. These results could be explained by the fact that these mutants own a selective advantage and thus establish on a new trade-off. After invasion of this mutation in the population, it could be possible to observe a new negative relationship.

At each level of measure, a novel term has been associated to trade-off (like intra-specific or intergenerational), which contributes to produce confusion around it. Diversity of trade-off detection originates from the ambiguity of the word trade-off itself because it encompasses both a cause and a consequence (Coste, 2017). Indeed, we classically use the term of trade-off to designate the compromise that an individual faces when it allocates its own energy between functions (the cause) but we also use this word to talk about the negative correlation in a population emerging from individual's differential alloca-

tions (the consequence) (Roff & Fairbairn, 2007). This leads to some misunderstanding regarding the existence of trade-offs in some contexts. Moreover, the observation of trade-offs is often completely dissociated from the study of the underlying mechanisms that regulate it and the evolutionary mechanisms that can act on it. Indeed, the negative relationship between traits emerges if there is genetic variance and therefore depends on the genotypes present in the population. Movements on the trade-off depend on whether the position is heritable or not, which will depend on the mutations that are maintained in the population or whether the individuals are sensitive to environmental noise. The genetic variance present in the population as well as the genotype space it occupies constitutes key elements for understanding trade-offs. Indeed, it seems necessary to understand how the relationships between genotypes and phenotypes (*i.e.*; the GP-map) evolve to understand the functioning of the trade-offs.

Trade-offs	Taxa	Species	Traits	Level of observation	References
Quantity - quality of offspring	Reptiles and amphibians	<i>Uta stansburiana</i>	Phenotypic	intra	Sinervo & Licht (1991)
		<i>Lacerta vivipara</i>	Phenotypic	inter and intra	Uller & Olsson (2005)
		<i>Physalaemus biligonigerus</i>	Phenotypic	intra	Camargo et al. (2008)
	Birds	<i>Parus major</i>	Phenotypic	intra	Smith et al. (1989)
		<i>Parus caeruleus</i>	Phenotypic	intra	Merilä & Wiggins (1995)
		<i>Porphyrio porphyrio</i>	Phenotypic	intra	Sanchez-Lafuente (2004)
	Insects	<i>Pararge aegeria</i>	Phenotypic	intra between male and female	Gibbs et al. (2005)
		<i>Parapulex cheprenis</i> <i>Xenopsylla ramesis</i>	Phenotypic	intra	Khokhlova et al. (2014)
		<i>Stator limbatus</i>	Phenotypic	Intra	Czesak & Fox (2003)
	Mammals	<i>Homo sapiens (pre industrial)</i>	Phenotypic	inter and intra	Walker et al. (2008) ; Gillespie et al. (2008)
		532 species of eutherians mammals	Phenotypic	inter	Charnov & Ernest (2006)
		<i>Clethrionomys glareolus</i>	Phenotypic	intra	Oksanen et al. (2002);
	Fish	<i>Oncorhynchus kisutch</i>	Phenotypic	intra	Fleming & Gross (1990)
		<i>Salmo trutta</i>	Phenotypic	intra	Jonsson & Jonsson (1999)
Current - future reproduction	Reptiles and amphibians	<i>Dermochelys coriacea</i>	Phenotypic	Intra	Rivalan et al. (2005)
	Birds	<i>Junco hyemalis</i>	Phenotypic	Intra	Ketterson & Nolan Jr (1992)
		<i>Fulica atra</i>	Phenotypic	Intra	Brinkhof et al. (2002)
	Insects	<i>Venturia canescens</i>	Phenotypic	Intra	Desouhant et al. (2005)
	Mammals	<i>Myodes glareolus</i>	Phenotypic	Intra	Rutkowska et al. (2011)

Trade-offs	Taxa	Species	Traits	Level of observation	References
Longevity-reproduction	Reptiles and amphibians	<i>Lacerta vivipara</i> ,	Phenotypic	Intra	Massot et al. (2011)
	Birds	<i>Empidonax occidentalis</i> , <i>Turdus migratorius</i> , <i>Troglodytes aedon</i> , <i>Junco hyemalis</i> , <i>Vermivora celata</i>	Phenotypic	Inter	Ghalambor & Martin (2001)
	Insects	<i>Drosophila melanogaster</i>	Phenotypic	Intra	Flatt & Kawecki (2007)
		<i>Anopheles gambiae</i>	Phenotypic	Intra	Dao et al. (2010)
	Mammals	<i>Eutherian mammals</i>	Phenotypic	Inter	Holliday (1994)
	Fish	<i>Poecilia reticulata</i>	Phenotypic	Intra	Abrahams (1993)
		<i>viviparous and oviparous fishes</i>	Phenotypic	Inter	Gunderson (1997)
Immunity - Growth (maintenance)	Reptiles and amphibians	<i>Ctenophorus fordii</i>	Phenotypic (immune activation)	Intra	Uller & Olsson (2005)
		<i>Nerodia rhombifer</i>	Phenotypic	Intra	Korfei et al. (2015)*
	Birds	<i>Pica pica</i>	Phenotypic (immune activation)	Intra	Soler et al. (2003)
		<i>Oceanodroma leucorhoa</i>	Phenotypic	Intra	van der Most et al. (2011)
		<i>Riparia riparia</i>	Phenotypic (immune activation)	Intra	Brzęk & Konarzewski (2007)
	Insects	<i>Spodoptera littoralis</i>	Phenotypic (immune activation)	Intra	Cotter et al. (2008)
		<i>Gryllus bimaculatus</i>	Phenotypic	Intra	Rantala & Roff (2005)
	Mammals	<i>Sigmodon hispidus</i>	Physiological	Intra	Derting & McClure (1989)
	Plants	<i>many species</i>	Physiological	Inter	Denancé et al. (2013)
		<i>meta analysis</i>	Genetic	Inter	Lozano-Durán et al. (2013)

Figure 2.4: example of the diversity of traits that can be traded off, the diversity of species involved, the diversity of the scale studied.

2.2 Origins and regulations of trade-offs

Trade-offs can arise from different mechanisms which may interact and give rise to other trade-offs. In this part, I will focus on the description of these mechanisms and their interactions.

2.2.1 Genetic level

Both direct-, indirect antagonistic pleiotropy and linkage disequilibrium can be (in theory) mechanisms responsible for trade-offs between traits (Sgrò & Hoffmann, 2004; Bennett & Lenski, 2007).

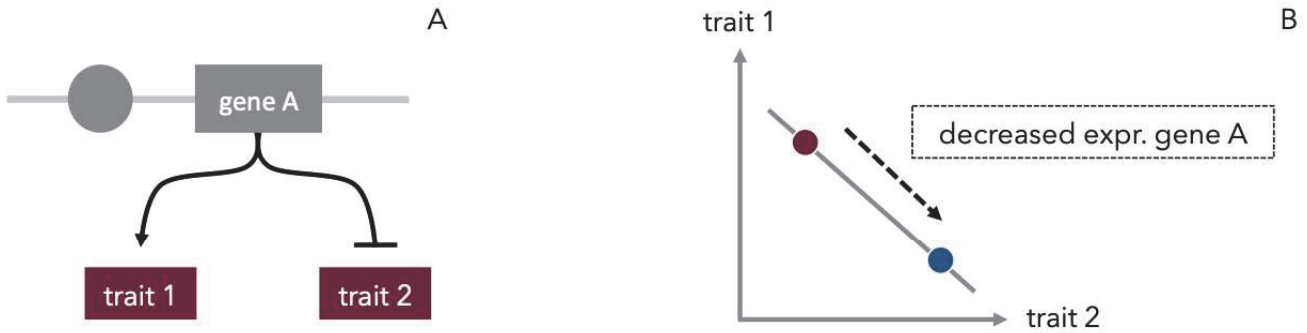


Figure 2.5: Direct antagonistic pleiotropy. **A-** One gene controls two different traits, which have an antagonistic effect on one another. An individual (purple dot) with a high gene A expression leads to an activation of trait 1 and an inhibition of trait 2. An other individual (blue dot) has a low gene A expression, leading to a light inhibition of trait 2 and a slight activation of trait 1. **B-** Consequence on individual position on the trade-off when a change in gene expression occurs.

Direct antagonistic pleiotropy is a mechanism acting at the genetic level. It corresponds to a particular situation where one gene codes for 2 traits (such gene is pleiotropic) positively correlated with fitness (Wang *et al.*, 2010). However, its action on both traits is antagonistic. If we consider an example illustrated in fig 2.5-A, the expression of the gene A has a *positive* impact on trait 1 (it increases its value, like an increase of the number of offspring if we consider the trait fecundity). On the other hand the expression of the same gene has a *negative* impact on trait 2 (it decreases its value, like a decrease in its ability to escape from a predator if we consider the trait survival). Conversely, reducing the expression of this gene may increase trait 2 and decrease trait 1. Hence, any change in this gene expression has an antagonistic effect on the two traits considered and creates a negative relationship between them.

Antagonistic pleiotropy is one explanation suggested by Williams in 1957 to explain senescence. He proposed that genes with antagonistic pleiotropic effects (good effect in early life) could be favored by selection (when selection efficacy is weak) even if they are deleterious at the end of organism lives. Thus, a little beneficial effect of a gene in the beginning of life can be selected for because it exceeds negative consequences, at the end of life, even if those are lethal (Kirkwood & Austad, 2000). Moreover, natural selection

has a weak effect on aged individuals if the reproduction is favored in early life (Haldane *et al.*, 1941; Hamilton, 1966; Charlesworth *et al.*, 1994).

However, this simple mechanism infers that one single gene would directly code for two key life-history traits, whereas quantitative genetics predicts that life-history traits are potentially the results of a multitude of genes (Linnen *et al.*, 2013). In an empirical study in *C.elegans*, Estes and colleagues did not find evidence of phenotypic life-history trade-offs being generated by antagonistic pleiotropy (Estes *et al.*, 2005; Roff & Fairbairn, 2007). A possible example of such mechanism is found in the unicellular (I did not find any example in multicellulars) *Escherichia coli* where a trade-off between stress resistance and vegetative growth is regulated by a transcription factor RpoS (detailed in the next paragraph) and is reinforced by a structural modification of the cell namely caused by RpoS. The size of porins (*i.e.*, transmembrane proteins forming channels able to diffuse molecules through cell membranes) is determined by the concentration of RpoS. If porins produced are large, then large molecules can enter the cell. Consequently, more nutrients can enter (increasing growth) but also more antibiotics (decreasing stress resistance). In the other case, small porins induce a decrease of growth and an increase of stress resistance (Ferenci, 2016).

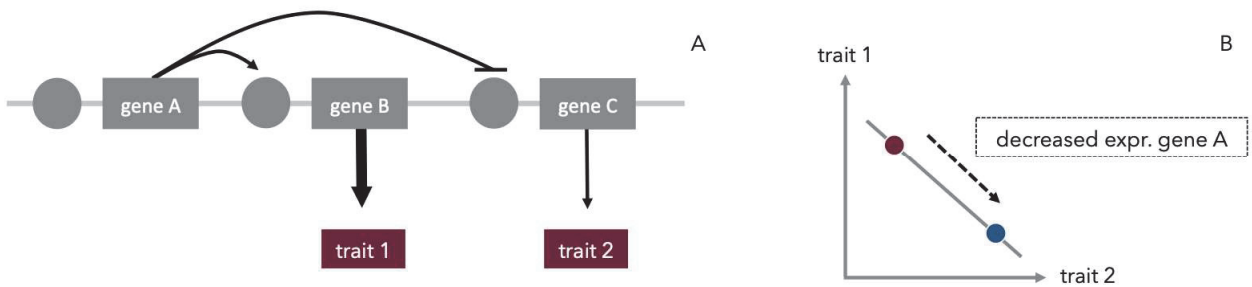


Figure 2.6: Indirect antagonistic pleiotropy. A- The gene A activates gene B and inhibits gene C. Genes B and C activate respectively trait 1 and trait 2. A individual (purple dot) with an high gene A expression leads to an activation of trait 1 and trait 1. An second individual (blue dot) has a low gene A expression leading to an activation of trait 2 and a inhibition of trait 1. B- Consequence on individual position on the trade-off when a change in gene expression occurs.

Indirect antagonistic pleiotropy, is a mechanism with intermediate level that could lead to the expression of a trade-off. In the case illustrated in figure 2.6-B, traits 1 and 2 are respectively regulated by genes B and C. These genes are controlled antagonistically by the expression of gene A. If an individual has a strong expression of gene A (purple dot on the illustration), then its expression of gene B is enhanced and it increases the value of trait 1. It also inhibits the expression of gene C and decreases the value of trait 2. And *vice*

versa if the individual has a low expression of gene A (blue dot). As for direct antagonistic pleiotropy, modification of the gene A expression changes traits values of individuals and generates a negative relationship. This type of mechanism leaves greater freedom in terms of the number of possible regulations between the genes and the phenotypic traits and can lead to the emergence of particular trade-offs as in unicellular organisms.

In these species, intra-cellular communication can be ensured by transcription factors (this is also the case for multicellulars). Therefore, the role of genetic expression in the regulation of trade-offs has been mainly described in unicellulars² (Mappes *et al.*, 2004; Jessup & Bohannon, 2008; Bayer *et al.*, 2009; Heineman & Brown, 2012; Maharjan *et al.*, 2013; Azevedo *et al.*, 2016; Ferenci, 2016). In microorganisms, the two most important life-history traits are vegetative growth (merging longevity, number of offspring and growth) and stress resistance (combining all functions that improve survival such as drying or antibiotic resistance and maintenance) because of their scissiparous lifestyle. For example in *Escherichia coli*, an allocation trade-off between stress resistance and vegetative growth is partly regulated by the concentration of a transcription factor named RpoS (sigma factor interacting with the RNA polymerase). In a context of high RpoS concentration, the expression of growth genes is inhibited while that of stress resistance is stimulated, and *vice versa* (Maharjan *et al.*, 2013). This regulating mechanism can be considered as "indirect antagonistic pleiotropy" because it implies multiple genes involved in the expression of two different life-history traits.

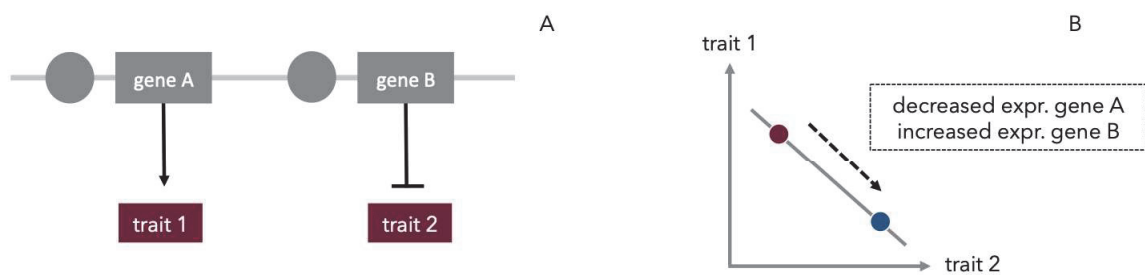


Figure 2.7: Linkage disequilibrium. Two genes are close on the chromosome and thus are likely to be expressed together (few chances for a crossing over between the two genes). The two genes encode for different traits, one activates trait 1 while the second inhibits trait 2.

Linkage disequilibrium (illustrated in fig 2.7) is defined as a nonrandom association between alleles at different loci (Hartl *et al.*, 1997) and is theoretically involved in the

²As might be expected, it exists example in the vertebrate molecular endocrine literature where a gene specifying a hormonal regulator positively affects expression of one gene but negatively affects expression of others (Hau & Wingfield, 2011).

emergence of trade-offs. A gene A codes for a trait 1 and increases its value while a gene B codes for trait 2 and diminishes its value. However, it seems unlikely that trade-offs can arise from such mechanism because this hypothesis would assume that two traits are encoded by two genes (with antagonistic effect) close to each other on one the same chromosome. In this context, a mutation (in case of bacteria, where promoters are shared) or several successive mutations (in case of multicellular organisms, 1 mutation for each gene) should have a similar effect to maintain the trade-off in population without changing the relationship. Nevertheless, these mechanisms are sometimes proposed as an explanation for emergence of trade-offs (Sinervo & Svensson, 1998). Indeed, genetic correlations are supposed to can cause trade-offs (Reznick, 1985) and they originate from linkage disequilibrium between two or more genes (or from pleiotropic effect) (Lande, 1982). Moreover linkage disequilibrium can be promoted by natural or sexual selection but can be broken down after environmental changes (Lande, 1981).

2.2.2 The differential allocation of resources

The differential allocation of resources was historically the first mechanism used to explain the presence of trade-offs between life-history traits. First enunciated by Levins & MacArthur and used by Cody in 1966, it is still the most intuitive way to understand the emergence (and maintenance) of trade-offs. The core idea is that each individual (no matter the species) has access to a finite amount of energy during its lifetime. The organism has to divide (*i.e.*, to allocate differentially) this quantity between all vital functions energetically costly, like maintenance, growth, mating, immune functions, courtship display (and more), which are components of fitness : survival and reproduction. However, all energy invested in one trait can not be invested in another trait. Hence, two traits competing for a same type of resource are linked by a negative relationship. Thus, allocation trade-offs can be resource- and context-dependent because phenotypic traits inter-connected share a same specific resource.

The simple notion of differential allocation of resource was modeled by Van Noordwijk & de Jong (1986) in their seminal paper. They considered only two traits (reproduction R and survival s , grey boxes in fig 2.8) and a quantity A of one resource acquired (yellow stars in fig 2.8). The allocation compromise can be determined by the energetic investment in one trait (for instance a proportion b (large yellow arrow in fig 2.8) of energy is allocated to R) because the other investment is deducted from the first one (the remaining energy

for s is $1-b$ and will be allocated to this function). From this very simple equation, all possible combinations of traits distribute along a linear negative relationship.

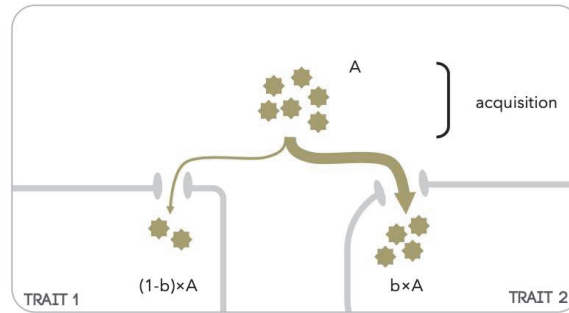


Figure 2.8: The differential allocation of resources inspired by the Y model of Van Noordwijk & de Jong (1986)^b. Yellow stars represent acquired energy (A) which is divided between two phenotypic traits (grey boxes). The energy devoted to trait 1 (R) is the remaining from investment in trait 2 (s).

^bIn this influential paper, Van Noordwijk and de Jong already described why positive correlation can arise where negative ones were expected (see more in section *Invisibility of expected trade-offs*). In the study of trade-offs, the allocation is not the only essential parameter. The acquisition of resource A also represents a main variable to consider. Even if different individuals allocate in a same proportion their energy, they can express different trait values just because some acquire more resources than others. Thus, positive correlations between traits can emerge while a trade-off is linking these traits.

This model is classically considered as one of the most mechanistic used in the study of trade-offs. While of great historical importance this model is still rather simple mechanistically. Indeed, it is difficult to imagine that a "bigger tube" will feed one trait rather than another (to be caricatural). Of course, we can wonder how this differential allocation actually works? Which mechanisms (in the real sense of the term) are behind this allocation? In multicellular organisms, the differential allocation of resources is often regulated by hormones (as represented in figure 2.9). These particular molecules play the role of internal messengers among several trait-specific organs and hence coordinate most organismal functions. They are involved in aspects of physiology that contribute significantly to stress response, fecundity, longevity, reproduction and behaviour. Hormones are even found in the most simplistic multicellular organisms (*Volvox carteri*, Starr & Jaenicke (1974)). They are considered as key mediators of trade-offs between life-history traits (Harshman & Zera, 2007; Flatt & Heyland, 2011).

In *Drosophila melanogaster*, as in many arthropods, the trade-off between survival and reproduction is directly controlled by the concentration of the juvenile hormone (JH). An increase in hemolymphatic concentration of JH causes a decrease in longevity (and immune functions) for the benefit of an increase in fecundity (Tatar *et al.*, 2001). This shift of hormone concentration purportedly modifies the differential allocation of resources

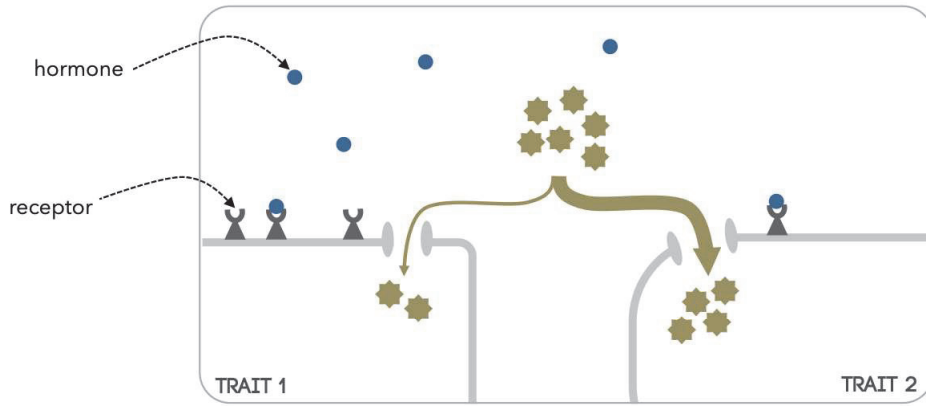


Figure 2.9: The differential allocation of resources regulated by hormonal dynamics. Hormones (blue dots) and receptors (grey cones) are expressed in organism. They can bind to each others and form complexes. Depending on hormones and receptors concentrations, a certain amount of energy is allocated between the two traits.

(Flatt *et al.*, 2005).

Populations of the cricket *Gryllus firmus* are distributed in temporary sand dunes which is a heterogeneous environment (Roff & Fairbairn, 1993). Each patch persists for some finite period and then, crickets have to reach a new inhabitable patch (Roff, 1994). Significant distances separate patches (and so, separate crickets from one another) and crickets need to fly to new patches (Roff, 1994). *G. firmus* exhibits two morphs in natural populations: the « short wing » morph (SW) with atrophied wing muscles and wings, which is unable to fly and to travel over long distances and the « long wing » morph (LW) with functional wings, which is able to disperse by flight and to reach other patches. The expression of wing length is naturally determined by a set of genes and this trait is highly heritable but expression of this phenotype is also sensitive to photoperiod and temperatures (Roff, 1986). SW individuals are unable to disperse on long distances but possess more ovarian tissue (and thus have an increased fecundity). This increase in fecundity is due to energetic economy possible due to the non-development of wing muscles and reduced production of flight fuel (*e.g.* lipids). It is possible that the differential allocation of energy is also regulated by JH concentration. During development, LW individuals produce less JH than SW individuals. The causal role of JH was tested in laboratory. A JH injection was realised on adults with LW morph, which lead to a post-developmental modification of phenotype. After the injection, wing muscles were atrophied and the collected energy was reinvested in increasing ovarian tissues and thus fecundity (Zhao & Zera, 2002). Nevertheless most recent work indicates that JH might

not be involved (Zera, 2016).

2.3 Interaction between genetic and physiological levels

identifying the hormonal mechanism allow one to understand how the allocation of resources occurs at the physiological level. However, we know that, in nature, hormonal concentrations are not modified via needle injections. This hormonal mechanism itself is controlled by a genetic regulation as well as by environmental regulation. Genes code for the expression of hormones and receptors but also for their respective conformations (as shown in figure 2.10).

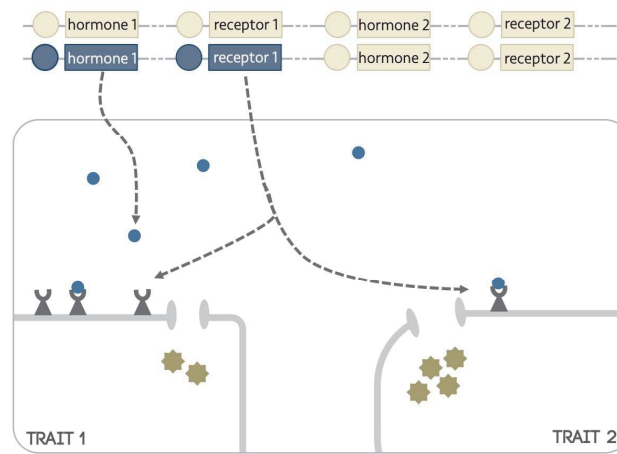


Figure 2.10: Hormonal dynamics regulating the allocation of resources are determined at the genetic level. Genes code for hormones and receptors concentrations and/or conformation. Hence, mutations can modify hormone-receptor relationships and then alter the trade-off.

An example of such mechanism is described in *Mus musculus*, where a genetic regulation is a previous step before physiological levels. As often, studies of mutants reveal an underlying genetic mechanism. In this species, snell dwarf mice are mutants of the Pituitary factor1 (Pit1) gene, which implies a dysfunction of a transcription factor leaving it inactive. This transcription factor is responsible of the activation of a target gene in a regulatory cascade. With this particular mutation, mice are unable to produce three hormones (growth hormone GH, prolactin PRL and thyroid- stimulating hormone TSH). Hence, snell dwarf mutants are less fertile than wild type from the same litter but they express significantly extended longevity (Bartke & Brown-Borg, 2004). The differential allocation of resources between reproduction and longevity, in this species, is regulated by the combined action of three different hormones themselves activated by a single transcription factor.

If we are interested in a phenotypic trade-off in multicellular organisms, the differential allocation of resources does not represent the explanatory mechanism of regulation of trade-offs. Obviously, the allocation of resources can be studied as a simple energetic division and variations in allocated proportions to traits (as it is done with dynamic energy budget models DEB (Jager *et al.*, 2013)). However, by definition the genotype space is totally different depending on what the mutations represent. If mutations directly modify the allocation of resources or if mutations have consequences on interactions between hormones and receptors which lead to change in the allocation, then the expected results of evolution will not be the same. In the case of a trade-off expressed in a population as a negative relationship between traits, the genetic variation present in the population is central and this present and future genetic variation relies on the genotype space. The emergence of the genetic variation and its maintaining can be understood if models consider in details endocrine mechanisms. Thanks to this, realistic genotype space can be generated and consequently displacements in this space (through mutations and/or recombination events) will be realistic too. Hence, the allocation of resources represents only an intermediate step between hormonal dynamics encoded genetically and the phenotype expressed, which can be subject to environmental variations.

The study of trade-offs does not have to be split between different scales (genetic, evolutionary, physiological). It is obviously necessary to concentrate on a level where one seeks to decipher precisely its working. Nevertheless, the description of a level should not be accompanied by a categorization of the trade-off. Indeed, it is a mind representation to divide trade-offs into different types since all scales are inter-connected. This integrative vision was foreshadowed by Ketterson and Nolan in 1992, already assuming the crucial role of hormonal dynamics in evolution of trade-offs. Thus, by considering that the trade-off observed at a phenotypic scale was the result of a cascade effect coming from the genotype, it was possible to envisage the evolution of trade-offs. They therefore suggested that particular mutations, such as a change in receptor density or in the affinity between an hormone and its receptor, could cause massive changes of trait values (Ketterson & Nolan Jr, 1992).

2.4 Invisibility of expected trade-offs

Despite the solid argument that trade-offs should in principle exist, it is sometimes necessary to reveal them (Zera & Harshman, 2001). Numerous studies did not detect any

trade-offs when they were expected to find some (see references in Table 2.4 with a star). Indeed, in some cases trade-offs are expected because they have been measured in a close species or simply because the theory has predicted their presence between certain traits. Different hypotheses could explain the non-observation of trade-offs despite their existence: first, trade-offs exist but individuals do not express variation in traits values (*i.e.*, each individual may divide its energy in the same proportion between traits leading to a same allocation, and without variance in the population the negative relationships can not appear). Second, the trade-off is possibly not measured at the right level to be revealed. For instance, fecundity sometimes should be measured as realized fecundity (*i.e.*, lifetime number of eggs layed Tisdale & Sappington (2001)) and not as the potential fecundity (*i.e.*, correlative approach based on size measurements Rhainds (2015)). Third, significant individual variations in resource acquisition can uncover a positive correlation between traits. Fourth, there may be a link between resource acquisition and allocation that actually cancels the trade-off.

As demonstrated by Van Noordwijk & de Jong (1986) and underlined by Metcalf (2016), trade-offs can be hidden due to variations in resource acquisition between individuals. Positive correlations between traits frequently appear where negative ones were expected because of acquisition variability. Indeed, individuals which acquire a larger amount of energy can allocate more to all their traits and express better trait values (Reznick *et al.*, 2000). In a population, if the variation in acquisition is large and despite variations in allocation (as described in figure 2.11-B), the relationship between two traits will appear positive, even if those traits are negatively inter-linked.

Therefore, one solution to reveal trade-offs is to control for individual acquisition in order to minimize this variation. At the present time, the most efficient manner to control for resource acquisition by individuals is to starve them. Different protocols have been developed to quantify the energy ingested but they have several disadvantages: they can only be developed in laboratory, they are costly and tricky to be implemented properly (Deshpande *et al.*, 2014). For all these reasons, trade-offs are most often detected in limiting environment in laboratory and in the field during bad year conditions (Roff & Fairbain, 2007; Droney, 1998). Many studies have compared the relationships observed in a population between two feeding regimes and often deduced that trade-offs disappear in good conditions (Reznick *et al.*, 2000; Marden *et al.*, 2003). These results have been interpreted as an evidence of absence of trade-offs but as previously demonstrated, the non detection of trade-off does not mean they are not present (Roff & Fairbain, 2007).

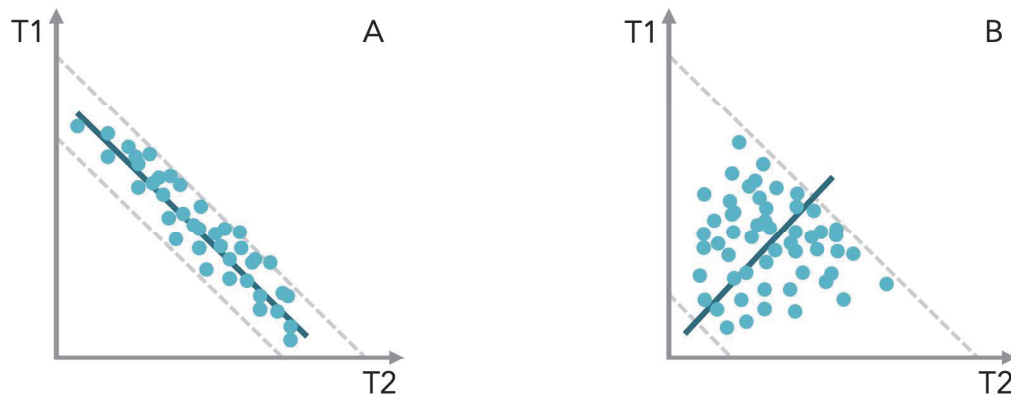


Figure 2.11: Variation in acquisition can conceal negative relationship between traits. In both cases, individual combinations of two abstract traits (T1 and T2) are plotted in blue dots. We consider that the distance between origin of the graph and the combination of trait of an individual represents a proxy of the amount of energy acquired by this individual. Hence, grey hashed lines represent the variation in acquisition. **A-** individuals have access to a similar amount of energy, which leads to a small variation in acquisition between them. They mainly differ in the allocation of this resource. A negative relationship between T1 and T2 is easily identifiable. **B-** individuals have access to various amount of energy. Some of them ingest a lot, the others only a few, which leads to an important variation in acquisition between them. The large variation in acquisition blurs the negative relationship linking T1 and T2. This figure is an adaptation from Van Noordwijk & de Jong (1986).

Moreover, the comparison between two feeding regimes could represent an interesting question but to answer it precisely would be challenging. Indeed, it would require to control precisely the amount and quality of resource ingested by individuals.

The evolution of trade-offs

Even if one studies a trade-off at a physiological scale by looking at how energy is allocated between different traits, genetic mechanisms of regulation are usually involved. These regulatory mechanisms, presented above, are therefore subject to mutation-selection processes. This is not without consequences on the values of traits expressed by the individuals of a population. Thus, via mutations acting on hormonal dynamics, crucial change in the shape of trade-offs can result from them (Ketterson & Nolan Jr, 1992). Furthermore, as mainly pointed out by Roff (Roff & DeRose, 2001; Roff *et al.*, 2003; Roff & Fairbairn, 2007; Roff & Fairbairn, 2012) but also by others authors (Houle, 1991; Chippindale *et al.*, 1996; Reznick *et al.*, 2000) understanding the evolution of trade-offs represents an essential question because trade-offs are involved in so many biological processes. In this part, I will first explore empirical studies that have identified changes in the form of trade-offs. Then, I will explain how we can theoretically envisage explaining this evolution by introducing my thesis work.

3.1 Empirical evidences that trade-offs evolve

One of the first study on trade-off evolution was conducted by Roff and colleagues in 2002. They compared 4 populations of crickets (*Gryllus firmus*) coming from different rearing conditions : 3 were directly sampled from the field and 1 was reared in laboratory conditions during 20 years. In this species, 2 morphs are naturally expressed and correspond to two extremes on the trade-off between dispersion and reproduction (illustrated on figure 3.1). This particular case forces the detection of the shape of trade-off to be linear and can only be characterized by the slope and the intercept. Roff and colleagues observed different intercepts for the 4 populations and the slope from the laboratory population was clearly dissimilar compared to the others. Even if the shape is probably environment-dependent, this result shows that trade-off can evolve rapidly.

In a second experiment, 10 years later, Roff was still interested in the same question

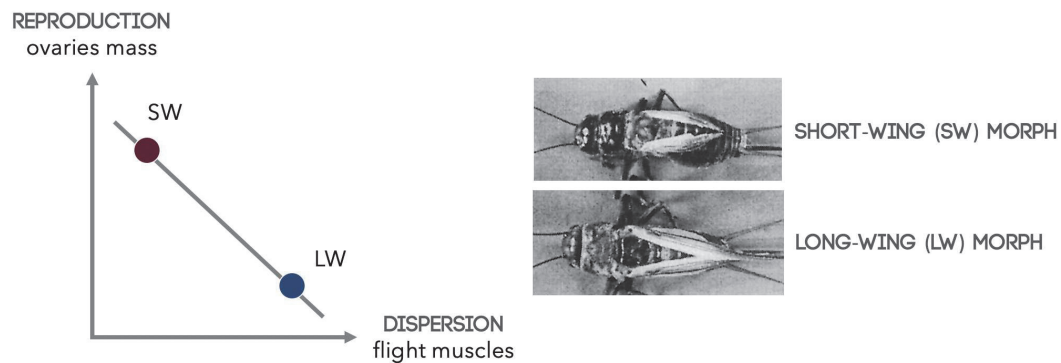


Figure 3.1: Schematic representation of the trade-off between dispersion and reproduction in *Gryllus firmus*. 2 morphs are naturally present in this species. Short-wing (SW) individuals with an small capacity to disperse (they can not fly but can walk) but large ovaries. Long-wing (LW) individuals with an important capacity of disperse (they can fly) but small ovaries. Pictures of the two morphes come from Zera (2005). There are no intermediate morphs. The individual variations in wing length and mass of the ovaries will form clouds of points around the 2 average morphs. Thus, the relationship that best explains these clouds will be a linear relationship.

with the same biological model (Roff & Fairbairn, 2012). Roff and Fairbairn conducted an artificial selection experiment in *Gryllus firmus* during 6 generations. They applied three different types of directional selection : increasing fecundity (ovaries mass), increasing flight muscles (dorso- longitudinal muscle mass) or increasing both. They observed an evolution of intercepts but not of slopes which matches perfectly their prediction made 10 years before. In Roff & DeRose (2001), they anticipated that (following quantitative genetics assumptions) a trade-off between a morphological trait (mass of flight muscle) and a life-history trait (fecundity) could evolve by changing the intercept but not the slope. The study of the intercept and the slope is particularly appropriate in this case of study. The model organism constraints the shape of the trade-off because of their 2 morphs, even if the changes are important, there will always be 2 clusters of points more or less exploded and a linear regression will remain the best fit to explain observations. They also specified that the evolution of trade-offs between two life-history traits "will show more complex responses". Indeed, the trade-off could have a different shape. Moreover, the applied artificial selection is debatable. Indeed, trait measurements need individual sacrifices: individuals could not be the parents of the next generation, other variants of the family were used.

Other types of observations raise the question of a potential change in the shape of trade- offs. A part of the field of demography is interested in the global question of senescence: why do organisms get older? how does it works ? and especially how to avoid

aging? (Kenyon, 2010). Consequently, there is a keen interest around mutants capable of expressing significantly extended longevity compared to their counterparts. Generally, when such a mutation is observed, it is associated with a decrease of fecundity or others traits (Walker *et al.*, 2000; Jenkins *et al.*, 2004; Wit *et al.*, 2013). These results suggest only a move along the trade-off. But some mutants seem to be able to express a higher longevity without impacting their other traits. These mutants appear to have escaped from the classical trade-off to reach perhaps a new form of trade-off. For instance, mutants with such ability are found in *Drosophila melanogaster* when the INDY gene is mutated. Marden and colleagues showed those mutants express a decrease in the mortality curve with no impact on their metabolic rate, flight velocity or age-specific fecundity but only when they are reared on "high caloric food" (Marden *et al.*, 2003).

A lot of these studies are linked with the presence or absence of food (with variation in its quality) and reveal that feeding behavior is determined by signaling pathways (Greer & Brunet, 2009; Kapahi *et al.*, 2004; Greer *et al.*, 2007). Hence, evolutionary biologists tried to modify these pathways in order to find the right adjustment leading to an immortal and fertile mutant. Lind and colleagues in 2019 finally found the "Holy Grail". They show that ageing could be regulated by what they called functional trade-offs. In their hyper-function hypothesis, they explain that suboptimal gene in late life causes ageing via excessive biosynthesis. In this case, biosynthesis would be energetically costly and would not have a beneficial effect in late life. By modifying insulin/IGF-like signaling in late life, they decrease excessive biosynthesis and significantly increase longevity without any cost on reproduction or on offspring. This work shows that when intermediary mechanisms (like excessive biosynthesis) are considered and deeply studied the evolution of trade-off can be understood.

3.2 In theory

However, for a long time, trade-offs have been considered as fixed (Tuomi *et al.*, 1983). Possibly because the difference between design constraints and trade-offs is sometimes blurred (Stearns, 1977) in the literature. Design barriers correspond to structural constraints and mainly emerge from physico-chemical laws that are absolutely static and inviolable. Nevertheless, trade-offs are the results of different mechanisms (as demonstrated in section *Origins and regulation of trade-offs*) partly genetically encoded and as such, they can evolve. Moreover, trade-offs can be summarized as the covariance between

traits such as the covariance part in the G-matrix. The stability of the G-matrix has been highly debated. Apparently, this stability depends mostly on the traits considered, on mutational correlation and on correlational selection (Jones *et al.*, 2003). In the particular case of fitness components (such as the traits involved in trade-offs), G-matrix is predicted to be instable both because of the adaptative landscape linked with these traits and multiples loci entailed in the expression of fitness components (Jones *et al.*, 2003). These theoretical and empirical works (Fong, 1989; Jernigan *et al.*, 1994; Land *et al.*, 1999; Roff *et al.*, 2002, 2004; Cano *et al.*, 2004; McGuigan *et al.*, 2004; Doroszuk *et al.*, 2008) on the G-matrix suggest that trade-off can evolve.

Trade-offs and in particular their shape, limit the evolution by restraining the combination of traits available in the short term. Indeed, the shape of a trade-off originates from the standing genetic variation and its evolution is controlled by the architecture of mutational neighborhood. Hence in the short term, this shape also restricts the combination of traits reachable by mutation. Therefore, the shape of a trade-off determines which position in the phenotype space is optimal (Maharjan *et al.*, 2013; Levins, 1968). However, empirical studies on trade-offs mostly ignored these crucial evolutionary parameters. They are detected with linear regressions which inform us on the slope but other shape's parameters (like the curvature or the length) are neglected while they can be decisive (Schaffer, 1974; Stearns, 1989).

When we (my supervisors and myself) wondered how to study the evolution of the form of trade-offs a fairly simple solution came to mind. It would be possible to generate a large variety of shapes of trade-offs and then look at what shapes persist and are preserved over generations. The first problem with such an approach is that it is obviously mathematically possible to generate functions with very different forms but that it is also highly probable that many of these forms can never emerge naturally in a population. The second problem lies in the determination of the neighborhood relationships between these shapes (genotype space) which are absolutely unknown and which should have been determined in a completely arbitrary manner.

For all of these reasons and in order to properly study and predict the shape of trade-off, it is essential to explicitly consider several layers of the GP-map. In theory, the integration of trade-offs in the GP-map to ensure a better understanding of adaptation, is a manner to add a synthetic degree of information previously missing. Indeed, trade-offs are highly dependent on the ecological context, because their shape summarizes information on availability of resources for instance.

It is in this integrative vision that my thesis work fits. I wanted to consider the different levels involved in the GP-map as well as the allocation of resources to study the evolution of the shape of trade-offs. All these layers are interconnected and play a major role in the evolution of these relationships. As demonstrated in an experiment of Lind *et al.* (2019) where they controlled excessive biosynthesis during adulthood in *C. elegans* which led to individuals with an extended lifespan without negative consequences on offspring production and performance (Maklakov & Chapman, 2019)), once the fine mechanisms were understood, they were able to control some of these parameters and to change the shape of the trade-offs. At first, I became interested in this question from a theoretical point of view. I have therefore created an evolutionary mathematical model that directly takes into account the different crucial levels:

phenotypic level: differential allocation, at the heart of the process, determines the trait values of individuals and their combinations place individuals in the fitness landscape.

genetic level: hormonal regulation is composed of interactions between hormones and receptors, whose expressions and conformations are genetically encoded. Hence, movements within the genotype space are explicitly modeled.

physiological level: emergent mechanism (*i.e.*, the differential allocation of resources) and maintenance mechanism (regulation of the differential allocation of resources via an hormonal dynamics).

With this model we could study the evolution of the shape of relationship between traits. Through the definition of shape parameters we found that the trade-off becomes shorter (decrease in standing genetic variation) and more concave in a particular environmental context. Indeed, we have shown that the cost associated with the storage of resources represents a major parameter in the evolution of trade-offs. This cost is rarely taken into account while it may differ between species or resources ingested. Besides, this model shows that changes in the ability to use the energy could lead to an escape from the former trade-off. Hence, through the combined action of mutation and selection, individuals are able to reach more optimal trait values and so achieve enhanced fitness. This work is detailed in the next part: *Hormonal pleiotropy and the evolution of allocation trade-offs*.

In a second step, I sought to integrate in this model environmental variations in the availability of resources. I also wanted to add more realism in the hormonal dynamics. Indeed, hormones are well-known for their responsiveness to environmental changes (Ketterson & Nolan Jr, 1992). To do so, we modified the genetic level. Hence, the hormonal production partly depends on the encoded genotype and partly on the resource availability. We observed the evolution of different endocrine mechanisms depending on the frequency of resource availability.

Third, I wanted to study the same question from an empirical point of view. Thus, I conducted an experiment where populations of *Drosophila melanogaster* evolved in artificially crafted fitness landscapes during 10 generations. I studied the classic trade-off between quantity and quality of offspring using fecundity during 24h and emergence rate as respective proxies. To this aim, I developed a framework where any fitness landscape can be set arbitrarily, controlling selection on these 2 life-history traits. I considered two fitness landscapes: one that is supposedly universally encountered in nature, where both traits are under directional positive selection, and one that has virtually never been encountered where the traits are under stabilizing selection. This work is described in the part: *Experimental modification of a fitness landscape to select for an escape from a classical trade-off in Drosophila melanogaster*.

Part II

THEORETICAL WORKS

Hormonal pleiotropy and the evolution of allocation trade-offs

This chapter is published in *Evolution* and was co-written with Laurent Jacob, Frédéric Menu and Etienne Rajon.

CHAPTER I OVERVIEW

In this chapter, we studied the evolution of the shape of the relationship between two abstract traits. We used the term trade-off to designate the negative relationship between 2 (or more) traits that arises at the population level, because our model includes an intern individual compromise where resources can either be allocated to one trait or the other. Our model, however, also comprise a regulatory mechanism for this resource allocation, as well as a compartment where resources can be stored at a cost; hormone- and receptor- conformation and expression regulate the allocation of resource which lead to a combination of trait values for each individuals. Hence, our model considers the three essential levels (as represented in Figure 4.1) for the emergence of trade-off relationships at the population level: the phenotypic level, and the underlying physiological processes and genotype (Stearns, 1989). We voluntarily chose to include the complex interaction at different levels to make the genotype-phenotype map more realistic, and relying on mechanistic assumptions. As explained in the previous part of my thesis, this is important as our aim is to study the build-up of genetic and non-genetic variance in a population, which may rely heavily on the mutational process. Moreover, it has been demonstrated that major life-history traits like reproduction and longevity are regulated by signaling pathways (implying hormonal regulation), and rely on resources that can be stored (Tatar *et al.*, 2003).

Receptors in our model work similarly to G-protein coupled receptors (GPCRs, here-

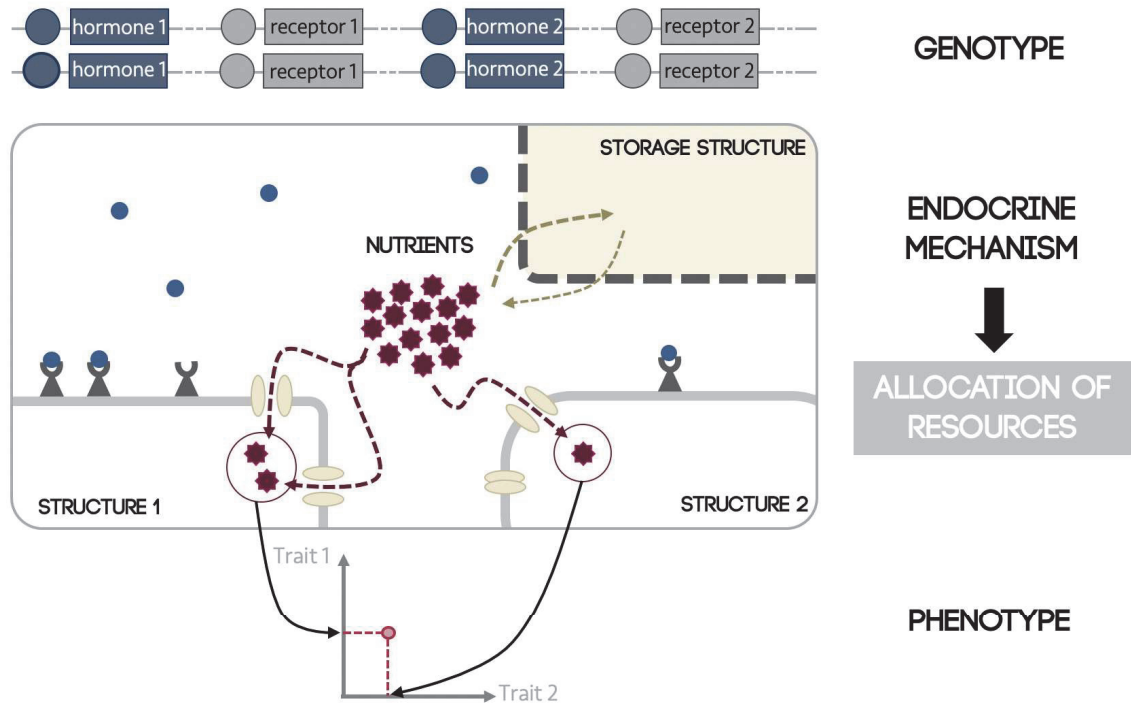


Figure 4.1: Illustrative representation of our model. The three crucial levels (genetic, physiological and phenotypic) are represented together to show their interlinked relationships. Hormones (blue circles) and receptors (grey cones) can form complexes, depending on their affinity. When a complex is formed, a nutrient transporter (beige ovals) is activated and energetic resource (pink stars) can enter the corresponding converting structure (each converts energy into one trait). Nutrients may diffuse in (if they exceed a threshold concentration) or out of the storage structure, with a cost such that only a part of the nutrients stored are released. Hormones and receptors are considered at equilibrium; once the unique meal has been consumed, each individual is described by a combination of traits which determine its fitness.

after) which are the largest family of cell-surface receptors. GPCRs are implicated in the mediation of many responses to environmental stimulus (such as odors, images or tastes) and also in signaling pathways (*e.g.*, glycogen and triglyceride breakdown). When a messenger molecule (such as an hormone) binds a GPCR, it blocks access to this receptor and triggers a cascade of effects via the release of a G protein. Then the messenger molecule is degraded and the surface receptor is available and functional after a short delay (because of receptor recycling). In our model, hormones are degraded after biding, and receptors are instantaneously freed instead of recycled for mathematical convenience. We tested the impact of receptor recycling (as shown Figure SI1-1) and obtained a correlation of 0.97 between models with and without recycling. Hence, we did not integrate recycling in our simulations.

One way to model mutations impacting the affinity between an hormone and a receptor could have been to assign random values to each couple upon mutations. We made the

different choice to assign conformations to hormones and receptors and compare these conformations to obtain an affinity. Indeed, it is the three-dimensional structures of these proteins that determine their interactions. However, precisely modeling these structures was absolutely beyond our capacity and we decided that each hormone and each receptor (proteins of the same length) could take 1000 different conformations (represented by integers). The closer the two numbers assigned to an hormone and a receptor, the higher their affinity. Despite the simplicity of this approach compared with modeling actual conformations, it captures two important features: an hormone and a receptor can be very distant from each other, and no mutation in the mutational neighborhood of each may increase their affinity, and two receptors (for instance) may have high affinities with an hormone, such that changing the conformation of the hormone would likely impede its affinity with both. The number of different conformations is, of course, arbitrary and this choice could be challenged later. This illustrates, we think, the value of our approach, which may allow considering new information without changing the structure of the model, simply by changing its parameterization.

Abstract

Recent empirical evidence suggest that trade-off relationships can evolve, challenging the classical image of their high entrenchment. For energy reliant traits, this relationship should depend on the endocrine system that regulates resource allocation. Here we model changes in this system by mutating the expression and conformation of its constitutive hormones and receptors. We show that the shape of trade-offs can indeed evolve in this model through the combined action of genetic drift and selection, such that their evolutionarily expected curvature and length depend on context. In particular, the shape of a trade-off should depend on the cost associated with resource storage, itself depending on the traded resource and on the ecological context. Despite this convergence at the phenotypic level, we show that a variety of physiological mechanisms may evolve in similar simulations, suggesting redundancy at the genetic level. This model should provide a useful framework to interpret and unify the overly complex observations of evolutionary endocrinology and evolutionary ecology.

4.1 Introduction

Evolutionary biologists have long known that heritable characters (or traits) usually do not vary independently from each other (Reznick, 1985; Stearns, 1989, 1992; Roff, 1993; Sinervo & Svensson, 1998; Flatt *et al.*, 2005). These correlations between traits may originate from correlated mutational effects, a phenomenon called pleiotropy, or from linkage between genes impacting different traits (Stearns, 2010; Wagner & Zhang, 2011). Different mechanisms may give rise to pleiotropy; for instance, a single mutation may alter a gene with multiple functions (Bennett, 1956; Grüneberg, 1938; Ostrowski *et al.*, 2005; Flatt & Kawecki, 2004), or a gene with a regulatory role that indirectly impacts different traits. The underlying mechanism may be even less direct, for example when a mutation increases the expression of one gene and, in consequence, decreases that of others (Stearns & Magwene, 2003), or when one increases the allocation of a resource to one trait, leaving less for others.

Pleiotropy is thought to hinder adaptive evolution, both because it restricts the available combinations of traits that can appear and because mutations are less likely favorable when they simultaneously impact many traits (Fisher, 1930a; Orr, 2005; Hine *et al.*, 2014). How pleiotropy evolves, and whether it is universal – *i.e.* every gene contribute, to some extent, to all traits – or restricted is the subject of an old but still ongoing debate (Wright, 1980; Welch & Waxman, 2003; Wagner *et al.*, 2008; Chevin *et al.*, 2010; Wagner & Zhang, 2011; Hill & Zhang, 2012; Wagner & Zhang, 2012; Paaby & Rockman, 2013). This question is currently investigated through systematic searches of genetic variants associated with changes in pleiotropy (Pavlicev *et al.*, 2013). Another promising avenue consists in deciphering how various levels of the genotype-phenotype map create constraints on multivariate evolution.

Pleiotropy commonly appears in the form of trade-offs, *i.e.* negative relationships between two or more traits scaled such that they correlate positively with fitness (Cody, 1966; Gadgil & Bossert, 1970; Stearns, 1989, 1992; Roff, 1993). Trade-offs have played a central role in evolutionary biology for more than 70 years (Lack, 1947). Yet the definition of this term remains ambiguous, possibly because it encompasses both the cause — for instance an allocation compromise — and the resulting negative correlation between traits (Coste, 2017; Roff & Fairbairn, 2007).

Trade-offs may have multiple causal origins involving many layers of the genotype-phenotype map. Empirical efforts have been devoted to deciphering their physiological

determinants, highlighting how changes occurring during the lifetime of an individual – for example, a plastic change in resource allocation – may incur a move along a so-called *physiological* trade-off (Zera, 2005; Hammers *et al.*, 2013). Notwithstanding, heritable variation also plays a clear role in generating differences between individuals within a population or even between populations or species, spread along *genetic/evolutionary* trade-offs (Stearns, 1989, 1992; Flatt & Kawecki, 2007). This common physiological/genetic dichotomy – where plastic/heritable would probably make more sense – should not hinder the fact that the physiological mechanism, whose constituents can mutate, is central to many genetic trade-offs.

Trade-offs are known to be important for evolution as they dictate which evolutionary movements are allowed on fitness landscapes (Verin *et al.*, 2017; Levins, 1968; Leslie *et al.*, 2017; Pásztor *et al.*, 2016; Flatt & Heyland, 2011). This is contingent on their inescapability, which is being questioned by the observation that individual mutations can depart from formerly established relationships between traits (Leroi *et al.*, 1994; Kenyon *et al.*, 1993). Moreover, actual changes in shape have even been observed after several generations in a new (artificial) ecological context (Roff *et al.*, 2002). Because trade-offs are most frequently observed in two phenotypic dimensions, such changes could be due to mutations with unobserved higher order effects. Nonetheless, the regulatory and physiological systems underlying trade-offs themselves being evolvable (Braendle *et al.*, 2011; Garland Jr & Carter, 1994), we here explore the alternative explanation that changes in trade-off shape may be caused by mutations impacting these systems.

Trade-offs may therefore be considered as comprising the largest part of – instead of all – heritable variation. Heritable phenotypic variation is likewise characterized as multidimensional in the field of quantitative genetics, resulting from a balance between mutations with unimodal symmetric effects on traits and selection. These assumptions are sometimes too simple to model the evolution of complex biological systems, for instance when a few genes have large and possibly epistatic contributions to the phenotype (Agrawal *et al.*, 2001; Carlborg & Haley, 2004), or when physiological processes create mutation biases. As we argue below, these criteria are both likely met for a widespread class of traits, namely those that rely on an energetic resource. Fortunately, another branch of evolutionary theory is devoted to these traits, which originated with van Noordwijk and de Jong’s seminal paper (1986). In these models, the relative allocation of a resource to one trait itself ‘mutates’, thereby creating a physiological dependency between traits. While obviously appropriate to model energy allocation, this framework still ignores the

underlying mechanism that regulates the allocation. Charlesworth (1990) has highlighted the important role of mutation on properties of the genetic variance that is maintained in a population, using a quantitative genetics model considering mutational biases arising from arbitrary functional constraints. The present study extends these theoretical developments by considering how mutation biases arise in a model where the regulation of resource allocation is explicitly considered.

Allocation trade-offs in multicellular organisms are presumably controlled by hormones (Zera & Zhao, 2004; Flatt & Heyland, 2011; Flatt & Kawecki, 2007; Ketterson & Nolan Jr, 1992, 1994). Hormones are involved in the dynamical control of the relative allocation to growth, survival or reproduction in life-history transitions (Finch & Rose, 1995; Zera & Harshman, 2001; Zera & Zhao, 2004). Furthermore, hormones are known to be key mediators of trade-offs in many taxa of multicellular species such as mammals (Derting & McClure, 1989; Oksanen *et al.*, 2002), birds (Ketterson & Nolan Jr, 1992), reptiles (Sinervo & Licht, 1991; Cox *et al.*, 2005), insects (Zera & Harshman, 2001; Flatt & Kawecki, 2007) and plants (Denancé *et al.*, 2013). This crucial role of hormones makes changes in the endocrine system good candidates to explain the aforementioned evolution of trade-off shapes (Ketterson & Nolan Jr, 1992). Here we study this evolution by considering how genetic variation builds up in endocrine systems. This genetic variation is converted into phenotypic variation as hormone-receptor interactions control the allocation of a resource to two trait-converting structures.

The genotype-phenotype mapping considered thus integrates several layers well described empirically (Zera & Harshman, 2001; Flatt & Heyland, 2011; Martin & Cohen, 2014). We argue that this is a necessary effort to study how genetic variation is maintained. Indeed, this allows epistasis – when the effect of a given gene on biological trait depends on one or more other genes (Moore, 2005) – to arise from mechanistic assumptions through actual changes in the conformation and expression of hormones and receptors. Standing genetic variation may thus evolve as populations move on the genotype space and reach new (qualitatively different) mutational neighborhoods, such that trade-off relationships can in principle evolve. We find that the evolving shape of a trade-off is highly dependent on a rarely considered parameter: the cost associated with resource storage. Consequently, in the simple ecological setting considered here, evolutionarily expected trade-off shapes should depend on the resource that is traded, and on ecological parameters that set the burden associated with storage structures. Despite evolutionary convergence at the phenotypic level (similar trade-offs evolved in simulations with iden-

tical parameters), we find little convergence at the genotypic level, suggesting that many solutions exist that yield similar phenotypes.

4.2 Model

4.2.1 Overview

We model the conversion of an energetic resource by specialized structures into two traits, with a conversion efficiency that decreases as the inward flow of the resource increases (see Figure 4.2). This flow is contingent on the dynamics of hormone-receptor binding at the surface of converting structures, which activates inward transporters of the resource. We assume that the resource intake by the organism ("meal" thereafter; each individual acquires in the population acquires an identical amount of the resource) takes place once the hormone-receptor dynamics has reached its unique equilibrium (studied in subsection *Hormone-receptor binding dynamics*). The model then considers the dynamical absorption of the resource which may enter each of two converting structures, or a storage structure insofar as the internal concentration of the resource is above a threshold. Past this threshold, the resource is instead released from the storage structure to maintain homeostasis. Storage comes with a cost, such that the amount of the resource released may be lower than the amount that had actually been stored (subsection *Energy allocation dynamics*). Resources entering a trait-converting structure immediately yield an increase in trait value, with a positive impact on fitness (subsection *Energy conversion into traits*). This physiological mechanism is controlled by four independent genes coding for two hormones and two receptors, whose expressions and conformations may change, respectively due to regulatory and coding mutations. These two types of mutation may impact the hormone-receptor dynamics, with potential effects on the resource allocation dynamics and thus on the traits values. We let this physiological mechanism change by mutation and recombination, and evolve under the influence of selection and drift as described in subsection *Evolutionary and population dynamics*, and analyze these simulation results as described in subsection *Estimates of shape parameters*.

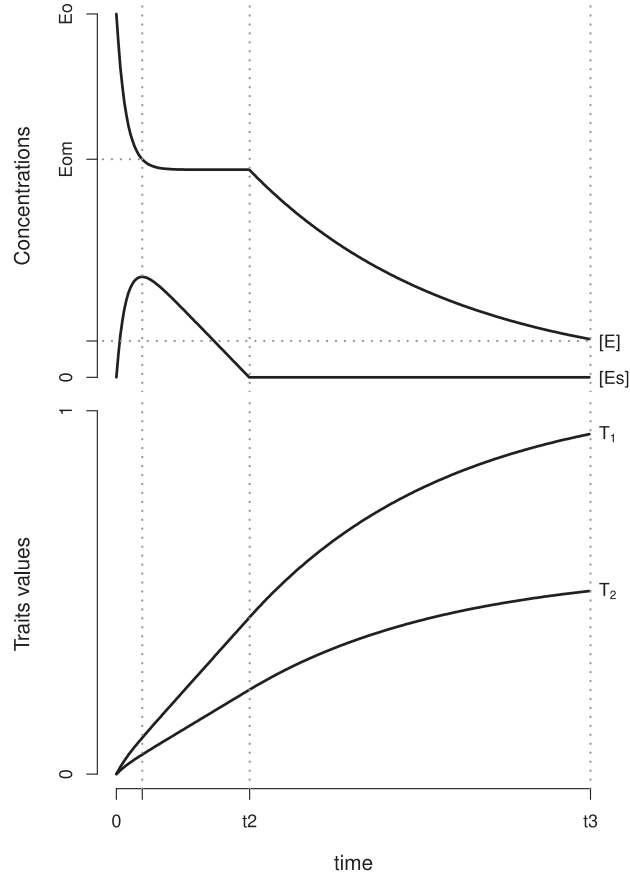


Figure 4.2: The final trait values, T_1 and T_2 , depend on the dynamics of the concentration of the resource circulating the organism, $[E]$, and stored, $[E_s]$. $[E]$ starts at E_o immediately after a unique meal, and decreases as the resource is both stored ($[E_s]$ increases) and used by the two converting structures (T_1 and T_2 increase). As $[E]$ decreases below a threshold E_{om} , the resource is released from the storage structure ($[E_s]$ decreases), which compensates for the energy used and converted into traits T_1 and T_2 . When no stored resource remains ($[E_s] = 0$), $[E]$ inevitably decreases (as T_1 and T_2 keep increasing), until it reaches a minimum threshold. The organism's final trait values T_1 and T_2 calculated at this point are used to assess its fitness. The hormones and receptors at the surface of the two converting structures control their resource intake, and thereby also the dynamics of $[E]$ and $[E_s]$, such that mutations of the expression and conformation of these proteins may change the organism's phenotype in a complex and unpredictable way. We used specific parameter values to obtain a clear illustration: $E_o = 1$, $E_{om} = 0.6$, $E_{min} = 0.1$, $a = 0.0001$, $C_{storage} = 0.1$, $\Omega = 0.1$, $b = 0.002$, $\omega = 0.1$

4.2.2 Hormone-receptor binding dynamics

Hormones are produced through the expression of two genes (identified by the subset $k \in \{1, 2\}$) by a specialized structure, followed by the export of their products. We consider peptidic hormones, whose conformations depends on single genes. Individuals are diploid, so two different hormones can be expressed by each gene ($p \in \{1, 2\}$). The internal concentration of the hormone expressed by the allele p of gene k is denoted $[H_{kp}]$.

Two structures converting energy into traits express receptors on their surface, which is assumed to remain constant throughout the energy allocation dynamics. These structures in our model may represent actual specialized organs in living organisms, like ovaries or testes for fertility (O'shaughnessy *et al.*, 1996), or receptor-equipped cells spread throughout the entire organism, like those forming the immune system for instance (Reppert *et al.*, 1994; Reiter *et al.*, 2000). The concentration of the receptor expressed by the allele q of gene i (with q and $i \in \{1, 2\}$) on structure j (with $j \in \{1, 2\}$) is denoted $[R_{iqj}]$ (we ignore the specific processes of production and degradation of receptors). Hormone H_{kp} and receptor R_{iq} form complexes at rate $k_{\text{on}_{iqkp}}$ (depending on their respective conformations, see *Hormone-receptor binding affinity*), and complexes dissociate at rate k_{off} . The concentration of complexes formed between receptor R_{iq} and hormone H_{kp} on structure j is denoted $[R_{iqj}H_{kp}]$. The hormone-receptor binding dynamics are described by the generic equations (4.1)-(4.5), where n_h , n_r and n_s are respectively the numbers of hormone- and receptor-coding genes, converting structures, and n_{ch} denoted the ploidy (here individuals are diploid and $n_{ch} = 2$). We set $n_h = n_r = n_s = 2$, such that a possible evolutionary outcome is that the energy allocation to each converting structures is regulated by one specific couple of hormone and receptor.

Free circulating hormones. The concentration $[H_{kp}]$ of hormone H_{kp} , increases as the hormone is produced at a rate α_{kp} (first term in equation (4.1)) and as hormone-receptor complexes dissociate at a rate k_{off} . Conversely, $[H_{kp}]$ decrease as complexes form at rate $k_{\text{on}_{iqkp}}$ – specific of hormone H_{kp} and receptor R_{iq} – and due to unspecific hormone degradation (last term), depending on the concentration S_D of free degradation sites and on the association rate k_d specific to degradation.

$$\frac{d[H_{kp}]}{dt} = \alpha_{kp} + \sum_{i=1}^{n_r} \sum_{j=1}^{n_s} \sum_{q=1}^{n_{ch}} \left((k_{\text{off}} \times [R_{iqj} H_{kp}]) - (k_{\text{on}_{iqkp}} \times [R_{iqj}] \times [H_{kp}]) \right) - k_d \times [S_D] \times [H_{kp}] \quad (4.1)$$

Free receptors. The number of free receptors increases as complexes dissociate and decreases as complexes form with each of the n_h hormones produced by the organism (equation (4.2)).

$$\frac{d[R_{iqj}]}{dt} = \sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} ((k_{\text{off}} \times [R_{iqj} H_{kp}]) - (k_{\text{on}_{iqkp}} \times [R_{iqj}] \times [H_{kp}])) \quad (4.2)$$

Hormone-receptor complexes. The concentration of hormone-receptor complexes increases as hormones bind receptors and decreases as complexes dissociate (equation (4.3))

$$\frac{d[R_{iqj} H_{kp}]}{dt} = k_{\text{on}_{iqkp}} \times [R_{iqj}] \times [H_{kp}] - k_{\text{off}} \times [R_{iqj} H_{kp}] \quad (4.3)$$

The concentration of receptor R_{iq} on structure j is constant ($[R_{iqj\text{tot}}] = [R_{iqj}] + \sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} [R_{iqj} H_{kp}]$), such that each dissociated complex releases a free receptor and each formed complex monopolizes a free receptor. Constant receptor concentration accounts for the allometric increase of organs with body volume (Charnov *et al.*, 2001), and we assume that the surface-to-volume ratio is sufficiently large that surface is not limiting.

Degradation structure. Our model includes a structure that degrades hormones non specifically (e.g. through endocytosis). The liver can be considered an example of such structure, which degrades the growth hormone (Baumann *et al.*, 1987), insulin (Duckworth *et al.*, 1998) and glucagon (Deacon, 2005). The dynamics of the concentration of free degradation sites $[S_D]$ and of degradation sites occupied by hormone H_{kp} , $[S_D H_{kp}]$ are described by equations (4.4) and (4.5). We further assume that degradation sites are instantly freed, as represented by the first term in equation (4.5).

$$\frac{d[S_D]}{dt} = \sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} (([S_D H_{kp}]) - (k_d \times [S_D] \times [H_{kp}])) \quad (4.4)$$

$$\frac{d[S_D H_{kp}]}{dt} = -[S_D H_{kp}] + (k_d \times [S_D] \times [H_{kp}]). \quad (4.5)$$

We set the total concentration of degradation sites $[S_D] + \sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} [S_D H_{kp}]$ to 9.9×10^{-4} M, and the rate of hormone internalization k_d to 4.10^{-5} s^{-1} (Pearce *et al.*, 1999); we did not allow these parameters to change by mutation. Other parameters in the model, however, can mutate and change the equilibrium concentration of the free hormone (*e.g.* the rate of production of each hormone), as described in the section *Evolutionary and population dynamics*. We randomly drew the values of mutable parameters at the beginning of each simulation from known distributions, and verified that the internal hormonal concentration at equilibrium is, initially, within the range $5.10^{-12} - 10^{-8}$ M, in accordance with experimental observations (Polonsky *et al.*, 1988; Zadik *et al.*, 1985).

Hormone-receptor binding equilibrium. Hormone-receptor binding dynamics eventually reach an equilibrium where $[H_{kp}]$, $[R_{iqj}]$ and $[R_{iqj}H_{kp}]$ are stable (equilibrium values are identified by a star in equations (4.6)-(4.8), obtained by solving the system formed by equations (4.1)-(4.5)).

$$[H_{kp}]^* = \frac{\alpha_{kp}}{k_d \times [S_D]^*} \quad (4.6)$$

$$[R_{iqj}]^* = \frac{[R_{iqj} \text{tot}]}{\sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} \left(\frac{[H_{kp}]^* \times k_{\text{on}_{iqkp}}}{k_{\text{off}}} \right) + 1} \quad (4.7)$$

$$[R_{iqj}H_{kp}]^* = \frac{[R_{iqj}]^* \times [H_{kp}]^* \times k_{\text{on}_{iqkp}}}{k_{\text{off}}} \quad (4.8)$$

Despite some simplifying assumptions detailed below, we consider that receptors in our model function like G protein coupled receptors, whose activation produces an internal signal for a limited amount of time, followed by their internalization and recycling into free receptors. Hormone-receptor complexes normally dissociate at a rate of the order of $10^{-4} - 10^{-5} \text{ s}^{-1}$ (Pearce *et al.*, 1999). We voluntarily over-estimated this rate ($k_{\text{off}} = 0.1$) to model the recycling that follows the endocytosis of hormone-receptor complexes. In our model, both the hormone and the receptor are released when recycling is complete. With this parameterization, a receptor is recycled within 10 minutes on average, which is biologically realistic (Von Zastrow & Kobilka, 1992). While mathematically convenient, considering that both the hormone and the receptor are released appears unrealistic, as recycling is most often followed by the degradation of the hormone. We verified that this assumption does not impact our results by performing additional numerical simulations

where only the receptor is recycled (see Figure SI1-1).

4.2.3 Energy allocation dynamics

Each individual takes a unique meal at t_0 which sets its internal concentration of an energetic resource $[E]$ to $E_o = 1$; resource acquisition is thus constant across individuals. As the acquired resource is consumed (*i.e.* allocated to traits) and stored, its internal concentration $[E]$ decreases until reaches an homeostasis threshold E_{om} below which energy is instead released from the storage structure. Storage comes with a cost $C_{storage}$ that decreases the overall amount of energy available.

The rate of resource consumption depends upon the activation of resource transporters at the surface of trait-converting structures. In our model, newly formed hormone-receptor complexes instantaneously stimulate these transporters and become inactive. The concentration of newly formed hormone-receptor complexes bound on formed on structure j per time unit at equilibrium equals:

$$a_j = \sum_{k=1}^{n_h} \sum_{i=1}^{n_r} \sum_{p=1}^{n_{ch}} \sum_{q=1}^{n_{ch}} (k_{on_{iqkp}} \times [R_{iqj}]^* \times [H_{kp}]^*) \quad (4.9)$$

which we multiply by a dimensionless constant $C_f = 1000$ to obtain the inward flow of energy for this structure.

The energy allocation dynamics consider temporal changes in $[E]$ as well as in $[E_s]$, the amount of the resource stored (divided by the same volume as $[E]$ and expressed as a concentration for simplicity). As described by the following ODEs (equations (4.10) and (4.11)), the internal concentration of the resource initially decreases as it enters the converting structures at a rate $a \times [E]$ (with $a = \sum_{j=1}^{n_s} a_j \times C_f$) and the storage structure at rate $b \times ([E] - E_{hom})$ (with $b = 0.01$). When $[E]$ decreases below the threshold E_{hom} , and as long as the stock of resource $[E_s]$ remains positive, the stored resource is released at a rate $b \times ([E] - E_{hom})$, thus maintaining homeostasis as the resource continues to be consumed by converting structures. We note that b designates both the storage and release rate which are considered equal in our model. Moreover, storage incurs a cost such that a part $C_{storage}$ of the resource is lost. The cost is paid at the time of storage, not release, which is mathematically equivalent but in accordance with the biological fact that storage often requires the formation of costly covalent bounds to produce large molecules (glycogens, fatty acids).

$$\frac{dE}{dt} = \begin{cases} -a \times [E] - b \times ([E] - E_{\text{hom}}), & \text{if } [E] > E_{\text{hom}} \text{ or if } [E] < E_{\text{hom}} \text{ and } [Es] > 0 \\ -a \times [E] & \text{otherwise} \end{cases} \quad (4.10)$$

$$\frac{dEs}{dt} = \begin{cases} b \times ([E] - E_{\text{hom}}) \times (1 - C_{\text{storage}}), & \text{if } [E] > E_{\text{hom}} \\ b \times ([E] - E_{\text{hom}}), & \text{if } [E] < E_{\text{hom}} \text{ and } [Es] > 0 \\ 0 & \text{otherwise,} \end{cases} \quad (4.11)$$

We solved these equations (see SI text 1) and obtained the temporal dynamics $[E](t)$ and $[Es](t)$ as illustrated in Figure 4.2.

4.2.4 Energy conversion into traits

The energy allocated to each structure is converted into an increase of trait value 1 (for structure 1) and trait value 2 (for structure 2). Traits are deliberately abstract, but their biological relevance is quite obvious. For example, energy allocation to reproductive structures can increase the quality and number of gametes, thus increasing fertility. Likewise, energy allocation to somatic maintenance or camouflage may increase survival. Here we assume that allocating a lot of energy to one structure (and thus for a given trait) per time unit decreases conversion efficiency. Such inefficiency is likely unavoidable, as converting a larger amount of resources in a short time period should require that larger structures be maintained, for instance. The observation that fast feeding larvae of *D. melanogaster* have lower fitness than slow feeding larvae may support this assumption (Foley & Luckinbill, 2001; Prasad & Joshi, 2003; Mueller *et al.*, 2005; Roff & Fairbairn, 2007), although it could also be partly explained by an increased efficiency of digestion in slow feeding larvae. The instantaneous increase in trait T_j is modeled as a Michaelis-Menten-like equation, such that conversion into trait is proportional to the influx $a_j \times [E]$ as influx goes to 0 and saturates to ω for infinitely large influx (see Figure SI1-8).

$$\frac{dT_j}{dt} = \frac{\omega \times a_j \times [E] \times C_f}{\Omega + a_j \times [E] \times C_f} \quad (4.12)$$

$\omega = 0.02$ is the maximum increase of trait value per dt , and $\Omega = 0.01$ sets the speed with which this function saturates. The expression of $T_j(t)$ directly follows from the dynamics of $[E](t)$ described above (see Figure 4.2). The combination of trait values

calculated at time t_3 is the phenotype of the individual, used to calculate its fitness (see below).

4.2.5 Evolutionary and population dynamics

The population is formed of $N/2$ females and $N/2$ males. Offspring production is modeled using a Wright-Fisher process (Fisher, 1930a; Wright, 1931), with discrete non-overlapping generations. In the neutral instance of our model, the probability that mother or father i is drawn equals $p_i = \frac{2}{N}$. In the model with selection, the probability p_i that parent i is drawn is proportional to its fitness W_i relative to other same-gender individuals in the population:

$$p_i = \frac{W_i}{\sum_{j=1}^{\frac{N}{2}} W_j} \quad (4.13)$$

where $W_i = T_{1i} + T_{2i}$. This definition of fitness produces an additive fitness landscape where the isoclines are linear. It is expected that trade-off relationships should align with these isoclines (and be linear) whenever selection plays a prominent role in their evolution. This choice of an additive fitness landscape is thus convenient, allowing us to detect deviations from this expectation by studying the curvature of trade-off relationships (see below). Nonetheless, we checked with additional simulations that considering a multiplicative fitness landscape – which is sometimes more appropriate, for example when considering the evolution of fecundity and survival in a semelparous species – has no impact on our general predictions (see SI Figure SI1-2, showing similar results as obtained with the additive landscape).

The genotype in our model comprises four independent genes encoding two receptors and two hormones. Each parent transmits one gamete to a descendant, formed by sampling, independently for each gene, an allele from one of the two homologous chromosomes. The female status in the next generation is attributed to exactly $N/2$ randomly sampled individuals; the other $N/2$ become males.

Hormone-receptor binding affinity. Allele q at the gene coding for receptor i determines its expression on each structure j , $[R_{ij\text{tot}}]$, and its conformation ΨR_{iq} . Similarly, allele p at the gene coding for hormone k sets its production rate α_{kp} and its conformation ΨH_{kp} . $[R_{ij\text{tot}}]$ and α_{kp} directly affect hormone-receptor dynamics in equations (4.1)-(4.8), and thereby the energy allocation dynamics. We consider that receptors and hormones can only take a finite number of conformation, corresponding to unique folding pathways that

mutations can modify by changing a protein's free energy landscape (Sapra *et al.*, 2008). These conformations (ΨH_{kp} and ΨR_{iq}) are modeled as positive integers in our model. They also impact hormone-receptor dynamics through their contribution to the specific on-rate constant, $k_{\text{on}_{iqkp}}$ defined by equation (4.14):

$$k_{\text{on}_{iqkp}} = \kappa \times e^{(-\rho \times |\Psi H_{kp} - \Psi R_{iq}|)} \quad (4.14)$$

The affinity between hormone H_{kp} and receptor R_{iq} is maximum when $\Psi H_{kp} = \Psi R_{iq}$, and decreases as the difference between these numbers increases.

Mutations. An allele's contribution to the protein's expression or conformation can mutate with probability $\mu = 10^{-4}$ per regulatory/coding sequence per generation. Mutations can change a receptor's concentration $[R_{iqj\text{tot}}]$ or an hormone's production α_{kp} and thus modify the hormonal dynamics and equilibrium. In this case, the mutant's value is multiplied by 10^γ , where γ is sampled from a normal distribution with mean 0 and standard deviation $\sigma_m = 0.5$. Hence, mutations on gene expression are as likely to increase as to decrease expression, but their lognormal distribution of effects induces an upward mutational bias: they multiply protein expression by 1.94 on average. This choice was motivated by mechanistic considerations about transcription factor binding, whose strength is known to increase exponentially with the number of nucleotides matching the optimal binding site (Lässig, 2007), and by the practical need to maintain positive gene expression.

We assessed the importance of this choice by running additional simulations with unbiased mutational effects sampled from a Gaussian distribution with mean 0 and standard deviation $\mu/4$, with μ the current expression rate of the mutated gene. The extremely rare mutations (probability of occurrence $< 3.2 \times 10^{-5}$) leading to a negative expression were considered to cancel it completely. This did not change the overall pattern obtained with biased mutations and selection, but it impacted the neutral simulations and (notably) reduced their mean standing genetic variance (SI Figure SI1-3).

Coding mutations can change the conformation of an hormone or a receptor by adding an integer sampled from a discretized normal distribution with mean 0 and standard deviation 1, and from which 0 values were excluded to avoid neutral mutations. Conformations were kept within the range $\{1; 1000\}$ by considering the extreme values, 1 and 1000, as immediate neighbors. Coding mutations impact protein's conformation and thus modify affinities between mutated protein and other components of the system.

Initial conditions. Each population at $t = 0$ is monomorphic, such that all individuals

share the same genotype and are homozygotes for all genes. We nonetheless introduced variation in the initial genotypes across replicate simulations, by mutating every coding and regulatory sequence once from an average starting genotype. This average genotype produces hormones at rate $\alpha_{kp} = 9.9 \times 10^{-10}$ M ($k, p \in \{1, 2\}$) and expresses $[R_{iqj\text{tot}}] = 9.9 \times 10^{-5}$ M receptors at the surface of structure j ($i, q, j \in \{1, 2\}$), and has hormones and receptors conformations $\Psi H_{1p} = 9$, $\Psi H_{2p} = 109$, $\Psi R_{1q} = 29$, and $\Psi R_{2q} = 129$ ($p, q \in \{1, 2\}$).

4.2.6 Estimates of shape parameters

As described in the previous section, the endocrine system can change by mutations and evolve. We expect that at least some of these mutations will have consequences on the shape of the trade-off between traits 1 and 2. We estimated these changes by fitting a circle to individual coordinates in the phenotype space formed by these traits. Because no causal relationship should be expected between trait 1 and trait 2, we performed an orthogonal regression to obtain estimates of the radius of the most fit circle $\hat{r}$ and the coordinates of its center (x_c, y_c) using a gradient descent method (Coope, 1993). We then used the fitted circle to quantify the curvature, the length and the position of the trade-off. As the method used is novel, we assessed its accuracy using datasets generated from circles with known properties (radius, center coordinates). We found that these properties are well estimated across the range of shapes obtained in our results (see SI Figure SI1-4).

The curvature increases as the radius of the fitted circle, or its logarithm $\log(\hat{r})$, decreases – obtaining $\log(\hat{r})$ is straightforward from the analysis. We established whether the trade-off is concave (curved inwards when looked from the origin) or convex by calculating the angle of each individual coordinate on the fitted circle, and checking that the median c belongs to $]3\pi/4, 7\pi/4[$ (in which case the trade-off is convex; see details in Figure 4.3). The length l – which is representative of the standing genetic variation in the population – is the distance between the two most distant orthogonal projections on the fitted circle. To obtain it, we first calculated the angle of each individual i as $\beta_i = \text{sign}(T_{2i} - y_c) \times \cos^{-1}(\frac{T_{1i} - x_c}{r_i})$, with r_i the Euclidean distance between (T_{1i}, T_{2i}) and (x_c, y_c) . Then we calculated $l = \hat{r} \times (\max(\beta) - \min(\beta))$. We then calculated the median angle among the values of β_i , which we used to calculate the Manhattan distance between the orthogonal projection of this “median” individual on the fitted circle and the origin $(0, 0)$. Note that this distance would equal the population mean fitness if

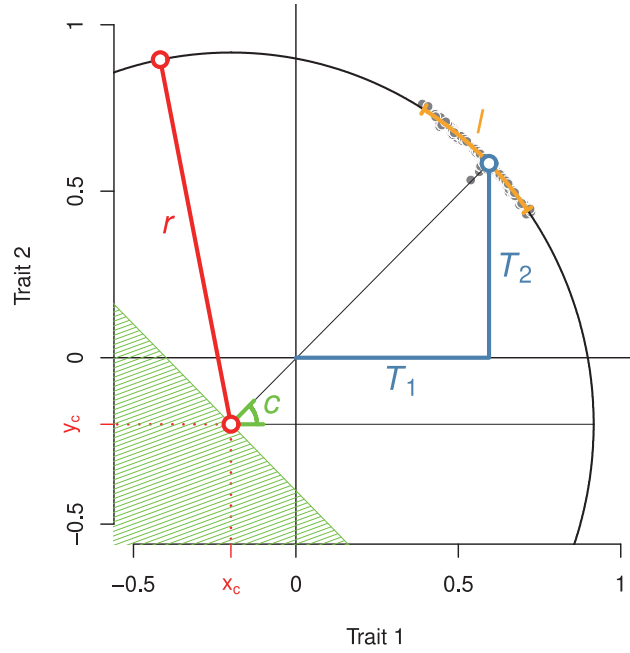


Figure 4.3: We extracted three descriptive parameters by fitting a circle to the phenotypes (trait 1, trait 2) at the end of our simulations. The parameters are l (the length of the trade-off – in orange), $\hat{r}$ (the circle’s radius – in red) and c (the median angle, from which we determine the concavity of the trade-off – in green). We minimized the squared distances of the orthogonal projection of individual datapoints to obtain $\hat{r}$ and the center of the circle (x_c, y_c) . Then we calculated the angle c as the median among the angles calculated for all datapoints. The trade-off is considered convex if c is between $3\pi/4$ and $7\pi/4$ (green area) and concave otherwise. We calculated d as the Manhattan distance (the sum of $T_1 + T_2$ in blue) between the origin of the graph and the median individual. l is the distance along the circle between the two points with the most extreme angles (see the *Material and methods – Estimates of shape parameters* section for a precise method description)

individuals were aligned on a fitness isocline.

4.3 Results and Discussion

4.3.1 Initial investigation of the optimal energy allocation dynamics

Our general aim of analyzing how trade-off relationships evolve involves a detailed analysis of how genetic variation builds up. This requires that we consider mutations of hormone- and receptor-coding genes, allowing populations to reach new mutational neighborhoods. Before going into this (necessary) complexity, it seems appropriate to bypass the genetic level and get a glance at which allocation strategy is optimal.

The allocation to trait T_j depends on the inward flux of the resource into the trait-converting structure j , a_j – in the full model, a_j depends on the concentration of hormone-receptor complexes formed at the surface of structure j . Let us consider an organism that

allocates resources equally to both traits, so $a_1 = a_2 = a$. The resulting trait value is obtained by replacing a_j with a in equations (4.9) to (4.12). Due to the equal allocation to both traits, and because selection is directional, maximizing trait value $T_1 = T_2 = T$ also maximizes fitness in this simple example.

We find that the optimal allocation rate is extremely low when storage is free of cost, because the resource can then be used extremely slowly and with maximum efficiency (see SI Figure SI1-5). The optimal allocation changes when we consider that storage incurs some cost, represented by parameter C_{storage} in our model. In nature, this cost may arise from two distinct sources. First, large molecules need to be produced for storage and later broken down at the time of release (Hers, 1976; Meyers, 1995; Alonso *et al.*, 1995); second, maintaining storage structures can be costly and the added weight and volume might have negative consequences for the organism's fitness. The cost of producing storage molecules is likely dependent on the resource: various types of molecules are ingested during a meal, with specific storage constraints (Williams *et al.*, 1963). Carbohydrates like glucose, for instance, are stored as glycogen, which is rapidly converted back into an energetic resource but requires a large amount of water – and thus a large volume and weight – for storage, contrary to lipids that are stored as fatty acids (Schmidt-Nielson, 1997). Specific advantages and disadvantages associated with each resource may translate into specific storage costs, likely in interaction with the ecology of the species considered. For example, the added weight might have a moderate fitness impact for marine species compared to aerial ones.

As the storage cost increases, we find that the optimal speed of allocation increases, as a slow consumption requires that some of the resource be stored. The optimal allocation dynamics is thus the result of a balance between the loss of efficiency due to fast resource consumption and the cost associated with resource storage, which changes with C_{storage} in our model. As shown below, this change in storage cost also has profound effects on the genetic variation maintained in our simulated populations.

4.3.2 Trade-off shapes do evolve

We now consider how the regulatory mechanism underlying resource allocation evolves, through mutations changing the expression and conformation of hormones and receptors, selection on the traits, and genetic drift. A representative example of the evolutionary dynamics when the traits are under directional selection is shown in figure 4.4-a. The

population is initially monomorphic, so it occupies a single point on the phenotype space with low values of traits 1 and 2. Then genetic variation builds up in the population, forming an almost linear relationship in the phenotype space ($t = 2000$ in Fig. 4.4-a). Around timestep 3000 trait 2 increases following a trajectory nearly parallel to the y-axis – in about half the simulations trait 1 instead increased. This change conveys a very sharp fitness increase because the population reaches a different isocline in the fitness landscape. Then the population roughly follows this isocline toward a point where trait 1 equals trait 2 ($t = 10000$ in Fig. 4.4-a). This move in the phenotype space is associated with a slight increase in fitness, due to the higher efficiency of resource conversion when the two trait-converting structures are equally employed.

In order to identify systematic changes in the shape of the trade-off, we fit a circle to the combinations of traits that coexist at any time point in our simulations (see Estimates of shape parameters in the Model section and figure 4.3). The trade-off curvature decreases as the circle’s radius $\hat{r}$ increases; we thus use $\log(\hat{r})$ as a negative proxy for curvature. We also calculated the mean Manhattan distance d (the sum of the two traits from the origin) of the population. By definition d is close to the population mean fitness in the non-neutral model, which explains why it increases during the simulation (Figure 4.4-b). We also estimate the trade-off length as the length of the arc circle joining the two most distant projections of datapoints on the fitted circle.

Individuals in the population are initially distributed along a fitness isocline, such that the trade-off is almost linear (as shown by the fitted circle’s high radius; Fig. 4.4-c). Then the curvature rapidly increases in all the simulations, toward $\log \hat{r} \approx 1$. At the end of the simulation, all estimated trade-offs are concave (Figure 4.4-b, SI Figure SI1-6) – see legend of Figure 4.3 for a precise definition. The mean trade-off length across simulations quickly stabilizes around 0.6 (Figure 4.4-d).

We ran neutral simulations, where individuals have equal fitnesses regardless of their phenotype, with parameters identical to those used in Figure 4.4 (Figure SI1-7). The difference in evolutionary outcomes is striking: in the neutral case, half of the trade-offs are convex – while all are concave under selection –, and trade-offs are more linear and longer on average than under selection (see details of the determination of convexity in the legend of Figure 4.3).

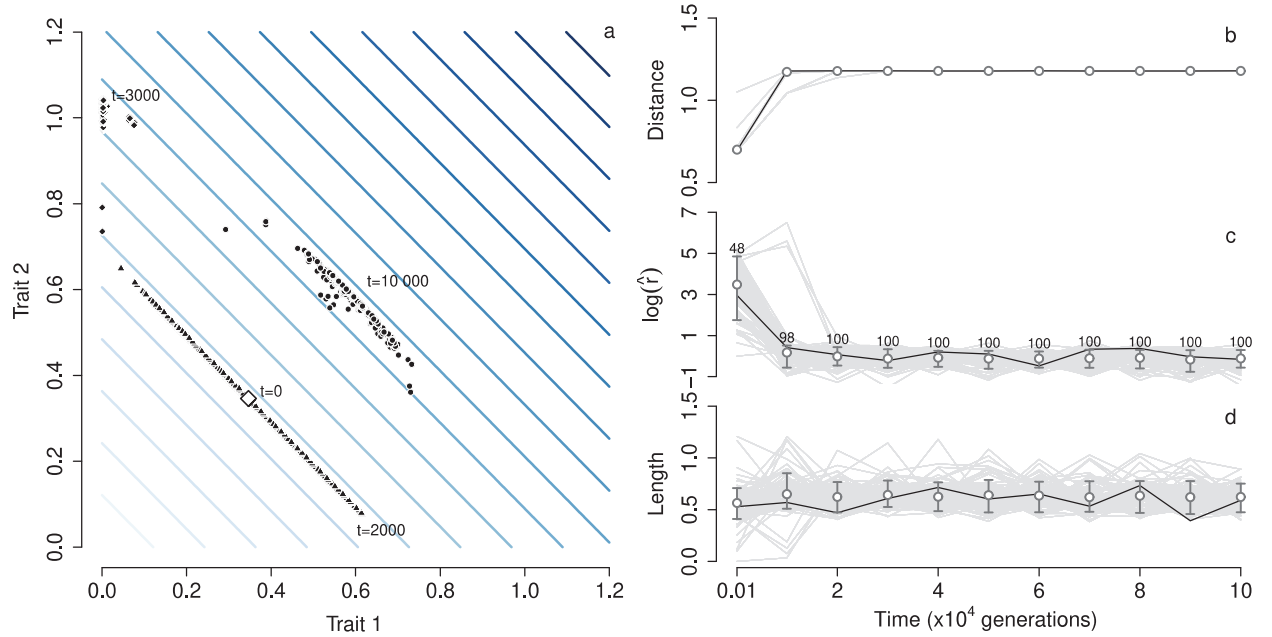


Figure 4.4: Trade-off shapes evolve in simulations where mutations change hormones' and receptors' conformation and expression, and selection acts depending on the resulting trait values. Panel a shows a few important timesteps in one representative simulation. In the background the fitness landscape appears, blue lines representing fitness isoclines. The more lines are dark, the more they correspond to better fitness values. We defined three parameters to study changes in shape systematically in an ensemble of 100 identically parameterized simulations, by fitting a circle to sets of points at regular timesteps (see text and *Material and methods* section- *Estimates of shape parameters*). Panel b shows the temporal dynamics of the euclidean distance of the median individual (sum of the two traits values). The natural logarithm of the fitted circle's radius (inversely related with the trade-off curvature) is represented in panel c, and the length of the trade-off (*i.e.* the distance along the circle between the two most distant projections) is represented in panel d. 99 replicate simulations are represented in grey in panels b-d, and the simulation in panel a is outlined in black. Dots and bar represent respectively the mean and quantiles (0.1 and 0.9) of each parameter's distribution (across simulations) at each timestep. Standard parameter values were used (as defined in the *Material and methods* section), and $C_{\text{storage}} = 0.5$.

4.3.3 The evolution of trade-off shapes depends on storage cost

We varied the storage cost between 0 (storage is free) and 0.9 (90% of stored resources are lost), and found that the evolutionarily expected shape parameters drastically change in response (Figure 4.5). As the cost increases, a larger part of the resources are inevitably wasted, which can be seen even in the neutral simulations as a decrease in the sum of trait values d generated from the resource consumed (Fig. 4.5-b). The decrease in the distance d is much less pronounced when the traits are under directional selection; in fact, d in the model with selection closely matches the prediction of the simpler analytical model derived above (compare triangles and circles in Fig. 4.5-a), suggesting that the genetic determinism does not prevent the optimization of the speed of resource consumption. Likewise, when the storage cost is high, selection favors rapid converters (as shown in Fig. SI1-8) that allocate a similar amount of energy to each trait. A deviation from this ideal allocation pattern would shift the speed of resource consumption of both structures away from their optima: one would receive a larger-than-optimum flux of resources and convert them less efficiently, while the other would get a lower-than-optimum flux, requiring costly storage. In the process, the sum $T_1 + T_2$ decreases as compared to its value when $T_1 = T_2$, creating a deviation from a linear relationship quantified by the curvature of the trade-offs – *i.e.* low values of $\log(\hat{r})$. The curvature increases when the storage cost increases (Fig. 4.5-b), which may partly be explained by slower resource consumption incurring direct storage costs under unequal resource allocation. As shown above, increasing C_{storage} also increases the optimal speed of resource consumption, in such a way that the efficiency of the resource-trait conversion becomes highly non-linear (see Fig. SI1-8). Allocating unequally to the traits is thus associated with a loss in the sum of trait values, more pronounced as C_{storage} increases. The trade-off length, representative of the population's standing genetic variation, also decreases with increasing C_{storage} (Fig. 4.5-c), likely due to extreme individuals on these highly curved trade-offs being efficiently purged by selection.

In the neutral case, selection does not optimize the speed of resource allocation and; in consequence, trade-offs tend to be linear on average. This explains that our method estimated that they are convex in about half the simulations (Figure 4.5-c and Figure SI1-6; see legend of Figure 4.3 for a short description of the method and the model section for more details). In contrast, trade-offs are systematically concave under selection when storage costs exceed 0.1. Nonetheless, the curvature of trade-offs (inversely related to the radius of the fitted circle) slightly decreases as the storage cost increases in the neutral

case. This is likely due to the upward bias in regulatory mutations, as the mean curvature remains constant when this bias is eliminated (Fig. SI1-3). Likewise, the trade-off length decreases slightly as the storage cost increases in the neutral model (Fig. 4.5-c), but much less so when the bias is eliminated. There remains a small decrease in standing genetic variance as the storage cost increases, possibly because the population is overall closer to the origin in this situation, restricting the range of positive trait values along the trade-off. This effect in the neutral case is so small compared to the effect of selection on genetic variance that it appears negligible.

We have performed additional sensitivity analyses to three parameters: the population size, the mutation rate and the level of gene expression noise (Figs. SI1-9 - SI1-11). All three parameters increase the variation among individuals and thus the length of the trade-off, and in the case of noise this increase in length coincides with a slight decrease in the curvature of the trade-off, which may have two distinct causes. First, more linear trade-offs may be selected as they make the organism robust to noise (Wagner, 2005; Draghi & Whitlock, 2015). Indeed, if the trade-off is more linear and aligns with the fitness isoclines, perturbations of gene expression levels still move individuals along trade-offs, but these moves have moderate fitness consequences. Second, noise has the effect of diminishing the strength of selection (Mineta *et al.*, 2015). Hence, as noise increases in our simulations, it is possible that selection on the curvature weakens, such that the curvature decreases towards the neutral expectation (see Figure SI1-9). Although we cannot entirely rule out this second hypothesis, the reduced variance among simulations under strong noise suggests that robustness may play some role in flattening trade-offs.

4.3.4 The level at which evolution converges

At this point we need to introduce the classical heuristic of genotype networks, which are made of nodes (each one a genotype) connected by mutations or recombination events (Smith, 1970; Draghi *et al.*, 2010; Rajon & Masel, 2013). Typically, a genotype has a restricted number of neighbors relative to the total number of nodes in the network. A population with non-zero standing genetic variation (SGV) occupies a certain number of nodes. Since SGV arises from short-lived mutations and recombination events (Barrett & Schluter, 2008; Lerouzic & Carlborg, 2008), the population likely occupies a small subset of the whole genotype space at any point in time. This subset defines the features of SGV, including a trade-off's shape. In the longer term, a population might make larger moves

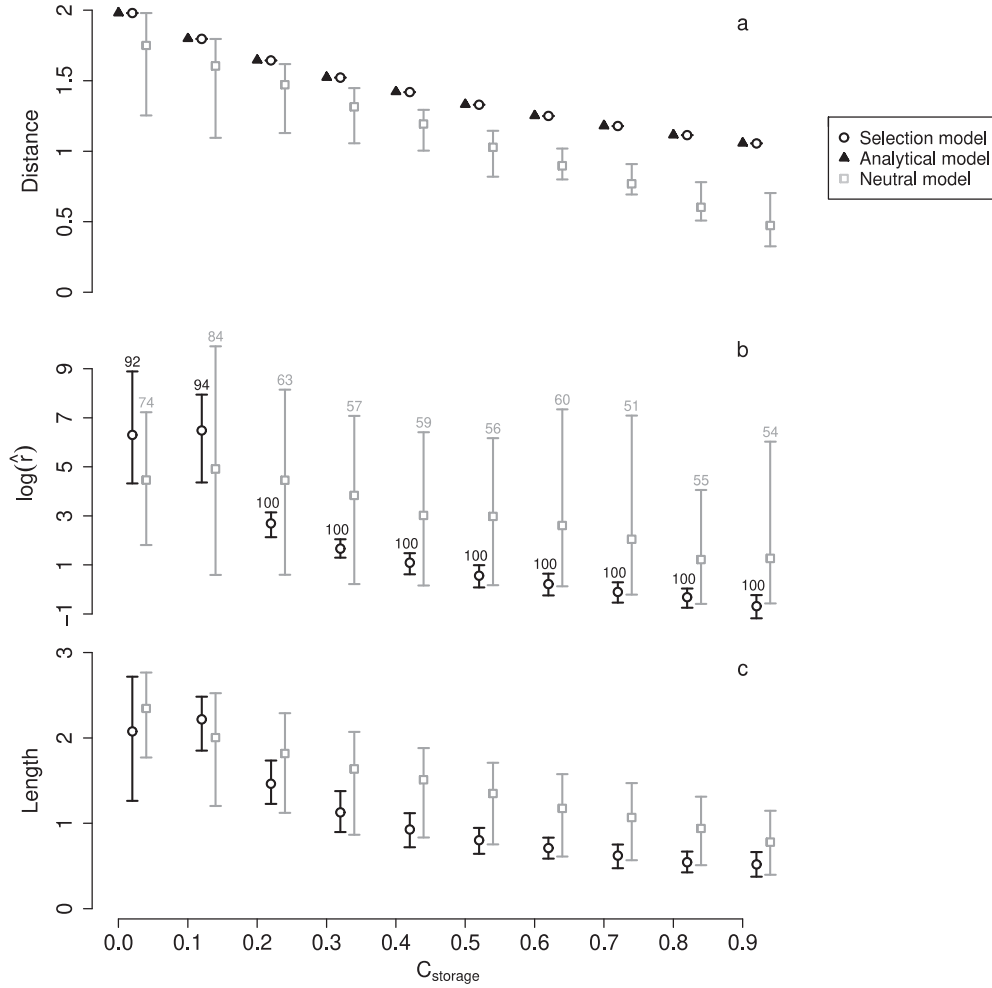


Figure 4.5: Evolutionarily expected shape parameters may depend on the cost associated with storage C_{storage} , due to selection. We analytically predicted the distance (sum of median traits values) and represented it with triangles. They perfectly overlap values obtained with the selection model (open circles). The impact of selection appears by comparing the evolutionary expectations of a model with directional selection on both traits (in black) with those of a neutral model (in grey; individuals have identical fitnesses in this model, independent of their trait values). Definitions for the shape parameters can be found in the text, in the *Material and methods* section *Estimate of shape parameters*, and in Figs. 4.4,4.3.

in the genotype space, changing its mutational neighborhood and thereby, possibly, the shape of its trade-off(s).

As we have seen, *e.g.* in Figure 4.5, evolution is clearly convergent at the phenotype level under selection – similar trade-off shapes evolve in similar contexts. We next ask whether evolution is also convergent at the genetic level, which would mean that organisms confronted to a similar problem have evolved identical molecular solutions (Garland Jr & Carter, 1994). In order to test this hypothesis, we performed a multivariate analysis on the variables characterizing the genotype in our simulation endpoints, that is the raw expressions and conformations of hormones and receptors. We performed a Principal Component Analysis (PCA), which revealed no apparent association between the variation in the genotypic variables and the storage cost (Fig. SI1-12). It indicates that the variation between populations in a similar context (*i.e.* with the same $C_{storage}$) is large compared to the variation between contexts. This means that evolution has found a variety of genetic solutions when confronted to a similar problem.

These results lead to the conclusion that evolution is not convergent at the genotype level. While this prediction contrasts with some empirical observations in unicellular organisms where genetic convergence can be common (*e.g.* Tenaillon *et al.* (2012)), it is consistent with empirical observation in a different protein confronted to similar selection pressures: hemoglobin. Indeed, a study in birds (Natarajan *et al.*, 2016) has revealed that convergent evolution at the phenotypic level – hemoglobin’s affinity for oxygen increases with altitude – but not at the genotype level, as different mutations are involved in different clades. More generally, our theoretical result is consistent with observations that convergence is more likely at the phenotypic level, which is sometimes interpreted as a strong role of selection over mutation (Lenormand *et al.*, 2016).

Moreover, our choice to model the evolution of peptide hormones, whose conformations rely directly on single coding sequences, may impact the structure of the genotype space. Peptide hormones are common – they include the growth hormone (Niall *et al.*, 1971), insulin (Barta *et al.*, 1981) and glucagon (Drucker & Asa, 1988) – but so are other important regulators like steroid hormones including testosterone (Shanies *et al.*, 1972), progesterone (Channing, 1966), cortisol (Goldsmith *et al.*, 1967) and ecdysone (Pankotai *et al.*, 2010). The expression and conformation of these non-peptide hormones may change by mutations that contribute indirectly through changes in metabolic pathways. This might reduce the number of possible conformations of non-peptide hormones and the probability that a mutation changes the conformation, which might contribute to the

apparent conservation of the endocrine system at large timescales (Tatar *et al.*, 2003; Barbieri *et al.*, 2003).

4.4 Conclusion

We have built a model for the evolution of the endocrine system and its consequences on a trade-off's shape. Selection plays a crucial role in this evolution: neutrally, a population would move on the genotype space towards locations where the trade-off is flat, while selection makes the trade-off curved and concave. The expected curvature, as well of the amount of standing genetic variation, should depend on physiological and ecological variables impacting the cost of resource storage.

Genetic variation builds up depending on mutational effect, which are themselves contingent on the structure of the genotype-phenotype map. In our model, mutations have cascading effects on phenotypic traits through changes in the endocrine system. The main benefit of this approach is that it make movements on the genotype space (either through mutation or recombination) and the effects of environmental noise more realistic. The debate over the introduction of mechanistic assumptions into evolutionary models dates from the origins of the modern synthesis, when Wright argued against Fisher in favor of physiologically realistic models to explain the prevalence of recessive mutations (Wright, 1929, 1934; Fisher, 1928). The major role of physiology in the evolution of dominance is generally accepted today (Bourguet, 1999), and Wright's proposal of introducing physiological details in evolutionary models has been followed to some extent in theoretical evolutionary genetics (e.g. Bost *et al.* (1999); Kacser & Burns (1973)). Our work suggests that this approach might also be fruitful in the field of evolutionary ecology.

Few empirical data are readily available to test our predictions, especially because trade-offs are often detected by fitting linear relationships. Roff and collaborators (2002) studied the trade-off between dispersal and reproduction in *Gryllus firmus*, and found that it had changed in one population raised in laboratory during 20 years (compared to natural populations). Unfortunately, variation along the studied trade-off is discrete with two morphs, which is an obvious obstacle to estimate the shape of the relationship. Another empirical issue is that large numbers of individuals should be required to estimate precise relationships. Indirect methods might thus be mandatory, especially in field studies; for instance, it should be possible to estimate the shape of the relationship by combining a method estimating the selection surface (*i.e.* the relationship between the traits and

fitness) and rough estimates of trait distributions.

Once we have resolved these issues, it should be straightforward to test, through intra- and interspecific comparisons of trade-off relationships, our prediction that their evolutionarily expected curvature should depend on characteristics of the resource that is traded and on the ecology of the species considered. For instance, glucose storage is likely more expensive than lipids storage, such that our prediction that trade-off relationships should be more curved for higher storage cost may be tested by comparing the relationships between traits relying on the two resources. Specific predictions could even be formulated if the actual costs are quantified in parallel. A comparison between species may also be relevant, since the costs associated with storing a given resource should change with the ecological context. For instance, storage should be more expensive for a flying species, which may generate a specific pattern in phylogenies towards more curved trade-off relationships following the evolution of flight.

Authors contributions: SB, ER and FM designed the study. SB performed the modeling and analyzed the results under ER's supervision. LJ contributed to the statistical analysis of simulation results. SB and ER wrote the manuscript, and FM critically revised several versions of it. All authors have read and approved the final version of the manuscript.

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Data archiving: The source code for simulations and results analysis is available on a public repository Dryad (DOI : [doi:10.5061/dryad.6t4s455](https://doi.org/10.5061/dryad.6t4s455) or at <https://doi.org/10.5061/dryad.6t4s455>). A preprint of the manuscript is available on bioRxiv.

4.5 Addendum

How heritability evolves

Trade-off shape parameters such as the curvature remain rarely estimated in field or laboratory experiments. Heritability, however, is a common metric used in these studies to quantify the amount of variation along a trade-off on which selection may act. We quantified narrow sense heritability h^2 as the ratio of the additive genetic variance to the whole phenotypic variance in our simulations. Strikingly, this parameter is almost independent to the cost of storage (fig. 4.6-a) once this cost is above 0.1. The expected value of h^2 is then close to 0.5, which corresponds roughly to the most expected value in a compiled dataset of morphological traits (see figure 3 in ref. Arnold (2014)).

While our predictions seem to match empirical observations on average, variance in observed values of h^2 is far greater than in our simulations, which is unlikely to be explained only by sampling noise. This illustrates that different traits may have different heritabilities, for instance due to particular ecological and population contexts. At this point, we are unable to consider this ecological complexity using our model of arbitrary traits evolving in an oversimplified context. Nonetheless, we can introduce a potentially important factor for the evolution of heritability that directly impacts the underlying physiological mechanisms: noise in gene expression. In figure 4.6-a as in the rest of the results presented so far, no noise was considered, such that the phenotype was entirely dependent on the genotype – h^2 is then lower than 1 due to dominance and epistasis. We modeled noise in gene expression by multiplying each gene expression by 10^ϵ , where ϵ is sampled from a normal distribution with mean 0 and standard deviation σ_n . As shown in figure 4.6-b, increasing σ_n decreases h^2 in a threshold-like manner: below $\sigma_n = 10^{-1}$, heritability remains close to its value in the absence of noise, while above this threshold h^2 rapidly vanishes – for comparison, mutations are modeled using a standard deviation $\sigma_m = 0.5$, such that if noise had the same effect on hormone production as mutations h^2 would be close to 0.

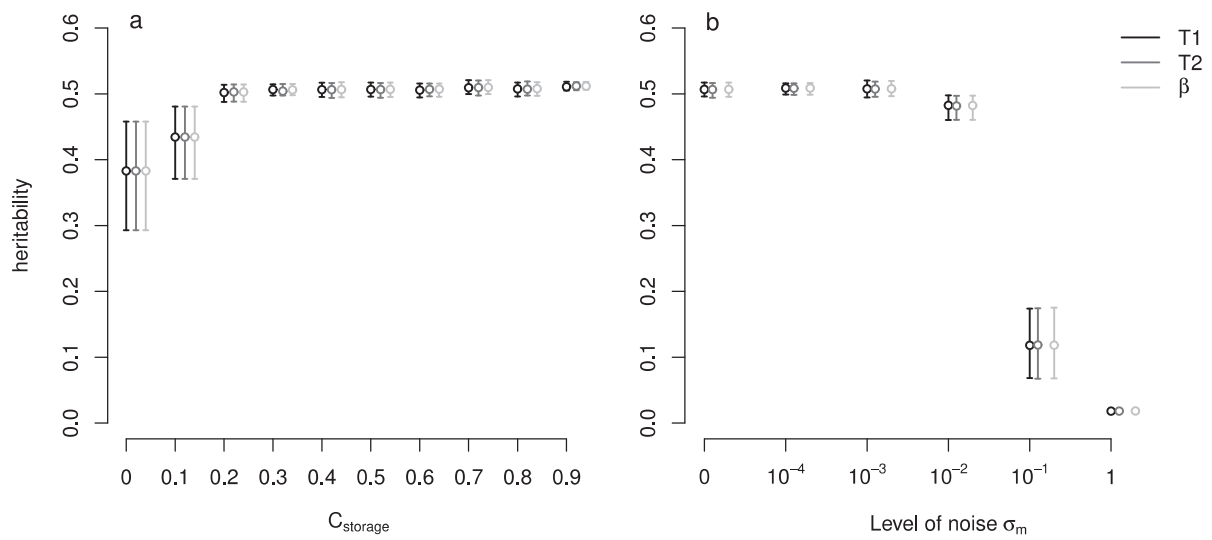


Figure 4.6: Evolutionarily expected heritabilities (narrow sense, h^2) are almost independent of the storage cost C_{storage} but are strongly impacted by increasing levels of noise (σ_n) in gene expression. h^2 was calculated as the ratio of the genetic additive variance to the phenotypic variance in populations evolved over 100000 generations, giving a distribution of values for each context ($C_{\text{storage}}, \sigma_n$) considered. We represented the means (dots) and quantiles (0.1 and 0.9, bars) of these distributions. Performing calculations on trait 1 (T_1), trait 2 (T_2), or on the angle of each individual on the fitted circle (β , see *Material and methods* section 4.2.6) has no visible impact on the results.

4.6 Supplementary information for the article “Hormonal pleiotropy and the evolution of allocation trade-offs”

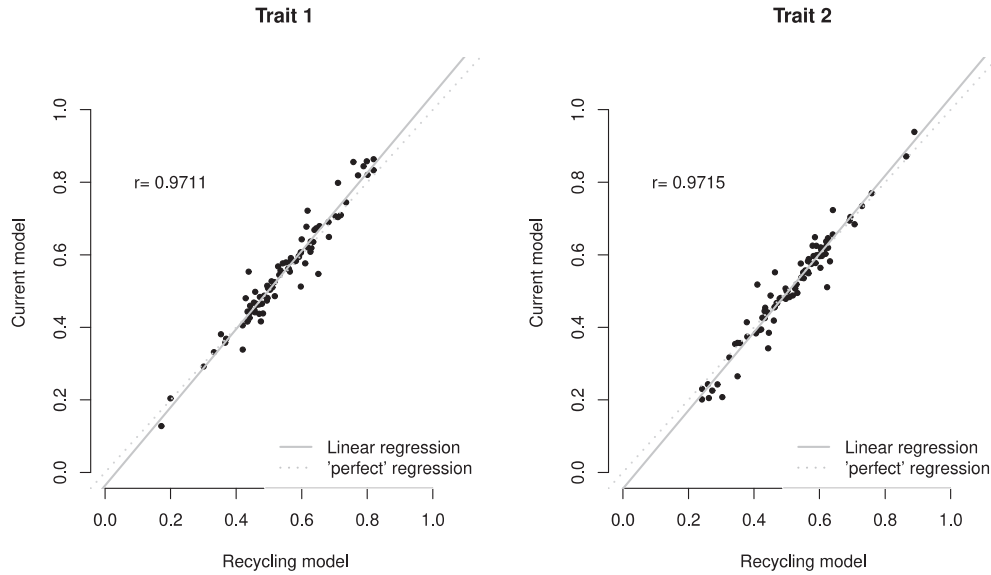


Figure SI1-1: Comparison of fitnesses obtained in our standard model with those obtained in a model where receptors only are recycled – instead of both the hormone and the receptor being recycled. In this latter model, eq. [1] becomes $\frac{d[H_{kp}]}{dt} = \alpha_{kp} - \sum_{i=1}^{n_r} \sum_{j=1}^{n_s} \sum_{q=1}^{n_{ch}} \sum_{p=1}^{n_{ch}} \left(k_{on_{iqkp}} \times [R_{iqj}] \times [H_{kp}] \right) - k_d \times [S_D] \times [H_{kp}]$, such that the term corresponding to the hormone released after complex dissociation is canceled. Dots correspond to different genotypes, which were randomly generated by simulating three mutations from the initial conditions defined in the main text. The line represents the situation where both models yield identical fitnesses.

SI text 1 – Solution for the energy allocation dynamics

Equations (10) and (11) in the main text yield three distinct phases for the energy allocation dynamics, as illustrated by figure 1.

Phase 1. As stated in the main text, phase 1 starts at t_0 (the meal) and goes on as long as $[E] > E_{\text{hom}}$, $E_{\text{hom}} = 0.08$ being a concentration threshold above which energy is stored. At t_0 , $[E] = E_0$ and decreases until $[E] = E_{\text{hom}}$ (t_1). From equation (10), we obtain

$$[E](t) = \frac{b \times E_{\text{hom}}}{a + b} \times (1 - e^{-(a+b) \times t}) + E_0 \times e^{-(a+b) \times t}. \quad (4.15)$$

We find t_1 from the equality $[E](t_1) = E_{\text{hom}}$:

$$t_1 = -\ln\left(\frac{E_{\text{hom}} \times a}{E_0 \times (a + b) - b \times E_{\text{hom}}}\right) \times \frac{1}{a + b} \quad (4.16)$$

Finally, substituting $[E](t)$ in equation (11) we obtain:

$$[E_s](t_1) = \frac{b \times (1 - C_{\text{storage}})}{a + b} \times \left(-a \times E_{\text{hom}} \times t_1 + \left(E_0 - \frac{b \times E_{\text{hom}}}{a + b}\right) \times (1 - e^{-(a+b) \times t_1})\right) \quad (4.17)$$

Phase 2. Here the resource is released from the storage structure until $[E_s](t) = 0$ (t_2).

At this point, we have:

In order to obtain t_2 , we assume that $[E]$ is constant during this phase, so $\frac{d[E]}{dt} = 0$ and $b([E] - E_{\text{hom}}) = -a[E]$. Therefore,

$$\frac{d[E_s]}{dt} = -a[E]. \quad (4.18)$$

From equation (4.18) we obtain

$$[E_s](t) = [E_s](t_1) + a \times t_1 \times \frac{bE_{\text{hom}}}{a + b} - a \times E_{\text{hom}} \times t, \quad (4.19)$$

which equals 0 at t_2 . We thus find t_2 :

$$t_2 = ([E_s](t_1) + a \times \frac{b \times E_{\text{hom}}}{(a + b)} \times t_1) \times \frac{(a + b)}{a \times b \times E_{\text{hom}}}. \quad (4.20)$$

Finally we calculate:

$$[E](t_2) = \frac{b \times E_{\text{hom}}}{a + b} \times (1 - e^{-(a+b) \times t_2}) + E_o \times e^{-(a+b) \times t_2} \quad (4.21)$$

Phase 3. Phase 3 begins when $[E_s]$ reaches 0, such that $[E]$ decreases until it reaches the critically low value $E_{\text{min}} = 0.01$ (t_3). During this phase, we find from equation (10) that

$$[E](t) = \frac{b \times E_{\text{hom}}}{a + b} \times \frac{e^{-a \times t}}{e^{-a \times t_2}}, \quad (4.22)$$

and we find t_3 by substituting $[E](t_3)$ by E_{min} in equation (4.22):

$$t_3 = \frac{\ln(E_{\text{min}} \times \frac{(a+b)}{b \times E_{\text{hom}}}) - a \times t_2}{-a} \quad (4.23)$$

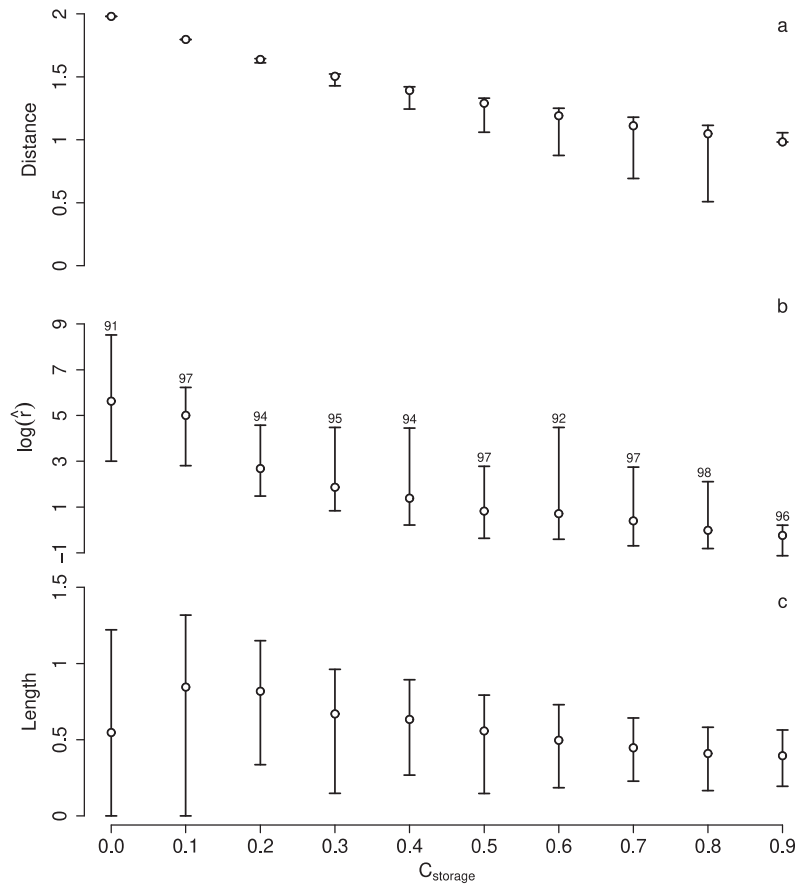


Figure SI1-2: Shape parameters for different storage cost in the model with multiplicative fitness. We observed the same pattern as with our current model : as the cost increases, populations reach lower trait values and express small and more curved trade-offs.

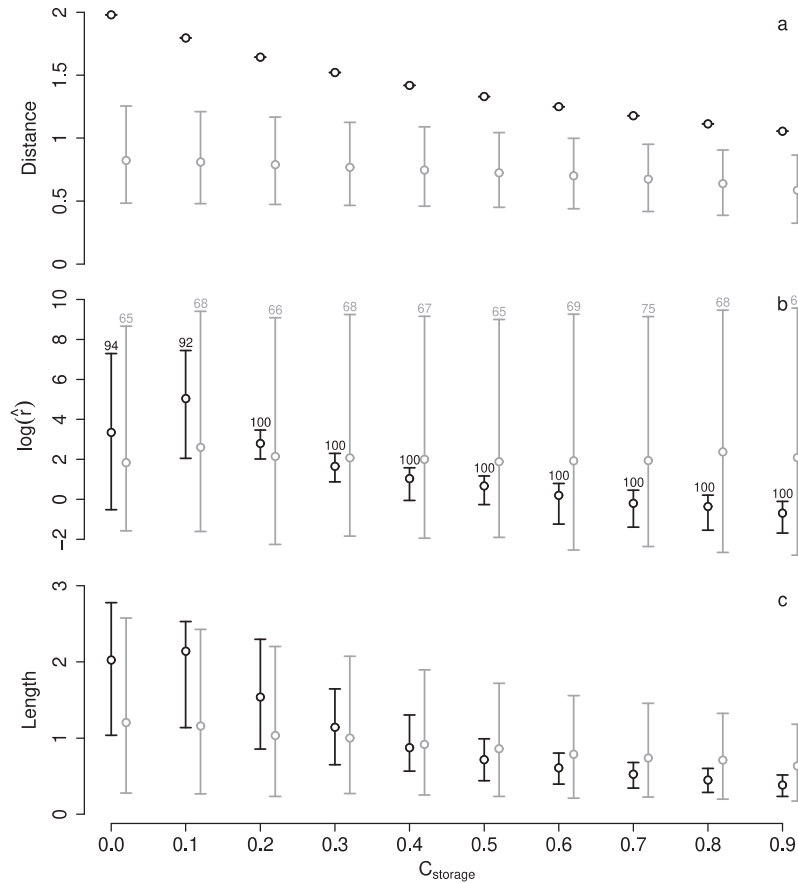


Figure SI1-3: Shape parameters for different storage cost in the model without mutational bias (model with selection in black , neutral model in grey). In this model, we ran 100 simulations per storage cost value with an adjustable Gaussian mutation effects. In order to avoid a bias induced by negative concentration values, we modified the mutational process. For each mutation, the concentration value of hormones or receptors is sampled in a Gaussian law (μ, sd) , where μ corresponds to the concentration value before mutation and sd equals $\frac{\mu}{4}$. The impact of selection appears by comparing the evolutionary expectations of a model with directional selection on both traits (in black) with those of a neutral model (in grey ; individuals have identical fitnesses in this model, independent of their trait values). Dots and error bars represent the mean and between-quantile difference ($q(0.9) - q(0.1)$) of the distribution of shape parameters calculated in 100 replicate populations. On panel b the percentage of concave trade-offs is added on the top of each error bars. The different tendencies observed in the standard model are also found with this distribution of mutational effect.

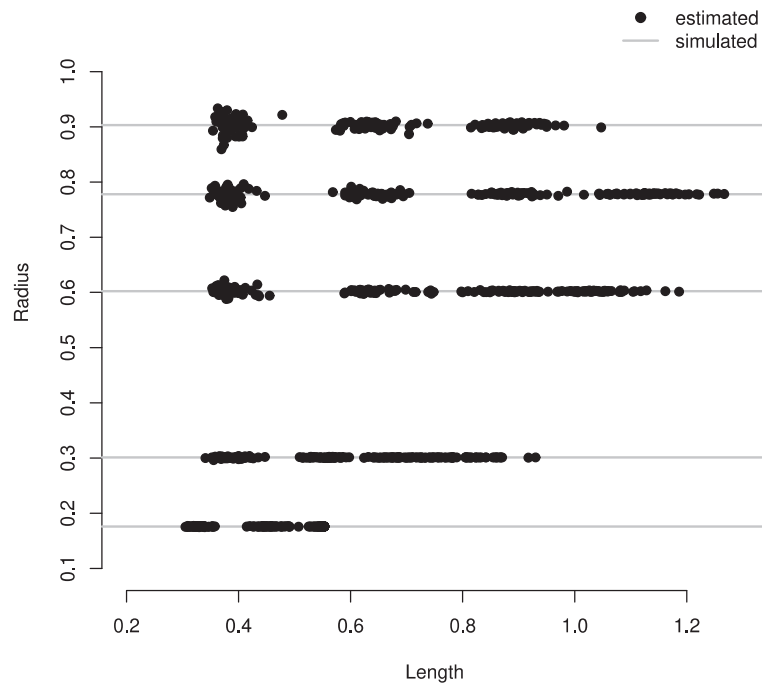


Figure SI1-4: Comparison of two shapes parameters simulated with those obtained with our circular regression method. We simulated 1000 datasets from datapoints spreaded on different circles for which we known the length, the radius and the center of the circle from which they are derived. Theses two comparisons show the robustness of our circular regression method.

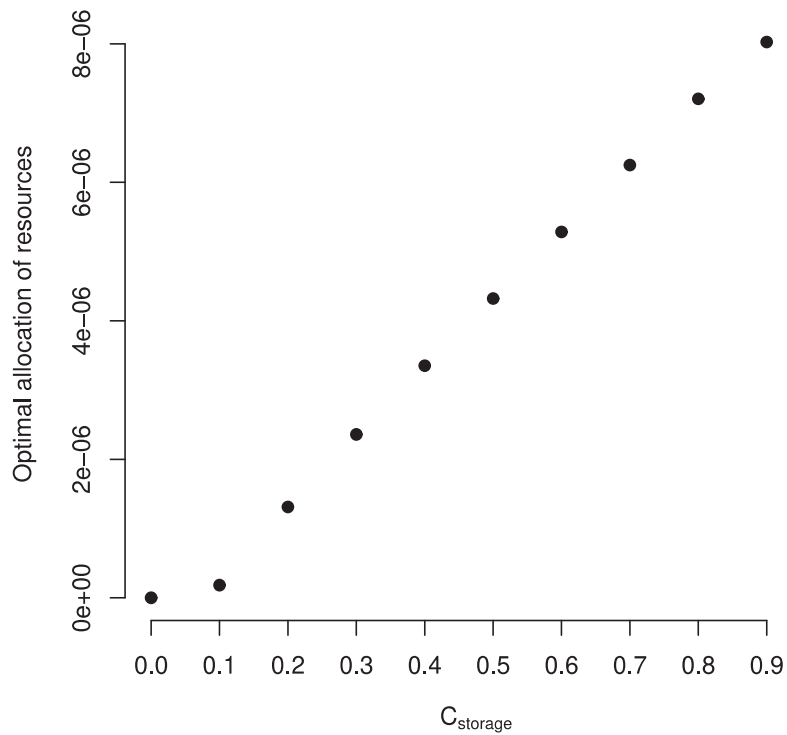


Figure SI1-5: Analytic predictions of the optimal allocation of resources (per unit of time) depend on the cost associated with storage. In an environmental context of weak (or null) storage cost, individuals can maximize their energetic conversion and slowly consume their resources. In an opposite case, where storage is highly costly, individuals have to compromise their speed of consumption between the cost associated with storage – which tends to increase the speed of consumption – and the energetic conversion – which can waste resources if allocation is too important.

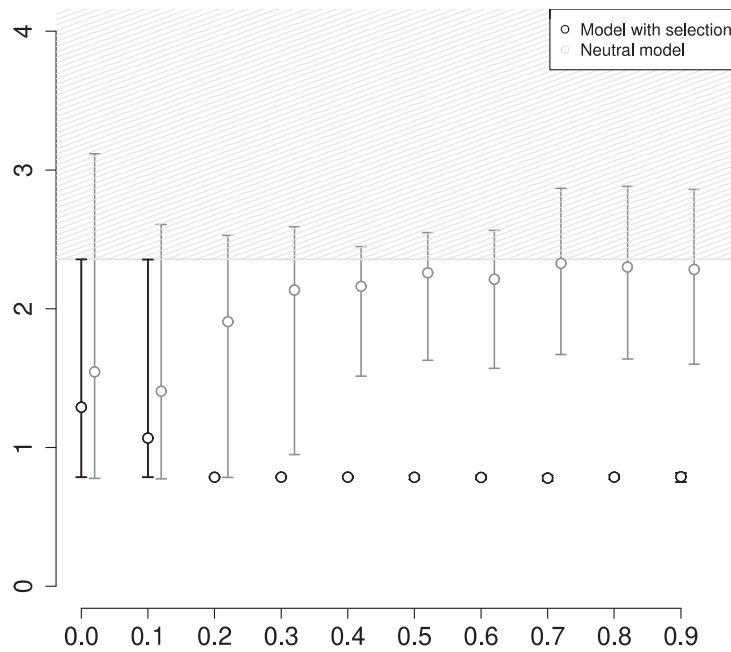


Figure SI1-6: The concavity parameters for different storage cost in our current model. The trade-off is considered convex if c is between $3\pi/4$ and $7\pi/4$ (the grey area). Dots and error bars represent the mean and between-quantile difference ($q(0.9) - q(0.1)$) of the distribution of the c shape parameters calculated in 100 replicate populations. The evolution of the c parameter is consistent with the percentage of concave trade-offs added in precedents graphs.

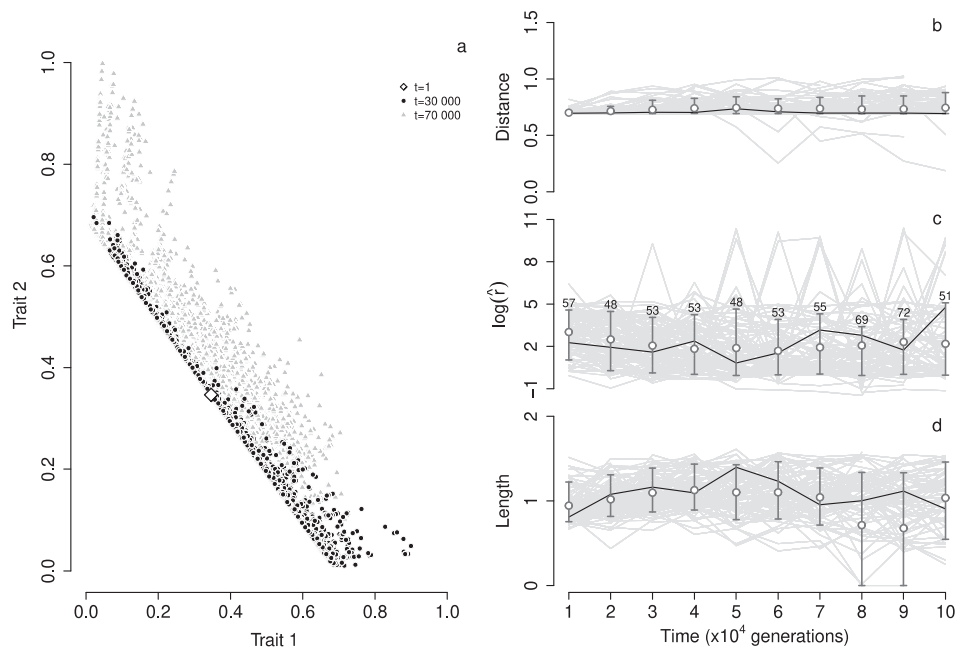


Figure SI1-7: Evolutionary dynamic for the neutral model. Panel b shows the temporal dynamics of the distance of the median projection on the fitted circle to the origin. The natural logarithm of the fitted circle’s radius (inversely related with the trade-off curvature) is represented in panel c, and the length of the trade-off (i.e. the distance along the circle between the two most distant projections) is represented in panel d. 99 replicate simulations are represented in grey in panels b-d, and the simulation in panel a is outlined in black. Dots and bar represent respectively the mean and quantiles (0.1 and 0.9) of each parameter’s distribution (across simulations) at each timestep. Standard parameter values were used (as defined in the Material and methods section), and $C_{\text{storage}} = 0.5$. On panel b the percentage of concave trade-offs is added on the top of each error bars. In neutral context, all simulations show convex trade-offs (contrary to simulations in with selection context).

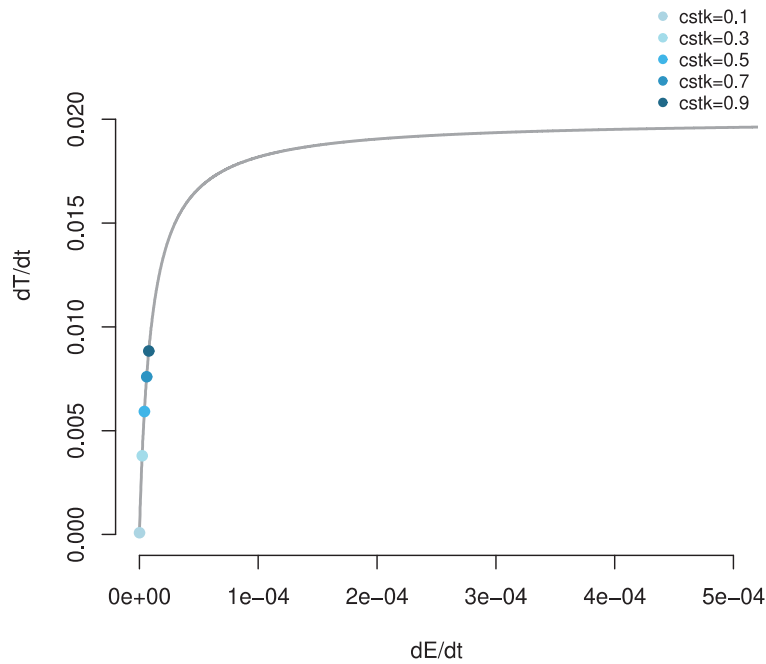


Figure SI1-8: The storage cost context influences directly the speed of resource consumption in order to maximize fitness. For each simulations with storage cost ($cstk$) $\in \{0.1, 0.3, 0.5, 0.7, 0.9\}$, we extracted the median a_j parameters (cf. Model B. Energy allocation dynamics) after evolution. Under a low storage cost, selection favors slow converters of energy. Indeed, in this context, they can maximize their energy conversion into trait value because they do not pay an important cost to store the energy (lightblue dot $cstk = 0.1$). On the contrary, when populations pay an huge cost of storage, the ability to rapidly convert the energy become advantageous (dark blue dot $cstk = 0.9$). Nevertheless, a trade-off appears under high storage cost because of the relationships between the amount of energy entering structures and traits values : it exists a limit of energy that can be correctly converted per unit of time, when this threshold is exceeded some of the energy is wasted.

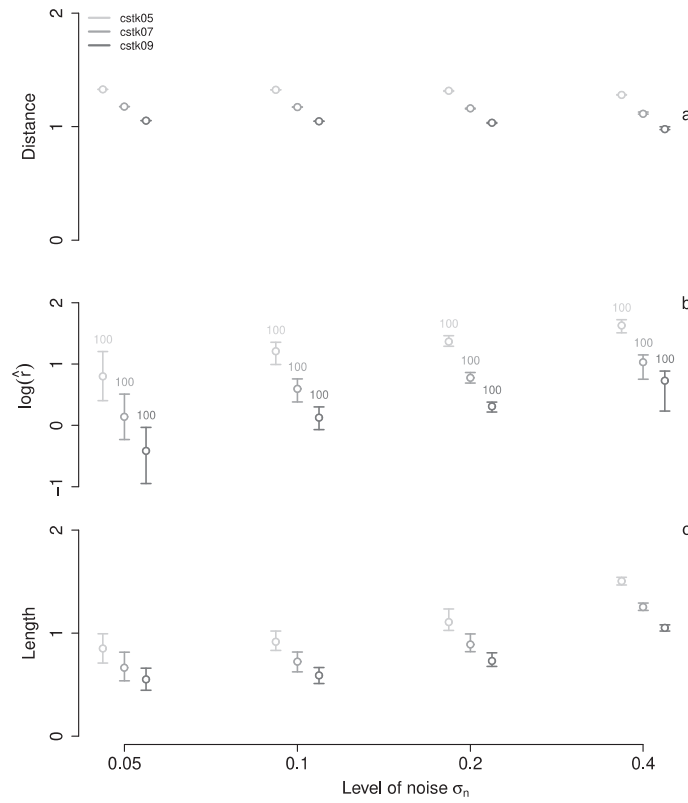


Figure S11-9: The trade-off shape changes in response to the level of noise in hormones and receptors gene expressions. The noise is modeled by multiplying each genetically encoded gene expression by 10^ϵ , where $\epsilon = \mathcal{N}(0, \sigma_n)$. Dots and error bars represent the mean and between-quantile difference ($q(0.9) - q(0.1)$) of the distribution of shape parameters calculated in 100 replicate populations. On panel b the percentage of concave trade-offs is added on the top of each error bars. Contrary to neutral simulations, trade-offs remain concave even in high level of noise context.

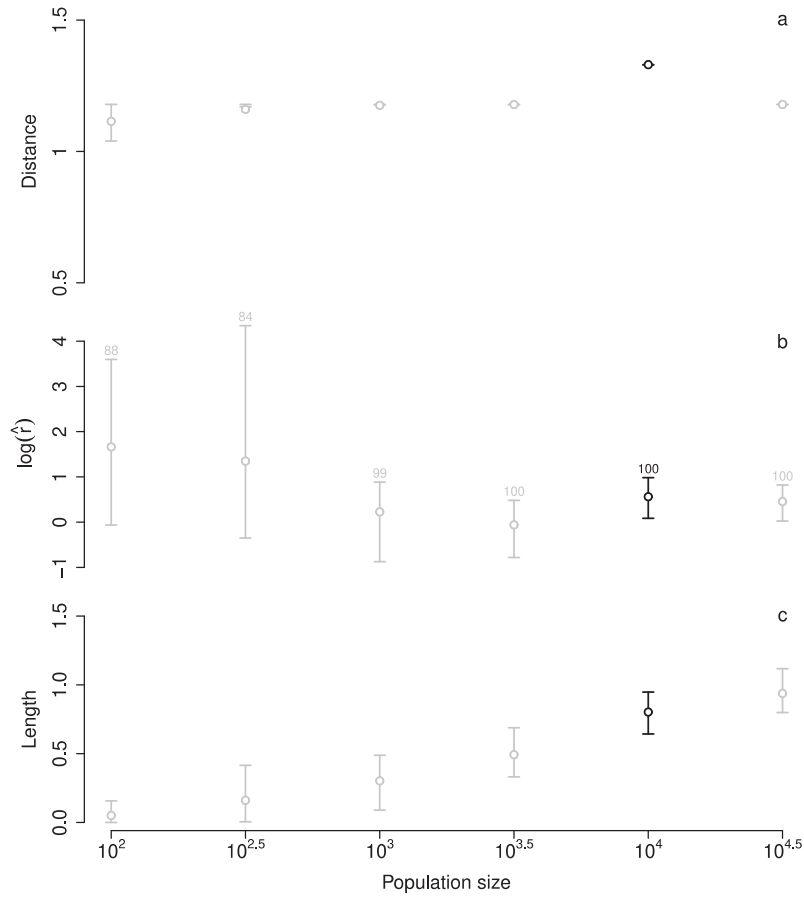


Figure SI1-10: Shape parameters for different population sizes. In black the population size used in our standard simulations. Dots and error bars represent the mean and between-quantile difference ($q(0.9) - q(0.1)$) of the distribution of shape parameters calculated in 100 replicate populations in a context of $C_{storage} = 0.5$. On panel b the percentage of concave trade-offs is added on the top of each error bars. As the population size increases, the trade-off length increases and the trade-off tends to curve (concave), the distance remains stable. Because of the growth of population size, more individuals can distribute on the entire trade-off and as a consequence increase its length and reveals its shape (concave).

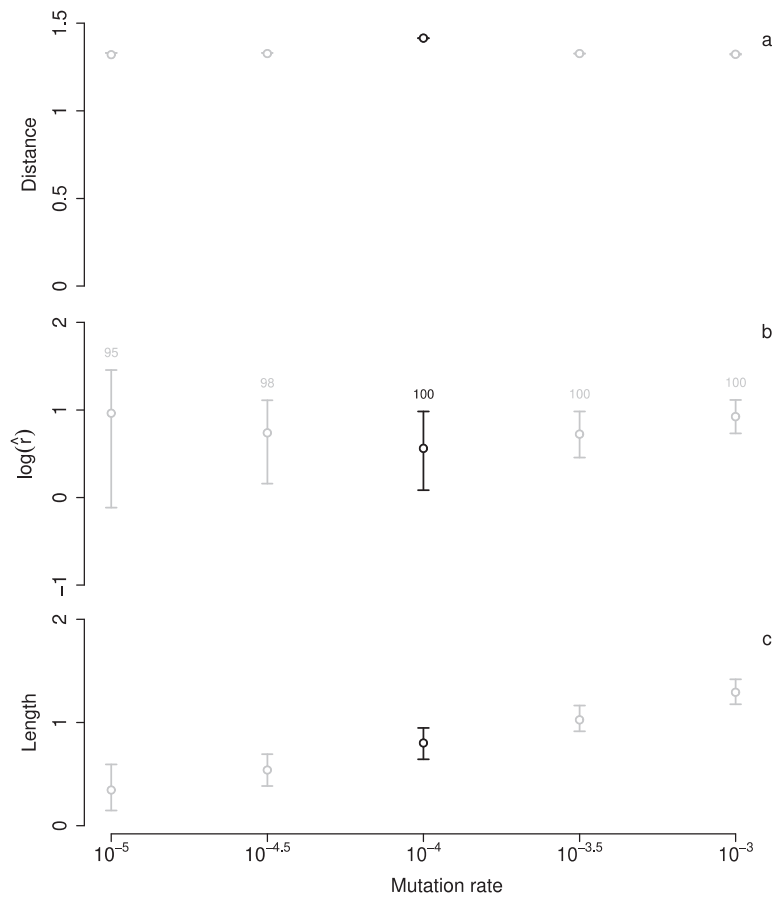


Figure SI1-11: Shape parameters for different mutation rate. In black the mutation rate used in our standard simulations. Dots and error bars represent the mean and between-quantile difference ($q(0.9) - q(0.1)$) of the distribution of shape parameters calculated in 100 replicate populations in a context of $C_{storage} = 0.5$. On panel b the percentage of concave trade-offs is added on the top of each error bars.

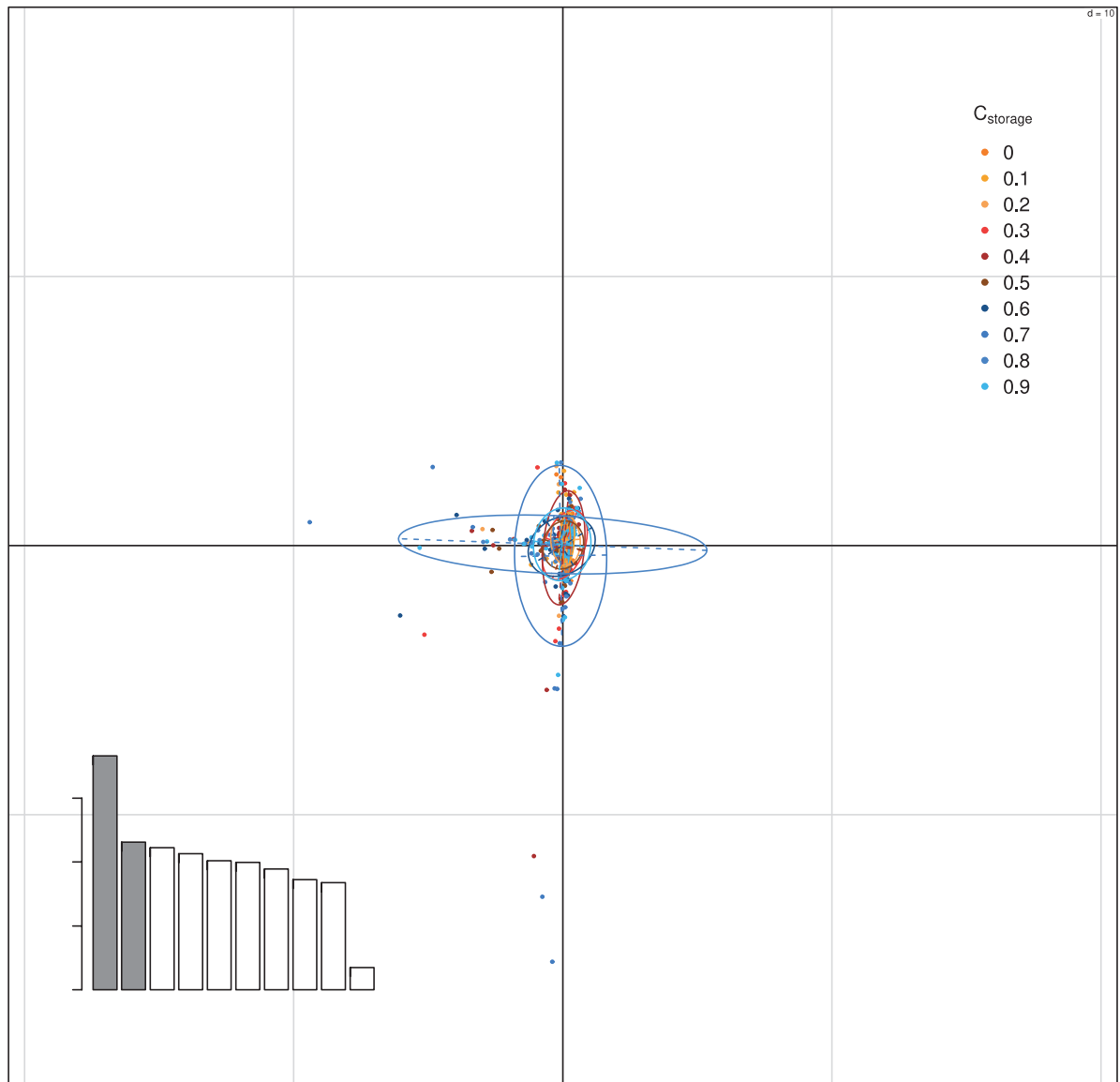


Figure SI1-12: The PCA shows no association between these variables and the context, here represented by the storage cost ($C_{storage}$). The two first axes of PCA are shown, whose relative contribution to the overall variance is shown in black on barplots in the bottom left.

Evolution of endocrine mechanism in response to variable environments and the consequences on two components of fitness

This chapter was co-written with Etienne Rajon.

CHAPTER II OVERVIEW

The results presented in this chapter are preliminary and exploratory. The structure of the model used here is similar to that in the previous chapter, except that we allowed a form of phenotypic plasticity to evolve, represented by the parameter Γ that allows the production of an hormone to change (positively or negatively, depending on the sign of Γ) according to the amount of food ingested. The environment can now be stochastic, such that food may not always be available. Finally, the traits considered are no longer arbitrary: we model the allocation of resources to survival (mortality can be reduced by eliminating metabolic waste products) and reproduction.

These choices make the model more complex at all levels - for instance, the population can now vary in size – but also closer to reality and, therefore, to making testable predictions. We had initially anticipated that a possible outcome of this work might be that individuals evolve in a variable environment to allocate massively to reproduction when food is available, and to survival otherwise. Although the results are not as clear and possibly require more exploration, this remains a possible outcome that could explain some puzzling results of extremely improved longevities observed under food starvation. Moreover, this work could potentially decipher the underlying mechanisms that may evolve in

variable environments, along with predicting which species should be subject to increased longevity under food starvation.

Eventually, the study of other parameters of the model (currently fixed) would be particularly interesting. For example, the study of the influence of the cost of storage might be key, as one may expect that the strategies evolving in a variable environment may only rely on storage when this cost is below a threshold. A more in-depth study of this model would also allow us to study the dynamics of allocation during the life of individuals. Early in life, organisms should invest mainly in survival while waiting to be sexually mature, which may occur in our model as individuals must have invested a minimal amount of energy in the reproduction to produce gametes. The lifetime dynamics of traits mostly remains a perspective of this work, however, because its proper modeling should take into account other traits (like growth) and the hormonal regulation of gene expression, which are both beyond the reach of this model.

5.1 Introduction

Variations in resource availability are one of the main extrinsic factors of mortality (along with predation and pathogens) that organisms face. Stable environments offering a constant renewal of resources, sufficient to maintain large populations, are rare. Furthermore, many populations of many species have been established in environments where they face significant variations in resource availability, driven by stochastically occurring climatic events (Menu *et al.*, 2000; Rajon *et al.*, 2009) or intrinsically pulsed resource systems (Yang *et al.*, 2008; Venner *et al.*, 2011). A major challenge to organisms is to be able to modify their behavior and/or metabolism when episodes of scarcity or abundance occur.

Endocrine mechanisms play an essential role in regulating complex traits such as development, homeostatic regulation or ontogeny (Zera *et al.*, 2007; Garland *et al.*, 2016). Intermediate molecules (*i.e.*, hormones) associated with surface receptors allow different organs or tissues to communicate. These hormones are considered key mediators of life history traits, because they regulate many crucial functions such as reproduction, growth and the immune function (Harshman & Zera, 2007). Their secretions depend on neuronal information, physiological stimuli and the concentration or presence of other hormones (Melmed *et al.*, 2015). Thus an endocrine system (including hormones, receptors and their interactions) in a particular environment generates a specific phenotype that can be plastic if the environmental conditions change (Lodjak *et al.*, 2015; Dantzer *et al.*, 2016). For instance, (Romero & Wikelski, 2010) studied the stress response of marine iguanas (*Amblyrhynchus cristatus*) in Galapagos islands during El Niño conditions which highly impact the food availability for marine iguanas. They had shown that the production of an high concentration of *corticosterone* in response to a stress (*i.e.*, starvation period) presents a selective advantage in this species (Romero & Wikelski, 2001). However, a prolonged exposition to *corticosterone* provokes some deleterious effects. Hence, an endocrine mechanism allowing a rapid decrease in *corticosterone* concentration after a stress is favored (Romero & Wikelski, 2010). This example demonstrates the crucial role of endocrine mechanisms in survival in fluctuating environments.

The recent field of evolutionary endocrinology aims at understanding how the links between endocrine mechanism and life history traits evolve (Zera *et al.*, 2007). Empirical studies in this field have been mainly concerned with variations in hormonal concentrations (Zera *et al.*, 2007; Cox *et al.*, 2016), which are relatively easy to obtain from samples of blood or hemolymph for instance. However, selection should also act on other components

such as receptor density, or binding affinities between hormones and receptors (Burns *et al.*, 2014; Rosvall *et al.*, 2016), which are likely highly heritable. Indeed, to react to environmental variations, organisms have to trigger cascade response beginning with increase (or production) of hormonal concentration (or a variation in other endocrine components). But the cascade effect efficiency depends the availability of receptors and on affinities between hormones and receptor. Indeed, if receptors are saturated or if hormones can not bind on receptors, then an increase of hormone concentration does not provoke a physiological response. Hence, by considering these parameters specifically, it should be possible to identify different endocrine mechanisms depending on the stability of habitats in resource availability.

We built an evolutionary resource allocation model containing all the ingredients necessary for the functioning and the evolution of an endocrine mechanism (hormonal productions, receptor densities, affinities between these proteins) where individuals have access to a quantity of resources varying over time, thus imitating the good and bad feeding periods that organisms face in natural conditions. The hormonal dynamics regulate the allocation of energy between the two components of fitness: reproduction and survival. Our model incorporates a new type of mutation (compared to the model in Chapter 1) that modifies the dependency between the production of a hormone and the quality of the environment encountered. The hormonal dynamics can thus be adjusted to the ecological context, throughout the lifetime of an individual, allowing it to modulate the allocation of resources between survival and reproduction.

5.2 Model

5.2.1 Overview of the initial model

In Bourg *et al.* (2019) we built an evolutionary individual based model. Individuals are diploid and contain two structures able to convert an energetic resource into trait values. They are described by their genotypes composed of four genes: two genes coding for two hormones and two genes coding for two receptors. Genes include two type of information on each protein: its expression and its conformation. Receptors are differentially expressed on the two converting structures whereas hormones are produced at a constant rate. Hormones and receptors can interact and form complexes when they bind together. Formation and dissolution of complexes depend on the hormone concentration and its

affinities with receptors as defined by their shape compatibility. All of these interactions produce an hormonal dynamic specific to each individual (see equation details in Part I). An equivalent amount of energetic resource is given to each individual. Upon hormone binding, energetic transporters are activated and resource can enter into converting structures. Hence, depending on the concentration of complexes on each converting structure, a differential quantity of energy is allocated between the two traits. During the allocation a portion of resources can be stored at a cost associated to the storage and then re-allocated. Once, the totality of energy is consumed, we obtain a combination of trait values for each individual. Mutations can impact the expression and/or the conformation of hormones and receptors. By cascade effect, theses modifications can alter the differential allocation of resource and the combination of trait values. The two traits modeled are under directional selection.

5.2.2 Hormonal dynamics

In the initial model, differential energy allocation began once the hormonal dynamics had reached equilibrium regardless of whether balance was very difficult to achieve. This hypothesis allowed us to obtain an analytic solution at the equilibrium of the hormonal dynamics. Very different dynamics, in terms of speed of reaction, could emerge and lead to a similar result. However, an individual who is able to quickly adjust its energy allocation in response to the environment is likely to perform better than an individual with a considerably slower time to adapt. In order to improve the realism of the model we integrate a part of variation in the hormonal production so that it is dependent on the quantity of energy available in the environment. In the previous version of the model the expressed phenotype was totally contingent with the genotype. Individuals now have an additional gene that will influence hormonal production depending on the amount of resource available in the environment. The Γ parameter (in red in the equation 5.1) is therefore also subject to mutations and thus fits into the equation of hormonal production.

$$\begin{aligned} \frac{d[H_{kp}]}{dt} = & \alpha_{kp} \times (1 + \textcolor{red}{\Gamma}_k \times \Delta_{\text{alim}}) + \sum_{i=1}^{n_r} \sum_{j=1}^{n_s} \sum_{q=1}^{n_{\text{ch}}} \left((k_{\text{off}} \times [R_{iqj} H_{kp}]) - (k_{\text{on}_{iqkp}} \times [R_{iqj}] \times [H_{kp}]) \right) \\ & - k_d \times [S_D] \times [H_{kp}] \end{aligned} \tag{5.1}$$

In the equation 5.1, the concentration $[H_{kp}]$ of hormone H_{kp} depends on the production rate α_{kp} and the association-dissociation dynamics of hormone-receptors complexes (similarly as the initial model). However the concentration $[H_{kp}]$ can be modulated by Γ_k which can increase or decrease the quantity of free circulating hormone H_{kp} . Adding this parameter makes the previously found analytic solution disused. More importantly, considering that the equilibrium solution is no longer achievable, the transient dynamics are themselves important here: a slow response to the environment can not be considered similar as a rapid one. The current model version simulates the dynamics of the endocrine system and of the energy allocation and storage using a classical Euler method. The rest of the equations of the hormonal dynamics are identical to the previous model.

5.2.3 Energy conversion into traits values

In the first model, the two traits were abstract and the conversion of energy into trait values was identical for the two traits considered. We now model the evolution of two main components of fitness: survival and reproduction. Fitness is no longer calculated, as it emerges from these trait values.

5.2.3.1 Reproduction

We consider a structure related to reproduction, such as a gonad. Depending on hormone-receptor binding, a given amount of energy enters the structure and is instantly used to increase the value of the trait as described 5.2.

$$\frac{d[\text{Repro}]}{dt} = \left(\sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} \sum_{q=1}^{n_{ch}} (k_{\text{onrepro}_{qkp}} \times [R_{\text{repro}_{qj}}] \times [H_{kp}]) \right) \times E \times C_f \times \Delta_{\text{repro}} \quad (5.2)$$

where the first term represents the sum of complexes formed on the reproduction structure. Each complex formed is multiplied by the concentration of free circulating energetic resource times a constant $C_f = 1000$ to obtain the inward flow of energy for this structure. The parameter $\Delta_{\text{repro}} = 0.8$ sets the efficiency of converting energy into reproduction material.

$[\text{Repro}]$ increases, and at some point reaches a threshold value $K_{\text{offspring}}$ that represents the minimum amount of energy necessary to produce an offspring (a sufficient number of gametes plus some provisioning). Whenever a pair of individuals in the population has

reached this threshold, they breed and form a new offspring inheriting half of the genetic material from each of its two parents. Recombination occurs as each parent randomly transmits one or the other of its two alleles for each gene). Following the production of an offspring, each parent loses the energy that was used for the production of the gamete (see equation 5.3).

$$[\text{Repro}]_{\text{parents}} = [\text{Repro}]_{\text{parents}} - K_{\text{offspring}} \quad (5.3)$$

5.2.3.2 Survival

Survival cannot be modeled by having capital allocated to this function. Indeed, that would ensure individuals to survive if they have invested an amount of energy above a threshold value. We assume that metabolism produces a quantity of waste ,such as reactive oxygen species, proportional to the energy entering each of the structures (corresponding to the first term of the equation 5.4). Mortality is then dependent on the concentration of waste contained in the organism.

$$\frac{d[\text{detritus}]}{dt} = \left(\sum_{k=1}^{n_h} \sum_{i=1}^{n_r} \sum_{p=1}^{n_{ch}} \sum_{q=1}^{n_{ch}} (k_{\text{on}_{iqkp}} \times [R_{iqj}] \times [H_{kp}]) \right) \times E \times C_f \times \Delta_{\text{detritus}} - K_{\text{on}_{\text{detritus}}} \times [\text{detritus}] \times [\text{maintenance}] \quad (5.4)$$

An individual can maintain a low waste concentration by investing in maintenance, which is the second trait modeled. Indeed, energy can be allocated to a maintenance structure, depending on the number of hormones and receptors binding on this structure as described in equation 5.5. The working of this structure is best understood as secreting elements that eliminate waste, with a parameter $K_{\text{on}_{\text{det}}}$ that sets the affinity between the two entities (second term of equations 5.4 and 5.5).

$$\begin{aligned} \frac{d[\text{maintenance}]}{dt} = & \left(\sum_{k=1}^{n_h} \sum_{p=1}^{n_{ch}} \sum_{q=1}^{n_{ch}} (k_{\text{on}_{\text{maintenance}_{qkp}}} \times [R_{\text{maintenance}_{qj}}] \times [H_{kp}]) \right) \times E \times C_f \times \Delta_{\text{detritus}} \\ & - K_{\text{on}_{\text{detritus}}} \times [\text{detritus}] \times [\text{maintenance}] - \sigma_{\text{maintenance}} \times [\text{maintenance}] \end{aligned} \quad (5.5)$$

Maintenance elements concentration is also affected by a constant $\sigma_{\text{maintenance}}$ which removes a proportion of maintenance elements to mimic their natural degradation (last term of the equation 5.5).

The previous equations set the concentration of waste at each instant, which is used

to calculate the instant mortality risk, modeled as a sigmoid function. This risk then multiplies the probability of still being alive at that point, $(1 - \text{mortality})$ (see equation 5.6).

$$\frac{dmortality}{dt} = (1 - \text{mortality}) \times \left(\frac{1}{1 + \nu_1 \times \exp -\nu_2 \times [\text{detritus}]} \right) \quad (5.6)$$

At each period of time (a hundred times dt), the survival of each individual is assessed as a Bernoulli process with probability $(1 - \text{mortality})$. Then the mortality of surviving individuals is reset to 0.

5.2.4 Evolutionary and population dynamics

The population is composed of N diploid hermaphroditic individuals. Hence, if they meet another member of their species they can mate by exchanging their gametes, producing an offspring and paying the same cost of reproduction. Production of descendants and death occur almost continuously, and thus generations overlap. Fitness naturally emerges from the combined action of survival and reproduction.

5.2.4.1 Mutations

Hormone and receptor expressions and conformations can mutate with probability $\mu = 10^{-4}$ per allele per generation. Mutations affecting hormone and receptor components are similar to the previous model. Γ can also mutate with probability $\mu = 10^{-4}$ per allele per generation. Γ is not directly encoded in an hormone-coding gene; we instead consider the latent variable γ , that is changed upon mutation by adding a value sampled from a normal distribution with mean 0 and standard deviation $\sigma_{m_\Gamma} = 0.05$. Our rationale for considering mutations of a latent variable is that we consider that the dependency of hormone expression on the environment is due to some regulatory change in gene expression in the hormone-producing structure following the sensing of nutrients in the digestive system. The action of this mechanism is thus likely bounded between inhibiting completely the expression of the hormone and enhancing it by some reasonable number. We therefore considered a sigmoidal response to σ that can approach -1 for negative σ (in which case expression is inhibited) and 1 for positive σ (expression is doubled, see equation 5.7).

$$\Gamma = \frac{-1 + \exp \gamma}{1 + \exp \gamma} \quad (5.7)$$

5.2.4.2 Temporal dynamics

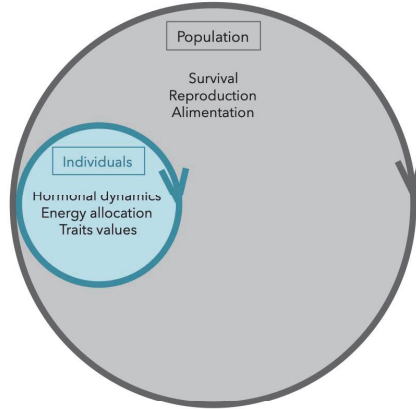


Figure 5.1: Temporal dynamics illustration.

Two nested temporal dynamics govern the evolutionary dynamics of these simulations (as represented in figure 5.1). First the individual scale (lasting 100 timesteps of size dt), during which after eating individuals allocate their energy between survival and reproduction according to their hormonal dynamics. This temporal loop runs for each individual and leads to new individual values for reproduction and survival. Individuals can not die or reproduce during the whole individual dynamic which represents a day. The bigger temporal dynamics takes place at the population scale. During this temporal loop, individuals can die (if their mortality is too high) or survive. They can also reproduce (or not if they have not reached the threshold value) and eat (if food is available in the timestep considered).

5.2.4.3 Environmental fluctuations : variations in resource availability

One of the central modifications of this new model is variation in resource availability. Resources can either be present in the environment (good conditions) or absent (bad conditions); the sequence of the environment is sampled at each timestep using a Bernoulli process, with probability p_{bad} . The average amount of resources available to the population is kept constant between simulations differing by parameter p_{bad} , such that the amount of the resource available under good conditions equals $\frac{0.1}{p_{\text{bad}}}$. When available, the resource is equally divided between the whole population, hence creating density-dependent population dynamics.

5.3 Preliminary results

5.3.1 Population dynamics in fluctuating and stable environments

We started simulations with 100 individuals per population and let them evolve for 200,000 timesteps. In the stable environment, populations rapidly increase in size and stabilize around 2640 individuals. As represented in figure 5.2, populations in the stable environment are virtually stable, and the impact of demographic variance is negligible. The others types of environment provided food resources at varying frequencies ranging from 0.4 (individuals have access to food each 4 timesteps on average) to 0.1 (individuals have access to food each 10 timesteps). All populations where the environment was most constrained (freq 0.1) went to extinction before 50,000 timesteps. Populations placed in intermediate environments remained until the end of simulation, but their average densities are below populations in a stable environment (for freq 0.4: 2400 individuals and freq 0.2: 1700 individuals). Populations with access to food with frequency 0.4 have fairly stable population densities over time, averaging between 2000 and 2600 individuals. The populations placed in the second most harsh environment show significant variations in their density over time.

5.3.2 Allocation and storage of energetic resources

In the following parts, we will not show the results for populations enduring the harshest environment (freq 0.1) because all simulations went to extinction. Hence, we will only compare 3 environmental conditions.

5.3.2.1 Storage dynamics

Energy storage dynamics are, on average, similar in the three environments. In the early stages, individuals accumulate energy even though there is a lot of variance among populations. Then the populations significantly reduce storage until they store very few resources. At the end of the simulations, there is practically no variance among populations of the same environmental conditions. Surprisingly, the populations under stable environment store more resources on average than the others at the end of the simulations.

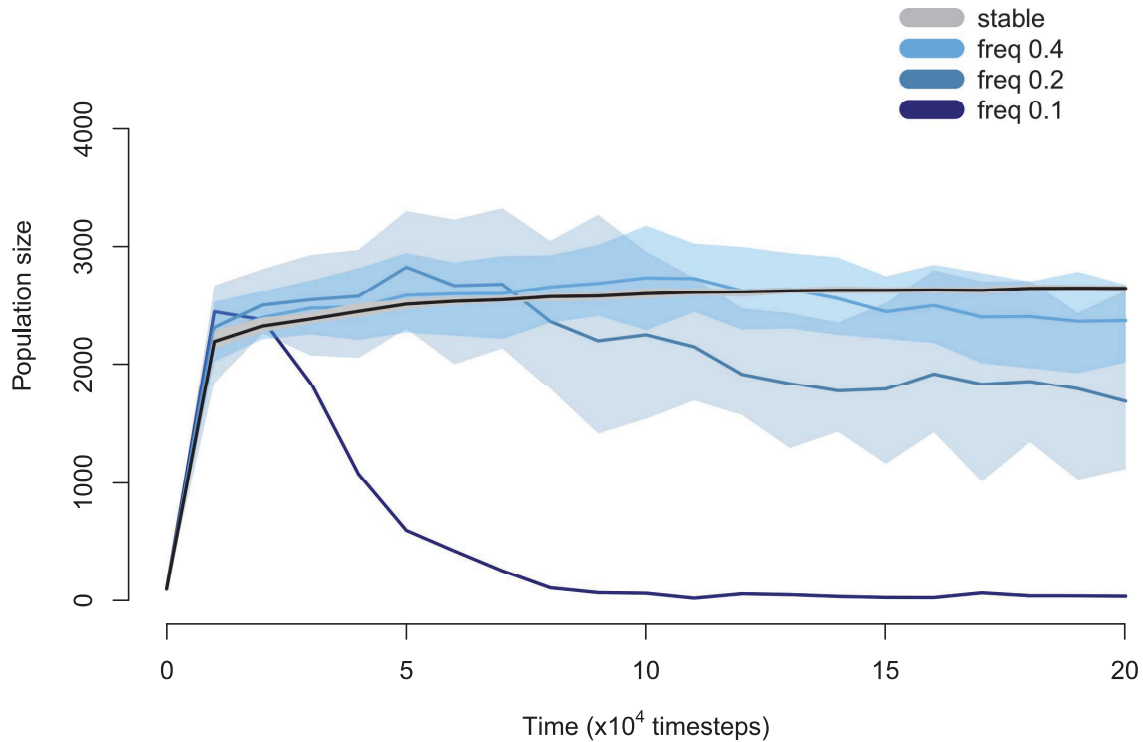


Figure 5.2: Temporal dynamic of population sizes across generations. Populations under stable environment quickly stabilize in size. Individual density for populations enduring fluctuating environments oscillate across time and are generally lower than populations in stable environment. All simulations for the highest level of fluctuation go to extinction. Lines represent variations in mean population size with time. Blue and grey areas represent quartiles (0.25 and 0.75) of population size depending on the environment encountered.

5.3.2.2 Differential allocation of energy between survival and reproduction

The dynamics of allocation to survival are quite similar for all three environments. At the beginning of the simulations, populations tend to invest a lot in survival. This investment decreases rapidly and stabilizes around 10,000 timesteps. Populations in stable environments invest the least in survival. Populations in fluctuating environments eventually show the same investment in survival on average (dots completely overlap on the graph); between population variances are larger in variable than in stable conditions.

The dynamics of allocation to reproduction change markedly with the frequency of good conditions. Populations in stable environments slightly increase their investment in reproduction over time and show very little among-population variation. On the other hand, populations in fluctuating environments strongly increase their investment in reproduction with a steeper increase for the highly constrained environment. On average, the investment at the end of the simulation is similar among populations evolving in fluctuating environments. We noted a shift in the allocation between the two traits. Starting at 50 000 timesteps, the survival allocation stabilizes and the allocation in reproduction

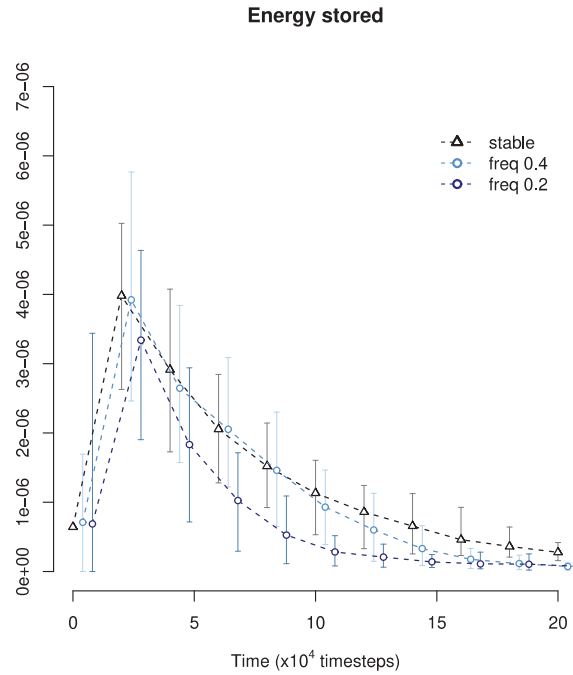


Figure 5.3: Storage dynamics. Black triangles represent the average energy stored for populations under stable environment. Light blue and dark blue dots respectively symbolize the average energy stored for populations in freq 0.4 and freq 0.2 environments. Errors bars associated represent quantiles (0.1 and 0.9). the three different environments present the same dynamic of storage.

begins to increase. This pattern is found in all three environments.

5.3.3 Endocrine mechanism underlying the allocation of resources

5.3.3.1 Evolution of the relationships between the endocrine mechanism and the availability of resources

The Γ parameter plays a role in the hormonal production in response to change in resource availability in the environment. The evolution of Γ follows the same pattern for the three environments. On average, the absolute values of Γ increases across time and its variance increases massively.

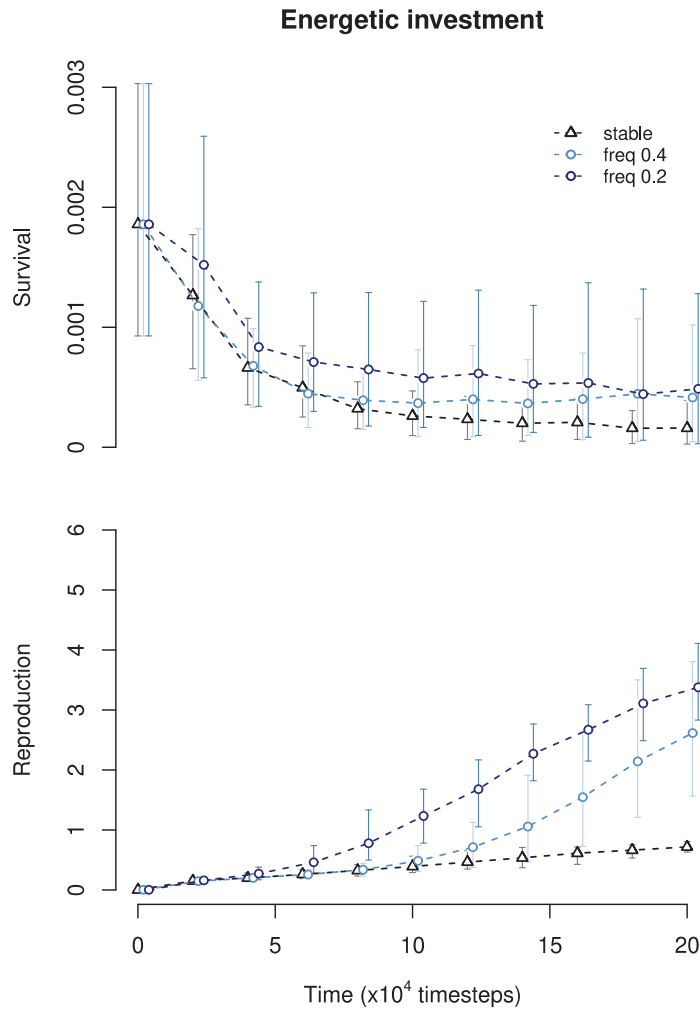


Figure 5.4: The differential allocation of energy between survival and reproduction. Black triangles represent the average reproduction- or survival-investment for populations under stable environment. Light blue and dark blue dots respectively symbolize the average reproduction- or survival-investment for populations in freq 0.4 and freq 0.2 environments. Errors bars associated represent quantiles (0.1 and 0.9). The three different environments present the same dynamic of survival allocation. Otherwise, the reproduction investment differs between environments with populations under stable environment investing less in reproduction.

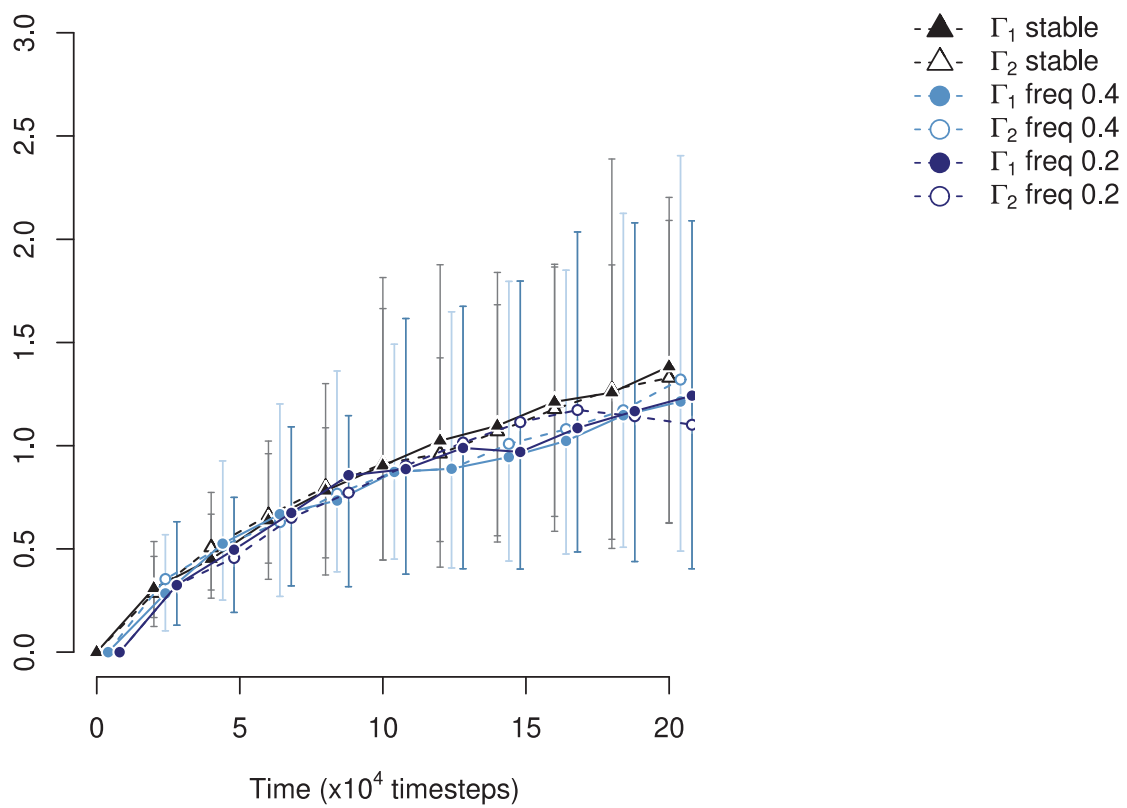


Figure 5.5: The evolution of the parameter Γ . Plain dots and triangles represent the average value of Γ_1 acting on the production of the hormone H1. White surrounded dots and triangles the average value of Γ_2 acting on the production of the hormone H2. Black triangles represent epsilon values for populations under stable environment. Light blue and dark blue dots respectively symbolize epsilon values for populations in freq 0.4 and freq 0.2 environments. Errors bars associated represent quantiles (0.1 and 0.9).

5.3.3.2 Affinities between hormones and receptors

Affinities are represented on the graph 5.6 as the minimum distance between all the combinations of hormones-receptors couples averages for all the populations. Hence, the more this distance increases, the less a hormone and its receptors will have a strong affinity and so the less time hormones will be bound. Results showed that over time, the affinity evolves to be weaker for the 3 different environments. Moreover, we observed large variances of these expressed affinities across populations.

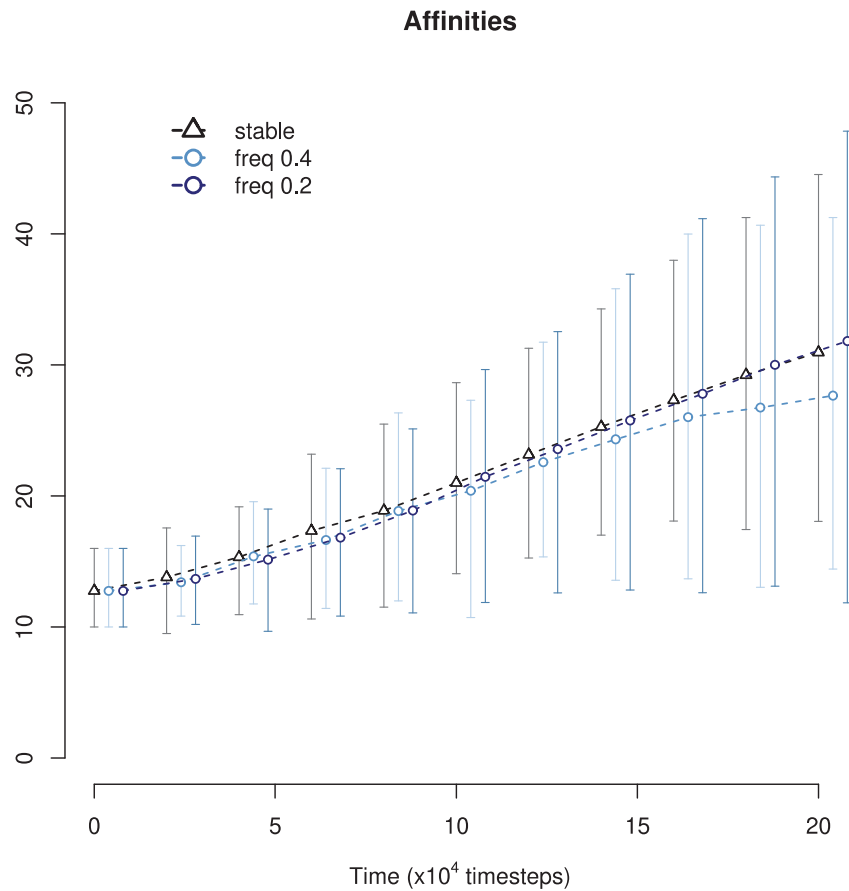


Figure 5.6: Evolution of best affinities between hormones and receptors. Black triangles represent the average best affinity between all the hormone-receptor combinations for populations under stable environment. Light blue and dark blue dots respectively symbolize the average best affinity between all the hormone-receptor combinations for populations in freq 0.4 and freq 0.2 environments. Errors bars associated represent quantiles (0.1 and 0.9).

5.3.3.3 Production of hormones and receptors

Regarding the evolution of hormonal production over time, we find the same pattern observed for the allocation to reproduction. Indeed, from 50,000 timesteps, hormonal production increases strongly for the populations in fluctuating environments (accentuated increase for the condition freq 0.2) and continues to increase gradually in the populations in the stable environment.

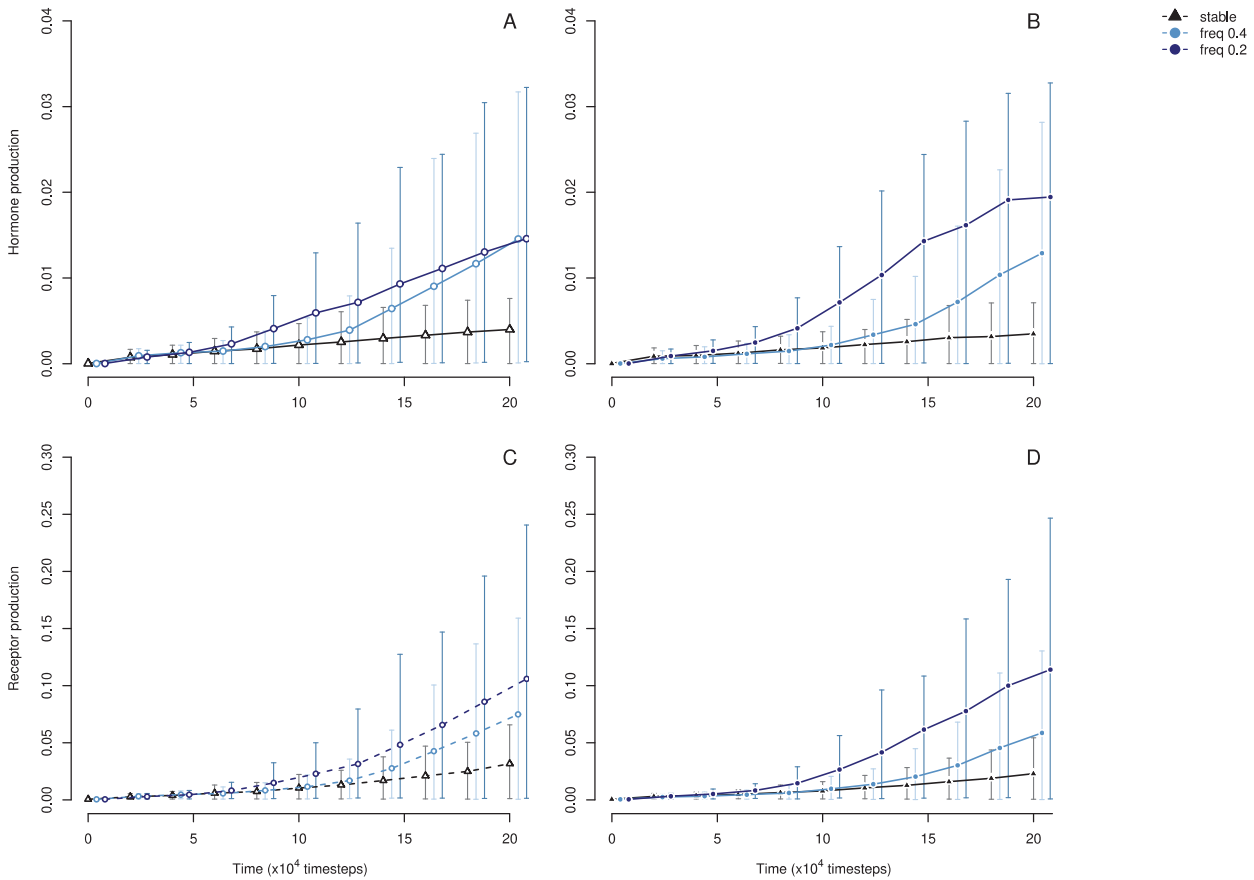


Figure 5.7: Hormone and receptor expressions. A- Hormone 1 production. B- Hormone 2 production. C- Receptor 1 production. D- Receptor 2 production. Black triangles represent the average expression of hormone or receptor for populations under stable environment. Light blue and dark blue dots respectively symbolize the average expression of hormone and receptors for populations in freq 0.4 and freq 0.2 environments. Errors bars associated represent quantiles (0.1 and 0.9).

The evolution of the production of the two types of receptors is represented in figure 5.8. The production of receptors evolves in the same tendency as the production of hormones. Indeed, there is an increase in production over time with production being more pronounced for populations in fluctuating environments. At the end of the simulation, stable populations produce, on average, two times less receptors 2 than populations in fluctuating environments.

The increase in receptor production is targeted at a particular structure. Over time, individuals tend to produce more receptors on the reproductive structure and reduce the expression of receptors on the structure of survival.

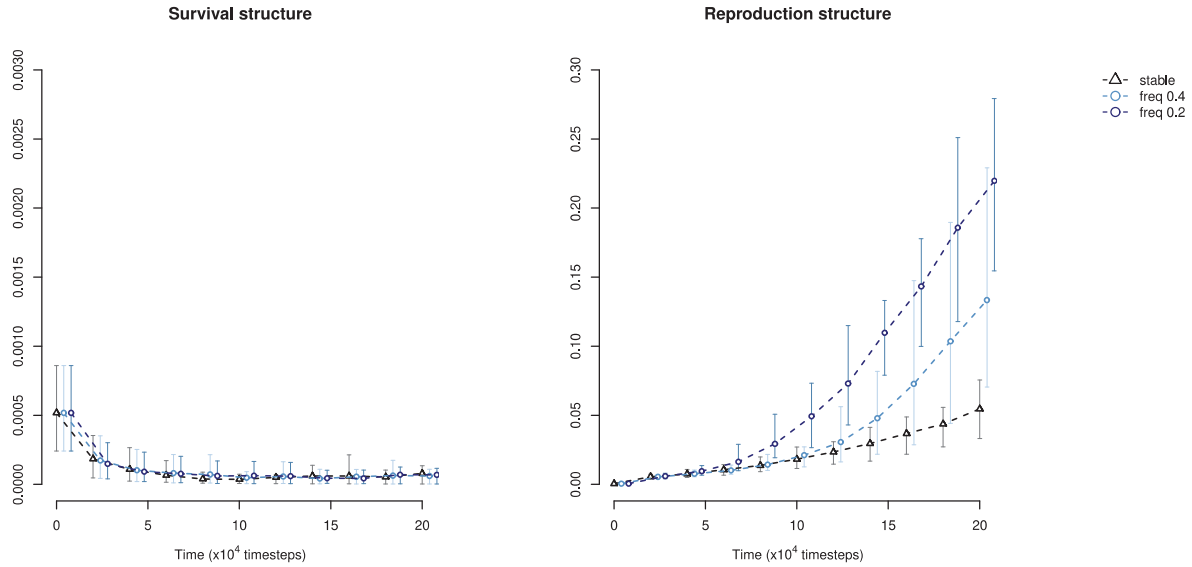


Figure 5.8: Evolution of receptors expressions on the two converting structure. Black triangles represent the average expression of receptor for populations under stable environment. Light blue and dark blue dots respectively symbolize the average expression receptors for populations in freq 0.4 and freq 0.2 environments. Errors bars associated represent quantiles (0.1 and 0.9).

5.4 Discussion and perspectives

I would like to restate that these results presented here are preliminary; as time was limited to both explore a wide range of parameter values, and run simulations on longer timescales. Indeed, simulations do not seem to have reached an evolutionary equilibrium. Some differences among the contexts considered are nonetheless visible after the 200 000 simulated timesteps.

As expected, we detect differences in population dynamics between the environmental contexts considered. Populations with stable resource availability should stabilize at higher density and should show less variation in size than populations enduring fluctuating environments. Even in environments with variations in food resources we expected to observe an average density stable associated with large variances. Some simulations might go to extinction due to a very bad succession of food availability, especially for simulations enduring the harshest frequency in food presence. As presented in figure 5.2, we obtained sub-differences among environmental conditions. However, although we expected that populations in the harshest environment (frequency 0.1) would survive, it appears that all simulations went to extinction.

The allocation of energetic resources between reproduction and survival were expected to be different depending on environmental variability. In stable conditions, individuals do not need to store resources. They can invest a majority of these in reproduction and maintain a limited investment in survival to ensure a reasonable degree of survival. In fluctuating environments, we expected that on average, the more the environment is variable in resource availability, the more individuals store energy in order to survive the unfavorable periods. Individuals displaying such strategies could correspond to iteroparous species. An alternative strategy could be that, individuals only allocate a minimum amount to survival at first in order to survive until an abundant food event. Once they reach this event, they invest all of their energetic resource in reproduction, even if they die soon afterwards (as semelparous species). Semelparity should easily emerge in variable environments because it does not represent an advantage in stable conditions. Indeed, in our model, if an individual expresses an efficient endocrine system, it can survive during a long time and still produce a lot of offspring.

Nevertheless, our preliminary results showed that populations in stable environment tend to store more resources than other environments. It remains unclear if this counter-intuitive result holds on longer timescales. Moreover, it could actually be an illustration

of the more pressing selection for the use of stored resources in varying environments; the more important storage in stable environments could simply be due to a sub-optimal exploitation of resources.

The implementation of plastic life-history strategies (for instance, allocating resources to fecundity only in good conditions) would require a plastic endocrine system able to rapidly respond to environmental changes. Non-zero values of the evolutionary variable Γ allows the organism to integrate an environmental stimulus – the amount of resources available – and accordingly modify the production of an hormone. Hence, genotypes with positive or negative values of Γ may be able to produce different phenotypes depending on the environment encountered. In a stable environment, we expect Γ to evolve randomly because the amount of resource available remains identical once the population density is stabilized. We could also presume that, in populations where the availability in food fluctuates weakly, the evolved values of Γ should not depart much from its expectation in a stable environment. Indeed, an optimized endocrine mechanism with a limited storage should compensate for the few bad periods encountered. Nonetheless, for populations evolving under strong environmental fluctuations, Γ is expected to be under selection for high absolute values, such that it should depart from zero (the initial value in all simulations) and should be noticeably more higher than for populations evolving in a stable environment.

Our results did not match all these predictions. In particular, we observe no significant difference in the evolutionary dynamics of Γ between the evolutionary contexts considered. Indeed, the increase of the average absolute value of Γ and the increase of variance associated seems to reflect a random walk of this variable, suggesting that it evolves neutrally. Here again, it is possible that this result is due to simulations remaining far from an evolutionary equilibrium. In particular, the high storage in all simulations might have prevented selection to operate on Γ in a variable environment, because a positive amount of stored resources may allow an organism to buffer the negative impact of food deprivation. A difference in evolutionary dynamics of Γ could well appear shortly after our simulations ended, since stored resources reach a critically low level after 200 000 timesteps.

However, Γ is expected to play a major role only if it co-evolves with the entire endocrine mechanism. Hence, the energetic allocation can only be shifted in response to environmental variations if energetic transporters are activated. As previously enunciated, energetic transporters are triggered when hormone-receptor complexes are formed. The

density of receptors and the affinities between hormone and receptors should evolve to allow a sensitive response to food variations. For instance, a receptor could evolve to be highly expressed on the reproduction structure and to have a strong affinity with an hormone normally produced in low concentrations. Hence, food availability would trigger a large production of hormone, leading to increased binding on the reproduction structure and, thereby, increasing energetic allocation to this trait. It is still possible that genotypes evolving in the most variable environments might have evolved the highest responsiveness, which we should be able to detect in the temporal dynamics of resource allocation. This should be linked to a detailed analysis of the relationship between receptors, hormones and Γ that have evolved in order to decipher which endocrine mechanism should evolve in each situation. It is possible that multiple combinations are suitable to produce a similar result.

In our simulations, the endocrine mechanism evolved essentially to express more receptors on the reproductive structure and to maximize the allocation of resource to this trait. It turns out that the survival of organisms seems to be maintained with a very small amount of energy to invest in survival. Organisms allocate to survival as a background task and are only minimally affected by variations in resource availability. This could explain why Γ evolves neutrally. It is likely that maintenance is more costly in reality than considered here, which we will take into account in future simulations by modifying parameters related to the dynamics of maintenance. Indeed, they are degraded at a steady rate (1 % per time unit) that could be increased in order to make investment in survival more essential.

In future analyses, it will be possible to study the impact of the evolution of these mechanisms at the population scale. We expect, obviously, that the shapes of trade-offs will differ according to the variability in available resources. In stable conditions, where individuals are the least constrained, they could express a large diversity of traits combinations without suffering from any cost. Thus the relationships detected should be long and probably linear. In slightly fluctuating environments, if populations evolve independently from the parameter Γ the trade-off is expected to be smaller and curved. A few optimal combinations could maximize fitness and thus, any departure from these could be costly, leading to an increasing curvature of the trade-off. In strongly fluctuating environments, the better strategy seems to be the semelparous one. As a consequence, the trade-off might disappear completely.

Part III

EXPERIMENTAL WORK

Experimental modification of a fitness landscape to select for an escape from a classical trade-off in *Drosophila melanogaster*

This chapter was co-written with Natacha Kremer, Frédéric Menu and Etienne Rajon.

CHAPTER III OVERVIEW

This chapter, reports the results of an experiment aimed at studying the evolution of the relationship between two life-history traits in *Drosophila melanogaster* : fecundity and juvenile survival of offspring. These two traits are in principle negatively linked, as increasing the number (quantity) of offspring should impact the amount of resources or parental care provided to each, hence their quality. This trade-off is one of the most commonly observed in nature (see *General introduction*). The two traits in question are both under positive directional selection: increasing one or the other improves fitness; but how much of the relationship observed depends on this selective context ? Or, if the selective context changed, would it be possible to “escape” the trade-off and reach other combinations of traits? As an attempt to answer these questions, I designed an evolution experiment where the fitness landscape can be defined arbitrarily. I used this framework and applied two different selective regimes during 10 generations: one mimicking the supposedly natural context of positive directional selection on both traits, and one favoring a combination of intermediate trait values.

After the 10 generations of artificial selection, populations in the two regimes of selection differed in their mean trait values. Moreover, the G-matrix evolved, especially due to a large reduction in the variance in fecundity in both selection regimes. Hence in the following chapter, we discussed the ability of a G-matrix to evolve and the flexibility and

orientation of the focal trade-off.

6.1 Introduction

Phenotypic traits (such as clutch size, age at sexual maturity or length of skull) that characterize organisms typically vary non-independently (Houle *et al.*, 1996). Among the correlations that have been observed between phenotypic traits, allometric relationships (*i.e.* relationship linking body size to shape, anatomy and physiology) and trade-offs constitute the most studied examples. How these relationships arise, and their subsequent consequences, is a major question in evolutionary biology (*e.g.*, (Roff, 1993; Stearns, 1992; Flatt & Heyland, 2011; Roff, 2002b; Wagner *et al.*, 2007; Brakefield, 2006; Brakefield *et al.*, 2009)).

The **G**-matrix is a commonly used representation of the heritable part of these relationships. It is a squared matrix with a size equal to the number of traits considered, and it includes the additive genetic variance – as defined in the field of quantitative genetics – on its main diagonal, and additive genetic covariances between traits off diagonal (Lande, 1979; Lynch *et al.*, 1998). The first theories about **G**-matrix evolution postulated that it might be stable over long timescales (Turelli, 1988). However, more recent work suggests that the **G**-matrix linking components of fitness is more likely to be flexible and its shape can evolve (Jones *et al.*, 2003; Arnold *et al.*, 2008). However, these predictions of quantitative genetics (regarding the evolution of the **G**-matrix) models might rely on necessary simplifying assumptions of the field, and do not account for the underlying constraints that could have a significant impact on phenotypic traits.).

In my first chapter, I presented models that include some of these constraints, such as the endocrine mechanism regulating the allocation of resources. This work indicates that the relationship between phenotypic traits can itself evolve, reaching an evolutionarily stable equilibrium that depends on the environmental context encountered.

Eventually, this endeavor to understand the evolution of phenotypic relationships theoretically should provide predictions for empirical studies of evolution of these relationships. In a remarkable example of such a study, Beldade and collaborators (2002) explored the evolution of the size of eyespots in the butterfly *Bicyclus anynana*. *B. anynana* naturally exhibits a specific wing pattern, with a small anterior eyespot and a large posterior eyespot. Natural populations exhibit variation of this pattern, along a negative relationship between the sizes of the anterior and posterior eyespots. While this pattern had long been

considered as resulting from a genetic constraint, Beldade and colleagues showed that the negative correlation can be broken down after only 11 generations of artificial selection for a positive relationship. This works implies that genetic correlations can be altered by artificial selection (Beldade *et al.*, 2002).

More recently, Delph and collaborators (2011) studied the genetic correlation between the female and male flower size in *Silene latifolia*. In this species a strong positive correlation ($r^2 \approx 0.9$) was observed between the size of female and male flowers. They applied artificial correlational selection (i.e., selection that directly affects traits correlations within a generation) during 5 generations and drastically reduced the correlation between male-female flower size. At the end of the experiment, only a small positive correlation remained ($r^2 \approx 0.1$) (Delph *et al.*, 2011).

Both of these studies (as well as others, see Rankin & Burchsted (1992)) show that the genetic correlations observed may not result from immutable functional constraints, but instead correspond to an evolutionary equilibrium. It is important to note, however, that the traits considered in these studies are morphological. Even if they are indirectly connected to fitness (wing patterns affect survival by mimicking an anti-predator device in *Bicyclus anynana* and the size of flowers are involved in reproductive efficiency in *Silene latifolia*), they are likely less closely linked to fitness than life-history traits, like clutch size for instance. Therefore, in this study we investigated life history traits more closely tied to fitness.

Roff and Fairbairn directly addressed the evolution of a trade-off between fecundity and dispersal in *Gryllus firmus* (Roff & Fairbairn, 2012). They applied directional selection, using sibling selection because trait measures required sacrifice of individuals, on ovary mass and on a major flight muscle separately and simultaneously during 6 generations. They confirmed a prediction made 10 years earlier that a trade-off generally evolves through changes in their intercept but not their slope (Roff *et al.*, 2002). Hence, in the particular context of a trade-off between life history traits, it appears that the genetic correlations might not be modified.

To our knowledge, no work has empirically studied the evolution of the form of the relationships between components of fitness (indeed, I do not consider the results of Maharjan *et al.* (2013) as an answer to this question, the fitted models are questionable and the study on bacteria raises the question of what is considered as a population). Thus, we focused on this particular question in this chapter. We studied the evolution of relationship between traits under constraints because they are supposed to be compromised. We

examined one trade-off among the most classically studied (Stearns, 1992): the trade-off between the size and the number of offspring (also called quantity-quality of descendant trade-off). As mentioned in the *General Introduction*, Lack in 1947 was the first to examine this trade-off when he worked on the variations of clutch size in European birds. The quantity-quality trade-off has been detected in numerous species (**reptiles and amphibians** : Sinervo & Licht (1991); Uller & Olsson (2005); Camargo *et al.* (2008); **birds** : Smith *et al.* (1989); Merilä & Wiggins (1995); Sanchez-Lafuente (2004); **insects** : Gibbs *et al.* (2005); Khokhlova *et al.* (2014); Czesak & Fox (2003), **mammals** : Gillespie *et al.* (2008); Walker *et al.* (2008); Charnov & Ernest (2006); Oksanen *et al.* (2002); **fishes** : Fleming & Gross (1990); Jonsson & Jonsson (1999)). The verbal model explaining the trade-off emergence is that a couple (or a female) can not produce an infinite number of offspring and provide them with enough energy or parental care to ensure their survival. For species without parental care, the allocation of energetic resources among offspring will be either low if equally distributed, or unequally distributed.

In our case, we studied this trade-off in *Drosophila melanogaster*. Our theoretical results from the Chapter I let us assume that an orthogonal evolution from the established trade-off is possible. So, we built a framework allowing us to create any arbitrary fitness landscape formed by two life history traits: fecundity and juvenile survival. We placed a genetically diverse population of *D. melanogaster* in two different selective contexts during 10 generations in *ad libitum* food conditions. One selective regime mimicked natural selection (*i.e.*, individuals with increased fecundity and increased juvenile survival are mostly represented in the next generation) and the other regime applied was one that fruit flies never encountered in nature: where we gave a selective advantage for individuals with sub-optimal traits values. We named this context stabilizing selection¹ hereafter in this chapter.

A preliminary investigation using a mathematical model that mimics the experimental design presented above (described in Box 1) indicates that populations under directional selection should not present significant change in their values across generations, while populations under stabilizing selection regime should evolve toward lower trait values. Contrary to our expectations, however, populations under directional selection clearly showed an improvement in both traits while populations under stabilizing selection exhibited a slight decrease of the combination of traits. To better understand this process,

¹We used this terminology to simplify the text even if in our case, we did not specifically apply stabilizing selection. Indeed, we actively selected for individuals with intermediary values but not for individuals with averaged values expressed in the population.

we subsequently studied the evolution of both the P- and G-matrix shapes across generations and determined that they can be modified.

Box 1 : Theoretical model to understand the evolution of the shape of negative relationships

Different fields of evolutionary biology (quantitative genetics, life history traits, etc) have studied shape changes in phenotypic relationships, frequently without considering underlying mechanisms. In the theoretical model presented in chapter I, we explicitly integrated regulating mechanisms and here we used this model to anticipate what can be expected theoretically from such an experiment. As a reminder, this model includes a genotype specifying hormones and receptors interact to regulate the allocation of resources to two traits. This differential allocation determines the combination of trait values expressed by the individual.

A few adjustments had been made to suit the experimental design (even if the model remains general, it was not built to fit any particular species or experiment). Individuals were fed *ad libitum* in the subsequent experiment. Therefore, in simulations, individuals ingested variable amount of resources. We created an initial population with large genetic diversity (as previously done in a experiment). To do so, we ran the classic model (without modification) during 100,000 generations in order to obtain populations with a stable established trade-off. We mixed 10,000 genotypes from 12 simulations and extracted randomly 1000 genotypes to start a new simulation with them. In this context, we let them breed for 3 generations to obtain the mixed original population. From this population, we used 50 individuals for each 100 replicate simulations. We applied 2 selection regimes: half of replicates were under directional selection and the other half was under stabilizing selection during 10 generations. However, these theoretical results do not represent predictions of the experiment presented in chapter III (traits considered are different) but represent an interesting tool to compare it with other theoretical works

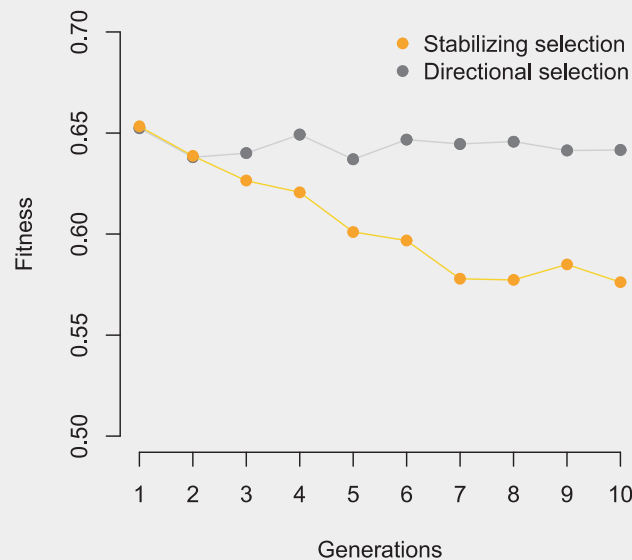


Figure A: **Predictions of fitness evolution across 10 generations for both selection regimes.** Dots are mean multiplicative traits values of 100 simulations mimicking the experimental design. The stabilizing selection regime is symbolized in orange and the directional selection regime is represented in grey. Directional selection prediction of fitness are relatively stable across time otherwise fitness of stabilizing selection is predicted to decrease with generations.

Fitness values are calculated in the same way (multiplication of T1 and T2 for each individual of each simulation then averaged) for the 2 selection regimes to favor their comparison. Under directional selection, fitness is expected to be stable (because the same selection is applied as in the simulations from which the individuals in the initial population are derived) or to decrease very slightly because of the random variation of resource acquisition in the population. Indeed, the hormonal dynamics have been previously selected to be very effective with a precise amount of resources. This trend is observed in simulation results with a slight decrease in average fitness over generations for directional selection simulations (see Figure A). However, under stabilizing selection, it is expected to decrease trait values more frankly since the applied selection favors individuals with intermediate trait values. The theoretical results are in agreement with these predictions: one observes a very clear decrease of the values of lines starting from the 7th generation.

To sum up, according to theoretical predictions, we expect to observe a change in trait values (i.e, a move in the fitness landscape) for replicates under stabilizing selection while we should not identify significant changes in trait values across generations under directional selection.

Figure 6.1: Box : Predictions of the evolution of fitness from a theoretical model

6.2 Methods

6.2.1 Biological material

6.2.1.1 *Drosophila melanogaster*: an historical model organism

The following study was conducted using the fruit fly *Drosophila melanogaster*, which belongs to the *Diptera* order. Wildtype fruit flies occur on all continents and naturally lay eggs on rotting fruit or other material like decomposed fungus. *D. melanogaster* is yellow-brown, with red eyes and transverse black rings across the abdomen. Charles William Woodworth is considered to be the first scientist to have raised *D. melanogaster* in laboratory conditions at Harvard between 1886 and 1888. He advised their use for genetic studies to his colleague William Ernest Castle. It was the work of Castle that inspired Thomas Hunt Morgan and motivated him to use this species for his studies on recombination. Few years later, TH Morgan discovered with his team the basic mechanisms of heredity, epistasis or gene mapping and received a Nobel Prize in 1933.

D. melanogaster exhibits a sexual dimorphism : males tend to be smaller and darker at the end of their abdomen. This species is considered as a model organism for different fields such as developmental biology, physiology, life history evolution and genetic. *D. melanogaster* has a short generation time, high fecundity, sexual dimorphism, endures anesthesia well, has only 4 chromosomes and its genome was fully sequenced in 2000, making it a very useful species for many experimental purposes.

6.2.1.2 *Drosophila melanogaster* life cycle

D. melanogaster develop in 10 days, from egg to adult, at 25°C (as represented in figure 6.2), which is the optimal temperature for fecundity. Under lower temperatures the development time is longer (19 days at 18°C - 50 days at 12°C) while it is shortest at 28°C (7 days; under higher temperatures the development time increases due to heat stress). Over its life cycle, the female lays around 1500 eggs that grow on rotting fruits. After 24h at 25°C, eggs become larvae (first instar) and continue their development through two molts (second and third stages). They are 5-6 days old when they encapsulate in the puparium and require a 3-4 days for metamorphosis to adult. Then adults emerge and females are sexually mature 8-12 hours after emergence.

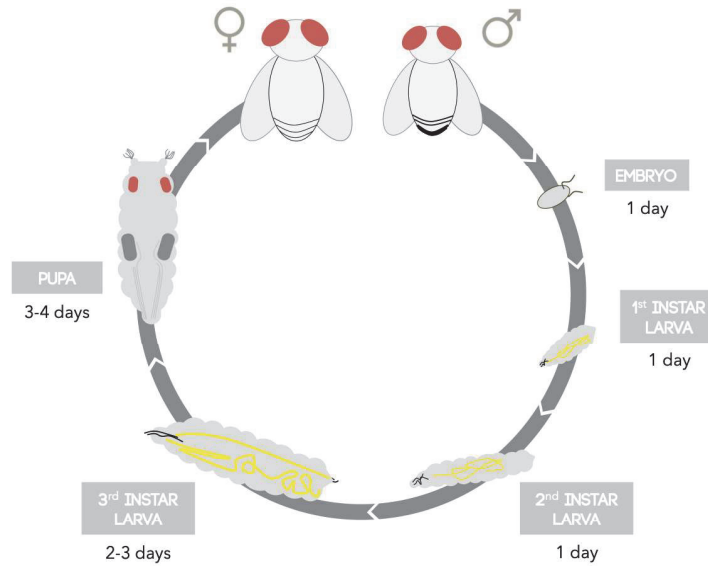


Figure 6.2: *Drosophila melanogaster* life cycle at 25°C.

6.2.1.3 Experimental evolution lines

In order to intensify selection and to reduce experimental time we formed an initial genetically diverse population. We randomly crossed individuals from 12 stock populations of *D. melanogaster* sampled in various countries from 4 continents (USA (2 lines), Argentina, Asia (2 lines), France (2 lines), Tchad, Saudi Arabia, Bolivia, Portugal, Madagascar). These stock populations had been maintained in standard conditions (25°C with a 12 hours light-dark cycle) for more than 10 years. After 3 generations of cross-breeding, we formed 8 lines each by randomly sampling individuals in this large genetically diverse population.

6.2.2 Experimental protocol

6.2.2.1 Measurements of life-history traits

We measured the evolution of fecundity and juvenile survival of offspring on females of 8 lines during 10 generations. During the whole experimental evolution, populations were maintained in controlled conditions in a climatic chamber at 25°C and 60% hygrometry, with a 12 hours light-dark cycle. Each line was composed of 50 individuals with a balanced sex ratio. Since the experiment extends over 6 months and the manipulations are heavy, the risk of an experimental problem is high. To limit these risks, lines measurements were divided into 2 experimental blocs ; each bloc contained 2 lines of each treatment (directional and stabilizing selection see below). To simplify the reading, measurements

are described below for one line and one generation.

Fecundity On the first day of each selection round, 25 tubes each containing a female and a male adults and a 2g pellet of standard food medium, consisting of agar and water, were formed. Mating pairs were kept for 3 days to ensure that they mated and that oogenesis was triggered. At day 4, we replaced food pellets, and 24 hours later we counted the number of eggs on each pellet which is hereafter called fecundity.

Juvenile Survival After egg counting, 25 eggs per females were placed on a new pellet to reduce larval competition. Eggs became pupae after six days of development. At which time, 20 of each² were isolated individually in separated parts of cryoboxes (RatioLab RATI5220014) filled with agar supplemented with sugar. Boxes were closed with foam (3 cm of thickness) and rubber band. Hence, flies received enough light to develop but they remained separated from other flies to keep them virgin. After 5 days in a climatic chamber (under standard conditions), we enumerated emerging flies from boxes and tubes. The sum of emerging flies divided by the number of eggs placed on the new pellet determines the juvenile survival value for each female.

6.2.2.2 Fitness landscape and Selection

The combination of the two trait values (fecundity and juvenile survival) corresponds to a female's coordinates on the fitness landscape. We used this position to determine the fitness value for each female and used this value to compute its relative fitness, which gives the probability for its offspring to be a part of the next generation. Fitness depends on the fitness landscape (their determinations are described hereafter). Next, we calculate the relative fitness value corresponding to the probability for female's offspring to integrate the next generation .

$$p_i = \frac{W_i}{\sum_{j=1}^{\frac{N}{2}} W_j} \quad (6.1)$$

Our experimental design is not specifically an artificial selection because we do not select systematically individuals assigned with the better fitness. We attribute a probability to integrate the next generation, which is proportional to the fitness value, but just "by chance" an individual with low fitness can be chosen to incorporate the new generation.

²1 pupae per section, the 5 remaining were conserved in their initial tubes

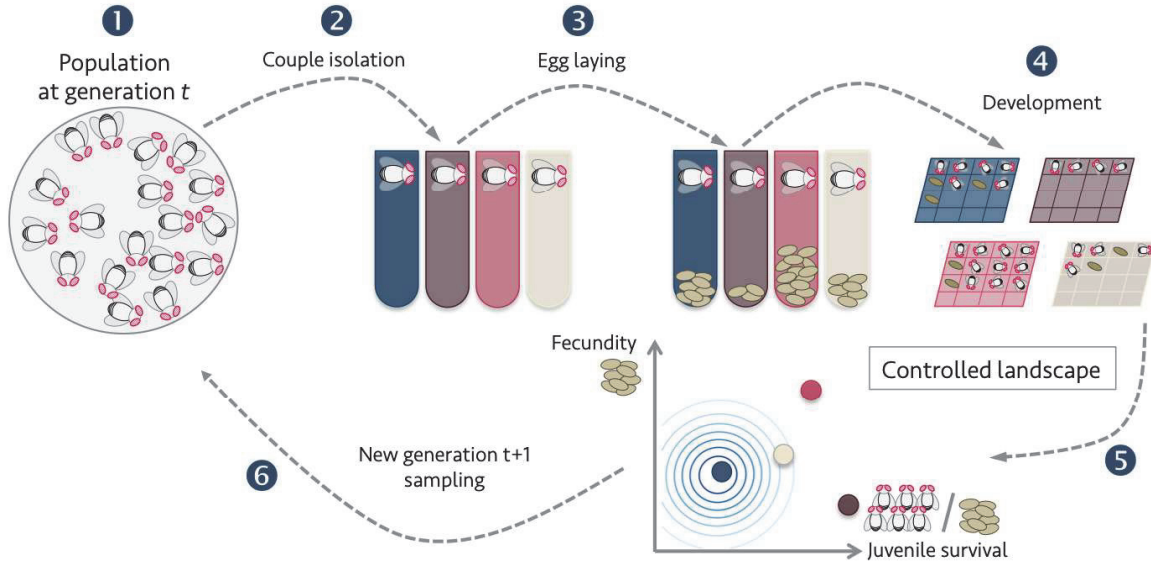


Figure 6.3: Schematic representation of the experimental design. (1) Initial population with a mixed genetic background. (2) Isolation of fruitfly couples in tubes. (3) Egg laying during 24h, then egg counting and isolation on new pellet. (4) Pupal development and adult emergence in cryoboxes. (5) Plot of female trait values in the fitness landscape applied. (6) Choice of offspring from selected females according to their fitness in order to form the next generation. In the directional selection landscape, the female from the pink tube should obtain a higher fitness because it layed more eggs with higher survivorship, and then its offspring should be selected to contribute to the next generation. Conversely, in the stabilizing selection landscape, the female from the blue tube has the higher fitness because it layed an intermediate number of eggs that had intermediate survival. Hence, blue female offspring should be selected to form the generation $t + 1$.

DIRECTIONAL SELECTION This protocol is supposed to mimic natural selection encountered by flies in nature. Indeed, we supposed that flies laying a lot a of eggs that are able to well developed have a better fitness than others. Thus, juvenile survival and fecundity should be under directional selection. The absolute fitness value was obtained by multiplying fecundity with juvenile survival value for each female.

$$W_i = F \times s \quad (6.2)$$

This selection had to be applied on females, however a mistake in the computing program makes that fitness values were attributed to each offspring. Hence, offspring from a large clutch with a good survival rate were more likely to be selected to be part of the next generation. Consequently, fitness landscapes were affected by this mistake but can be determined *a posteriori*. By multiplying the original equations with $\min(F, 20) \times s$ we can obtain the real fitness landscape applied during the experiment (see equation 6.3, where $\min(F, 20)$ signifies that the minimum value between fecundity or 20 is chosen to calculate W_i). Overall, it adds natural selection on individuals because the most perform-

ing (with a large brood in good shape) are more likely selected. But this modification is not proportional with the fecundity value because of the experimental design, where we isolated 20 pupae maximum for each females. That is why at maximum 20 offsprings for one female can be chosen to integrate the next generation. Despite the fact that fitness landscapes were not exactly what we originally anticipated, we applied two different selective regimes: a directional selection and a stabilizing selection. Then, the actual fitness value for directional selection were obtained with the following equation.

$$W_i = F \times s \times \min(F, 20) \times s \quad (6.3)$$

4 lines were placed under directional selection during 10 generations and formed our control lines.

STABILIZING SELECTION Under stabilizing selection, we actively selected individuals moving away from this relationship and closed to a novel optimum. The absolute fitness value for each female was determined according to the proximity of the coordinates to the optimum in the fitness landscape:

$$W_i = e^{-\left(\frac{(F_i - \mu_F)^2}{\sigma_F^2} + \frac{(S_i - \mu_S)^2}{\sigma_S^2}\right)} \times \min(F, 20) \times s \quad (6.4)$$

6.2.3 Statistical Analysis

Statistical analysis have been conducted in R (version 3.5.2). We used the package *glmmTMB* to study the evolution of traits separately across generations. This particular package fits linear and generalized mixed models with different distributions including zero-inflated Poisson (Brooks *et al.*, 2017). We compared two nested models (see equations 6.5-6.6) with Akaike information criterion (AIC) in order to relatively determine which statistical models explain most of our data.

$$\text{Full model : } \textit{trait} \sim \textit{selection} : \textit{generations} + \textit{generations} + (1|\textit{replicat}) \quad (6.5)$$

$$\text{Simpler model : } \textit{trait} \sim +\textit{generations} + (1|\textit{replicat}) \quad (6.6)$$

Estimations of heritabilities, **G**-matrices and **P**-matrices have been realized within the Bayesian framework using the R package *MCMCglmm* (Hadfield *et al.*, 2010). We implemented a multitrait animal model to decipher variations of fecundity and juvenile

survival in response to artificial selection. These variables were respectively fitted with a Poisson and a binomial family. Pedigrees contained identity of mother and father for each individuals (except for founder individuals) and trait values associated only for females. We used expanded priors to speed up the convergence (Hadfield *et al.*, 2010). We ran MCMC chains for 2,000,000 iterations with a burn-in period of 30,000 iterations and a thin interval of 500. The convergence of chains was assessed visually and the detail of auto-correlation and effective sample sizes are given in Supplementary information. From animal model outputs we extracted **G**-matrix estimations :

$$\mathbf{G} = \begin{bmatrix} G_{11} & G_{12} \\ G_{21} & G_{22} \end{bmatrix} \quad (6.7)$$

where G_{11} being additive genetic variance of fecundity, G_{22} additive genetic variance of juvenile survival and G_{12} (and G_{21}) corresponding to genetic covariance between the 2 traits (de Villemereuil, 2012). Heritabilities were obtained by dividing the estimation additive genetic variance of a trait by the estimate of phenotypic variance. From **G**-matrix estimation, we computed genetic correlations r_a as $r_a = \frac{G_{12}}{\sqrt{G_{11} \times G_{22}}}$.

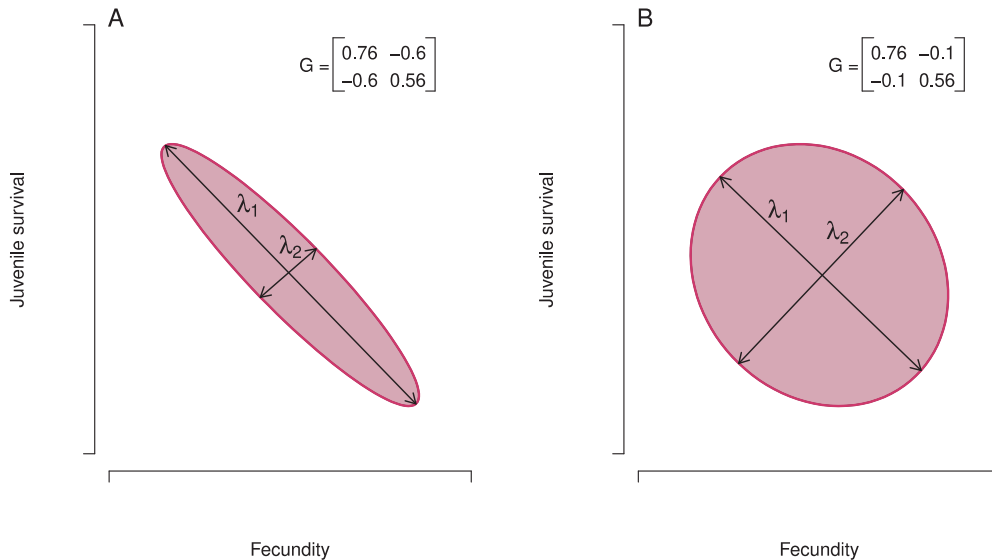


Figure 6.4: Examples of different shapes of G-matrix. (A)- **G**-matrix with an important negative covariance between the two traits. In this case the parameter $\epsilon = \frac{\lambda_2}{\lambda_1}$ is small and the matrix is cigar-shaped. (B)- **G**-matrix with a small negative covariance between the two traits. Here, the parameter $\epsilon = \frac{\lambda_2}{\lambda_1}$ is closed to 1 and the matrix is round.

As described in equation 6.7, **G**-matrix contains variances and covariances of two (or more) traits and can be represented in two different manners : as a matrix or as an

ellipse (see figure 6.4). Genetic values can be seen as a cloud and thus can be illustrated graphically. In the following chapter, when we state the shape of the **G**-matrix, we actually refer to the shape of the associated ellipse. This graphical representation facilitates the visualization of interactions between the **G**-matrix and the fitness landscape and variations of the shape of the **G**-matrix can be observed (Arnold *et al.*, 2008).

We characterized the size, the shape and the orientation of the **G**-matrix to study its evolution (Jones *et al.*, 2003). The measure of the overall size of the **G**-matrix corresponded to the total genetic variance of both traits ($\Sigma = G_{11} + G_{22}$). The shape of the **G**-matrix can vary from a circle to cigar shaped, depending on the genetic correlations between traits as described in figure (figure 6.4). Hence, the shape parameter named ϵ was determined by the ratio of λ_1 and λ_2 , which are the eigenvalues of the **G**-matrix (the smaller value always divided by the larger). The orientation is represented by the angle ϕ between the leading eigenvector and the x-axis, is expressed in degrees.

6.3 Results

During the experiment we faced a few issues with one experimental bloc. Fruit flies laid a very small number of eggs in the first two generations and died prematurely once they were isolated by couples. This caused a delay in starting the experiment for the bloc 1. As a consequence, the genetic background may be different for the 2 blocs, due to the 2 additional generations with random mating in the first bloc.

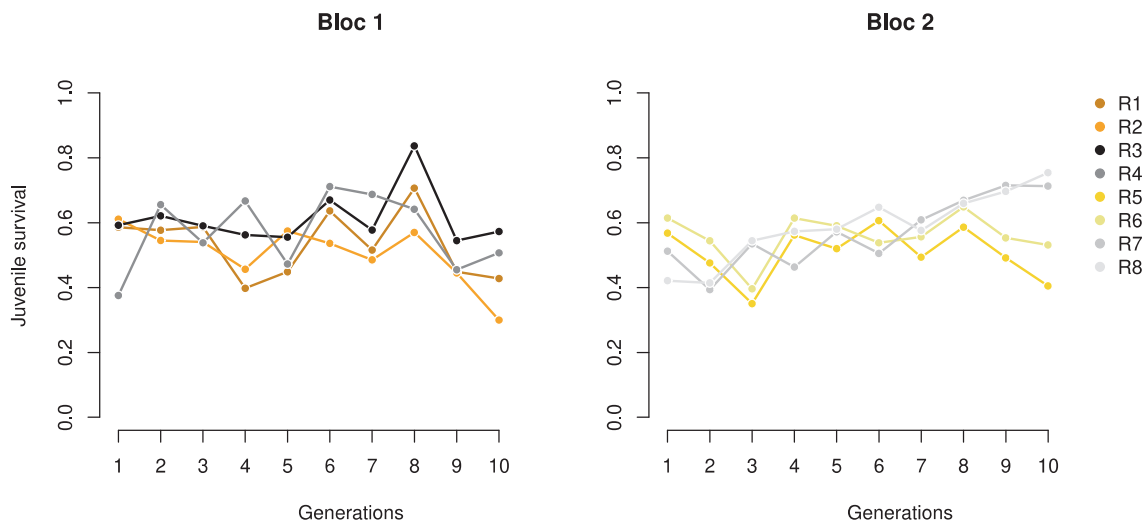


Figure 6.5: Evolution of juvenile survival across generations. The left panel represents only replicates from bloc 1 and right panel represents only replicates from bloc 2. Dots correspond to averaged juvenile survival at one generation for one replicate. Lines between dot symbolize the evolution of juvenile survival between generations. Replicates under stabilizing selection are represented in orange declination whereas grey declination depicts replicates under directional selection. We noted a clear decrease of juvenile survival for all replicates in the bloc 1 at generation 8, which pursuits to the next generation with low survival rate expressed too.

Moreover, larvae from bloc 1, exhibited an unexplained delay in emergence at generation 9. In normal conditions, pupae emerged after 4 days of isolation in boxes, to ensure the virginity of females and to control mating. Due to the delay in emergence, we chose to leave them in isolation boxes for an additional 7 hours period to obtain mature individuals. Even with this additional time, a very few number of fruit flies had emerged. Hence, we noted an important decrease of juvenile survival for all replicates of this bloc (see figure 6.5). At generation 10, we anticipated the problem and we isolated pupae outside boxes in tubes to observe and control their emergence. Once again, we observed a delay (9 hours) in emergence and obtained low juvenile survival estimates (figure 6.5). We still completed the experiment without shifting the end date originally planned. We observe a strong decrease in fitness for the replicates which suffered from the experimental

issues (no matter the selection regime (Supplementary Information figure SI2-1), which will require specific additional statistical analyses. We thus decided to remove results from the bloc 1 from the analysis; results presented hereafter in the chapter correspond to bloc 2. Results from the bloc 1 are presented in Supplementary Information.

6.3.1 Evolution of traits values

The figure 6.6 illustrates the evolution of phenotypes across time in the fitness control landscapes. Each dot corresponds to mean trait value for a replicat at one given generation. We graphically discern that the evolution for replicates under stabilizing selection is quite winding with back and forth moves in the landscape. However, we can distinguish an increase of fecundity and a decrease of juvenile survival between the beginning and the end of the experiment. In the case of directional selection, replicates move toward the optimum (dark blue zone in the landscape) across generations with a more uniform trajectory. We observe an increase on both juvenile survival and fecundity.

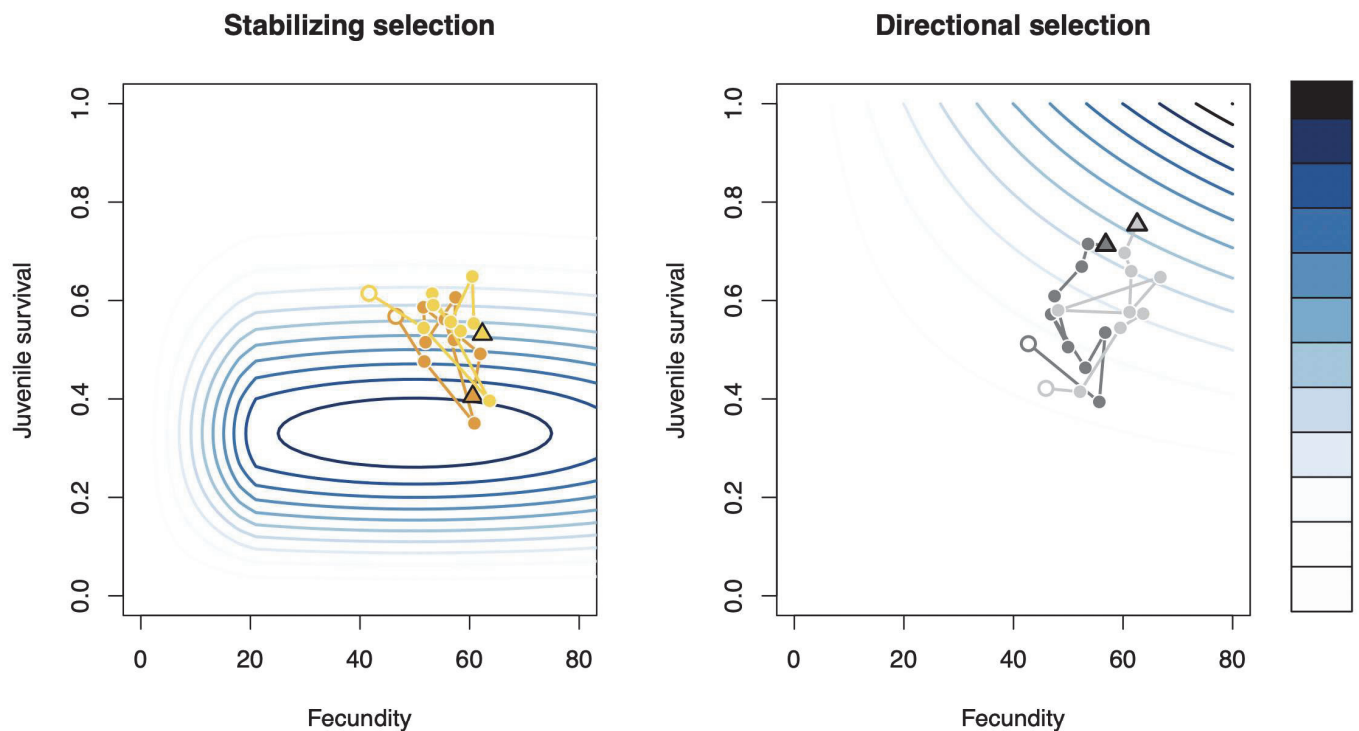


Figure 6.6: Evolution of fecundity and juvenile survival across generations according to the selection regime applied (stabilizing selection in orange and directional selection in grey). Dots represent the mean value for replicates of each selection regime (2 replicates per selection regime) at each generation. First generations are symbolized with white circles and last generations are represented in dark triangles. Lines are trajectories for each replicate across generations. Background colors represent fitness landscape (scale band on the right side : the more dots are closed to dark blue the better fitness values are).

In order to identify specific changes in fecundity and juvenile survival between selection regimes, we studied trait's evolution across generations separately. Generalized linear mixed models (GLMM) were fitted and compared with AIC using *glmmTMB* package in R (see Methods section *Statistical analysis*). We compared full and simpler models to identify the selection regime's effect. For both traits full models obtained better AICs. Full models included the generation and the interaction between selection regime and generations in fixed effects and replicates in random effects. Both stabilizing and directional selection showed an increase in eggs laid with generations (figure 6.7-A). Even if models indicate a significant difference between the two selection regimes, fitted slopes remained sensibly similar (Table 6.1). However, the juvenile survival evolution revealed important difference between selection regimes (figure 6.7-B). Under directional selection, juvenile survival significantly increased with generations while it decreased under stabilizing selection (Table 6.1). First, we notice that selection had an impact of trait values for both selective regimes (despite the graphical evaluation which seemed less clear for replicates under stabilizing selection). Second, selection did not act in the same way for the two traits.

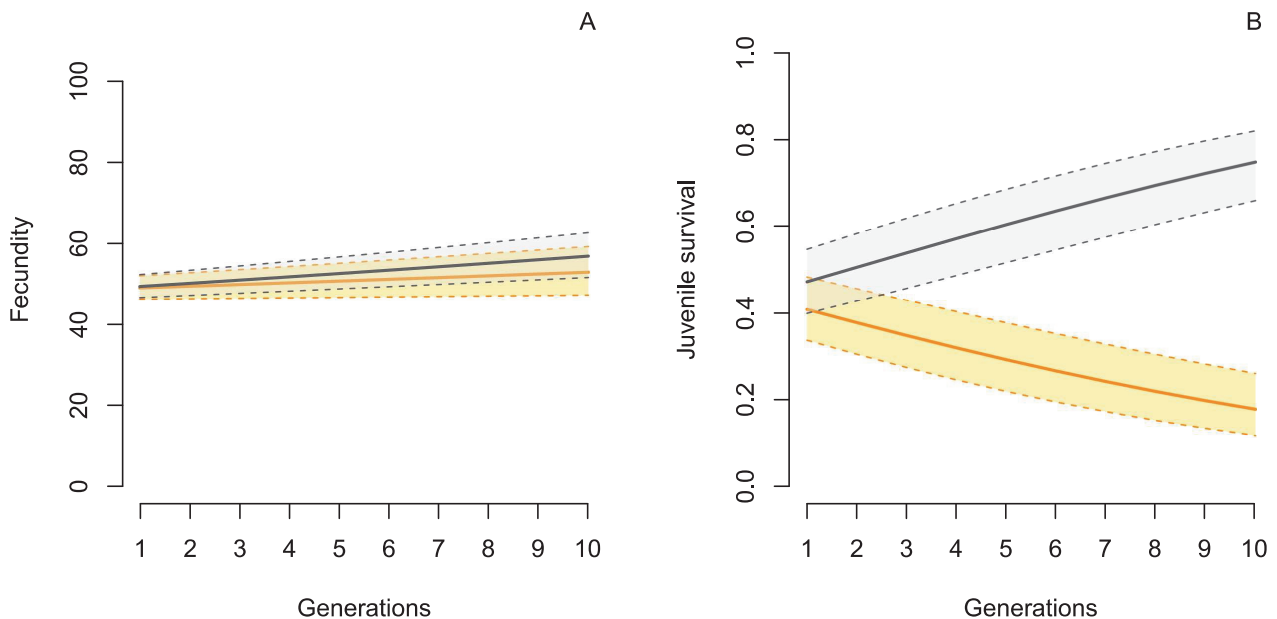


Figure 6.7: Response to artificial selection across generations for both traits (A: Fecundity, B: Juvenile survival) and both selection regimes (grey: directional selection, orange: stabilizing selection). Solid lines correspond to estimates of full models (see Table 6.1 for equation's details) and dashed lines represent confidence intervals at 95%.

Table 6.1: Estimates of generalized linear mixed models and AIC for both traits separately. A zero-inflated Poisson distribution was used for fitted fecundity models and a classic binomial distribution was used for fitted juvenile survival models. For both traits AICs were better with full models.

	Fecundity			
	Int	Generations	selection:generations	AIC
Full model	3.88**	0.0155***	0.00837**	11465.2
Simpler model	3.88***	0.019***	NA	11469.8
	Juvenile survival			
	Int	Generations	selection:generations	AIC
Full model	-0.24	0.133***	-0.128***	18201.4
Simpler model	-0.23***	0.0669***	NA	18425.7

6.3.2 Heritabilities

We wanted to understand why selection did not impact both traits in a same manner. Indeed, the difference of selection of both traits could be due to difference in heritabilities between these traits. Heritability is often used as a measure of evolutionary potential and is defined as the ratio between additive genetic variance and phenotypic variance (Hansen *et al.*, 2011). Heritability h^2 can vary between a very broad range of values ($h^2 = 0.001 - 0.9$) (Mousseau *et al.*, 1987). For life history traits, the median value of heritability is $h^2 = 0.25$ (Estes & Arnold, 2007), which means that estimations of this parameter closed to 1 are rare.

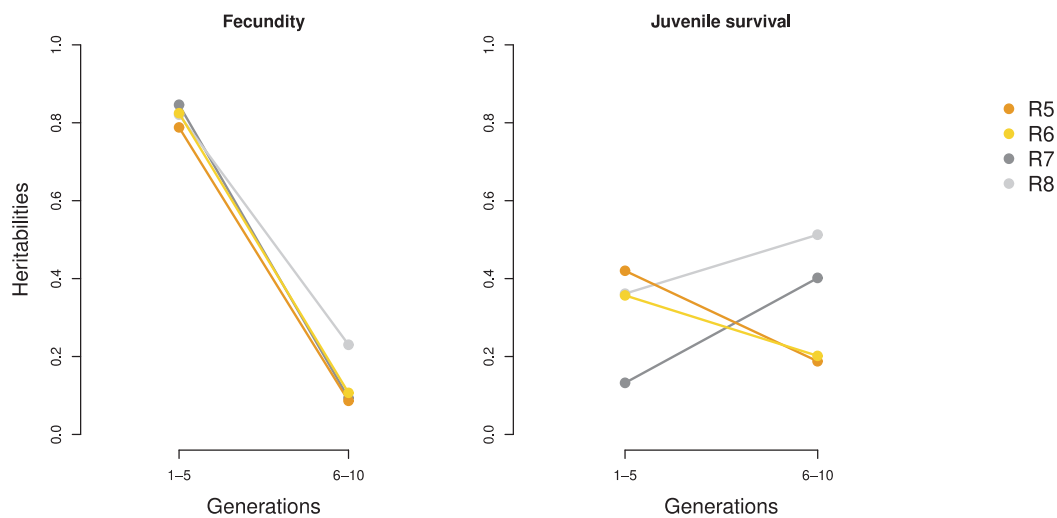


Figure 6.8: Comparison of heritabilities between generations 1-5 and 6-10. Dots correspond to heritabilities for fecundity (first column) and juvenile survival (second column) for each replicate and are linked with their associated values at the next generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection.

From the variance estimates from animal models - which take into account the relat-

edness of individuals in a data set along with their trait values - we estimated an heritability for both traits and between the two generation groups. At the beginning of the experiment, the heritability of fecundity was very high (mean=0.82, sd=0.02) for all replicates. It decreased significantly at generations 6-10 (Student test, p-value= $7.163e - 05$, mean=0.13, sd=0.06). This decrease of heritability might be explained by the erosion of standing genetic variance (figure SI2-2-B), together with a comparatively lower reduction in phenotypic variance. Heritabilities of fecundity were not distinguishable between selective regime.

Heritabilities estimates of juvenile survival at the beginning of the experiment appeared more biologically realistic (around 0.3) than estimates of fecundity. These estimated heritabilities differed between selective regimes (Student test, p-value = 0.04243). Moreover, heritability decreased for generations 1-5 to 6-10 for replicates under stabilizing selection while it increased under directional selection.

6.3.3 Positive genetic correlations between fecundity and juvenile survival

Above, we considered the evolution of fecundity and juvenile survival separately. Now, we consider their conjoint evolution and correlation across time. To do so, we separated our dataset in two sub datasets: the first one containing generations 1 to 5 (generation group 1-5 hereafter) and the second one containing generations 6-10 (generation group 6-10 hereafter). Genetic correlations r_a were calculated from estimations of genetic variances (as presented in *Statistical* section).

Intuitively, we expected to detect a negative genetic correlation between fecundity and juvenile survival at least at the beginning of our experiment because they were supposed to form a trade-off. However, we could not identify a general negative correlation between juvenile survival and fecundity either at the beginning or at the end (excepted for the replicate R5) of our artificial selection (figure 6.9). Indeed, during the first five generations, genetic correlations estimated were positive and widely spread (from 0.21 to 0.65). For the five last generations, genetic correlations were also mainly positive (exception for replicate R5). We could not identify clear differences between selective regimes, but correlation coefficients seemed to increase under directional selection and decrease under stabilizing selection

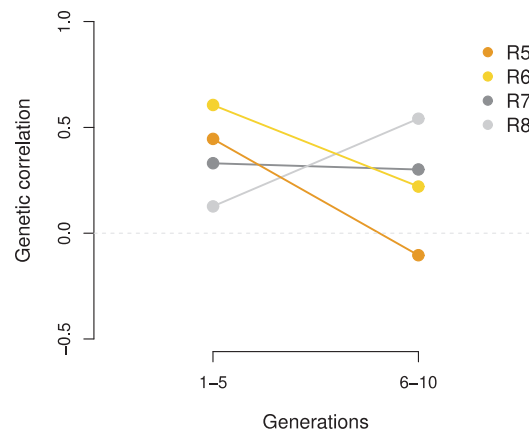


Figure 6.9: Genetic correlations between fecundity and juvenile survival among generations 1-5 and 6-10. Dots correspond to genetic correlation for each replicate and are linked with their associated values at the next generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection. Gray hashed line emphasizes the frontier between positive and negative values.

6.3.4 Impact of the artificial selection on $\mathbf{P}$ - and $\mathbf{G}$ -matrices

The $\mathbf{G}$ - and $\mathbf{P}$ - matrix can be useful tools to study joint changes in genetic and phenotypic variances and covariance between traits. Hence, we determined $\mathbf{P}$ -matrices that correspond to phenotypic variance covariance matrix of fecundity and juvenile survival at every generation, and the $\mathbf{G}$ -matrices (additive genetic variance covariance matrix) for only generations 1 and 6, using estimates of the animal model as above. We estimated $\mathbf{G}$ -matrices with Bayesian framework including pedigree information (6.10). Analyses were performed on each replicates separately. Variance values are expressed at the latent scale for $\mathbf{P}$ - and $\mathbf{G}$ -matrix to facilitate their comparison.

At generation 1, $\mathbf{G}$ -matrices (in blue) depicted important genetic variance on the two traits and a positive covariance between them. Although, we did not expect a positive relationship between these traits, we expected to detect some genetic variance on the two traits due to the initial population mixed from 12 different fruitflies lines (see subsection *Experimental evolution lines*). After 5 generations of selection, the erosion of additive genetic variance in fecundity reported above is the most visible feature of $\mathbf{G}$ -matrices in all selection regimes/ This decrease was associated with a significant decrease in additive genetic variance for juvenile survival for replicates under stabilizing selection and an increase for replicates under directional selection (See Supplementary Information - figure SI2-2.)

Regarding the evolution at the phenotypic scale, we expected $\mathbf{P}$ -matrices to exhibit

important phenotypic variances at generation 1 but, as represented in figure 6.10; fecundity did not show large variance. This is surprising because **G**-matrix is a component of the **P**-matrix which is supposed to display larger variances due to environmental factors. The fact that **G**-matrices presented larger variances than **P**-matrices is probably due to that **G**-matrices are estimated with animal models using pedigree information. Hence, fathers information are taken into account in the estimations of **G**-matrices whereas phenotypic measures have only been made on mothers. At generation 5, we also observed a rapid erosion of the variance in fecundity in **P**-matrices (they had become slimmer) and an overall decrease in variance for juvenile survival (although both phenotypic variances seem to "shake" in time).

Conversely, during the last 5 generations of selection, **P**-matrices showed an increase of juvenile survival variance (the **P**-matrix is extended) without change on fecundity variance. We can also note that traits did not seem to covary (likely due to the variance in fecundity being very small).

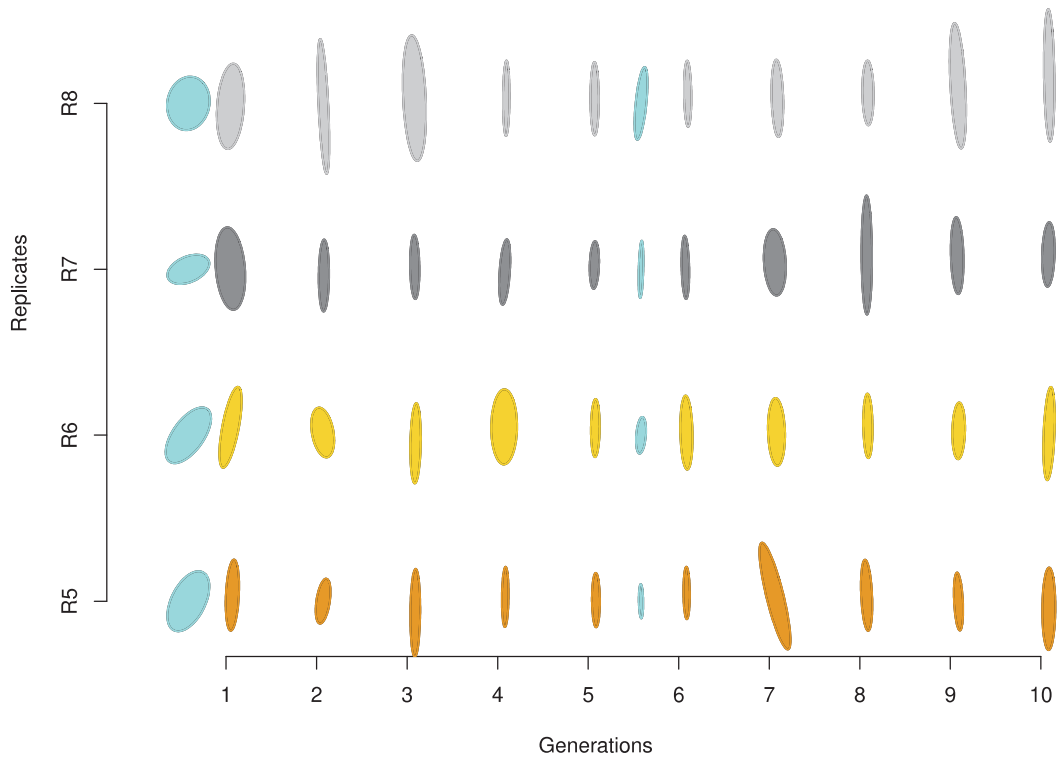


Figure 6.10: Evolution of the shape of phenotypic variances. Shapes of the $\mathbf{P}$ -matrices for each generation and each replicat are represented at the latent scale. Replicates under stabilizing selection are in orange and replicates under directional selection are in grey. In blue, $\mathbf{G}$ -matrices estimated with the animal model at generation 1 and 5. The horizontal axis of the ellipse indicates genetic variance in fecundity while vertical axis indicates genetic variance in survival.

6.3.5 G-matrix shape parameters

Previously, we studied G-matrices graphically in order to compare it with **P**-matrices. **G**-matrices can also be described with a set of parameters to study quantitatively their overall shape evolution (Jones *et al.*, 2003). Hence we estimated the size (Σ which the total sum of genetic variance), the shape (ϵ which is a ratio between the eigenvalues – see figure 6.4 for a graphical representation) and the orientation (ϕ which is the angle between x-axis and the leading eigenvector). The evolution of the first parameter (the size Σ) was consistent with previous results showing a decrease of genetic variance in both traits across generations (also found in figure 6.11-A). The second parameter, ϵ , represents an index of the global shape. A small ϵ means that the matrix has a high eccentricity, such that it is very cigar-shaped (see *Statistical analysis* section figure 6.4-A). On the contrary if ϵ is high then the matrix will have a more circular shape (as shown in figure 6.4-B). We detected a change of shape between the 2 groups of generations, the G-matrix tended to be more cigar-shaped after selection (excepted for R6, where *epsilon* remained approximately constant). Finally, the estimated orientation confirmed what had been observed graphically in figure 6.10. All matrices tended to be similarly oriented around 90°, which means that there was small variance in fecundity but a high variance in juvenile survival.

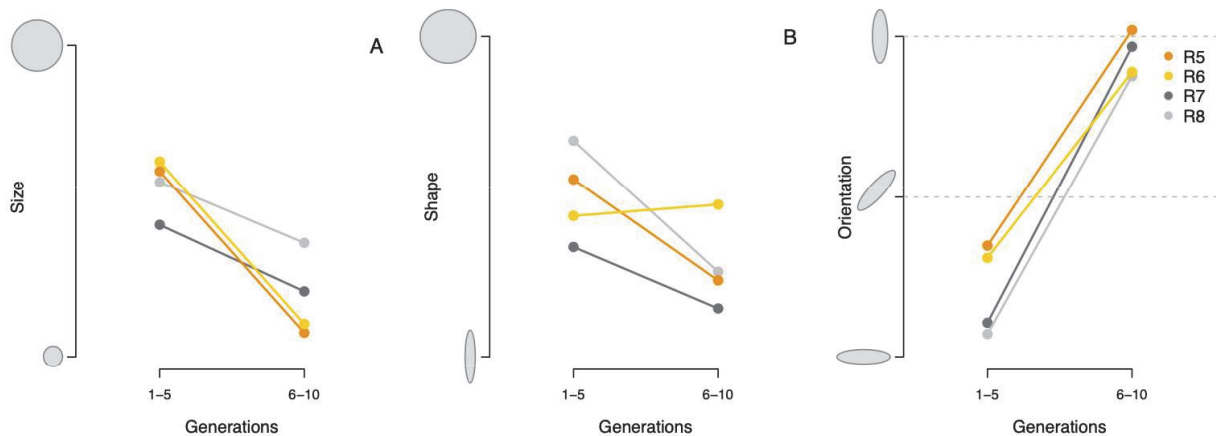


Figure 6.11: Evolution of the G-matrix form between generations 1-5 and 6-10. Dots correspond to size (A), shape (B) or orientation (C) for G-matrices for each replicate and are linked with their associated values at the next bloc generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection.

6.4 Discussion

6.4.1 Comparison between preliminary and experimental results

We ran theoretical simulations by modifying our model to better fit the experimental design (see Box 6.1). These results suggest that under directional selection trait values of individuals should remain stable (or decrease slightly) with little improvement. On the other hand, simulations under stabilizing selection indicate that a movement in the landscape closer to the new optimum is possible. Our experimental results confirm the latter expectation but not the former: fitness improved in populations in both selective regimes, and more effectively under directional selection.

These differences could result from the absence of heritability on acquisition in the model. On the contrary, in the experiment individuals can ingest variable quantity of food and it was demonstrated in *G.firmus* that acquisition is more heritable in *ad libitum* food context (King *et al.*, 2011).

6.4.2 Differential impact of selection on fecundity and juvenile survival

We studied a relationship between phenotypic traits supposed to be under constraints. Hence, we expected these traits not to evolve independently since they were interconnected by a supposed trade-off. Indeed, as a reminder, the trade-off between quality and quantity of offspring is found in many taxa (see *Introduction*). So why did we not get the expected results?

First, our estimations related with the fecundity variances are quite surprising. Indeed, fecundity slightly increased across generations but differed moderately between selection regimes (see figure 6.7). Moreover, we observed an impressive erosion of the genetic variance on this trait very early in the experiment, which caused a drastic decrease of the heritability between the 2 generation groups. Surprisingly, we detected the same pattern on all the replicates. The fact that the erosion of the genetic variance of fecundity was found on all replicates suggests that the experimental protocol could be partly responsible for this effect. Indeed, during the first generations, we assumed that individuals had to accommodate to the experimental protocol and we observed an important adult mortality. Adult survival has rapidly improved over the generations. Nevertheless, the few

performing individuals at generations 1 and 2 were necessarily selected to participate in subsequent generations (*i.e.*, the protocol we developed, applied a sort of "natural selection" even under stabilizing selection because we could not choose individuals associated with very low trait values to participate to the next generation because by definition they were not performing and produced few viable offspring). Hence, independently of the selective regime applied, a very few number of individuals contribute to reproduction, which could have reinforced the variance erosion.

Nevertheless, we observe a slight increase in fecundity (the number of eggs laid during 24hours) under directional selection. We observed during the experiment that fruitfly females can lay up to 100 eggs during 24h, which let a significant potential for trait value improvement. However, our fecundity measurement was quite stringent. Hence, females had only a few days to start their oogenesis and had only one male for fertilization and females can refuse mating. In addition, the time window of 24 hours was particularly drastic but inescapable for the protocol that we had developed. We could not reasonably count the total number of eggs laid per female during their lifetime and then select breeders for the next generation based on these values. For these reasons, it is possible that, on average, under these particular conditions, females may not be able to significantly increase their overall offspring production. In addition, due to the shape of the fitness landscape applied in the stabilizing selection study, females could lay between 35 and 70 egg and still maximize their fitness if they had produced an associated juvenile survival of 0.3 (see figure 6.6). Therefore, selection on this trait under stabilizing selection was not necessarily intense. A reduction of variance could be the results of a strong selection (which is not the case here) or could be due to genetic drift. Hence, we supposed that genetic drift might drive the erosion of the fecundity variance.

Despite the impacts of the experimental protocol on fecundity, we could expect co-variation between juvenile survival and fecundity. In the case of an absence of selection on fecundity, a non-evolution of juvenile survival could be expected. However, significant difference between selective regimes were found over time regarding juvenile survival (see Table 6.1). A possible explanation could be that variation in acquisition had an important role to play. As detailed in the Introduction Part (section XX), a negative relationship between traits can be blurred if individuals acquired different amounts of resource (*i.e.*, an individual with twice as much food as another may simultaneously increase both of its traits, so the relationship between the two individuals appears positive). The easy manner to control food ingestion is to starve individuals, however this solution was not feasible

because the females needed to lay viable offspring. We chose to propose *ad libitum* food to ensure their survival and thus we could not control the amount of food ingested by each females. Moreover, under directional selection, increasing resource acquisition in resource could represent an effective solution to increase trait values efficiently. Thus directional selection might encourage and increase variation in resource acquisition inter-individually. Therefore, the important variance in juvenile survival might be the result of the variance in the acquisition of mothers' resources. However, from the start of the experiment, none negative correlation was detected.

6.4.3 The evolution of the G-matrix between components of fitness

In natural populations or experimentally, G-matrix estimations revealed notable similarities suggesting that G-matrices were stable across long timescales (Arnold *et al.*, 2008). However, this stability might emerged from environmental conditions shared by many populations like strong and persistent pattern of correlational selection or a large population size (Arnold *et al.*, 2008). Indeed, some comparative empirical studies detected change in G-matrices between populations of a same species or in experimental design with stressful environmental conditions (Fong, 1989; Jernigan *et al.*, 1994; Land *et al.*, 1999; Roff, 2002a; Roff *et al.*, 2004; Cano *et al.*, 2004; McGuigan *et al.*, 2004; Doroszuk *et al.*, 2008). These studies were realized on morphological traits (except for Doroszuk *et al.* (2008) where they compared on the evolution of total fecundity and life span between 2 environmental conditions) on short time scales. In these contexts, population sizes were reduced which is favorable for a change in the size of the G-matrix (*i.e.*, with a smaller population comes less genetic variance) and a change in the orientation of the G-matrix as theoretically demonstrated by Jones *et al.* (2003). In our study, populations are placed in a peculiar situation where we controlled the fitness landscape and we actively selected on components of fitness. Our results showed that G-matrices evolve across generations : their orientations became vertical, their size decreased (as predicted by Arnold *et al.* (2008)) but also their global aspect tended to cigar-shaped. Hence our results support a hypothesis made by Jones *et al.* (2003) on the evolution of G-matrix between components of fitness. They assumed that these matrices should be more flexible and respond more quickly to selection, especially in relation to the genetic determinism of these traits, probably regulated by many genes.

Nevertheless, we did not detect difference between selective regime which is quite surprising. Indeed, the shape of G-matrix is supposed to be determined by the interaction between the M-matrix (*i.e.*, mutational effects variance-covariance matrix) and the fitness landscape (Jones *et al.*, 2007). Here, we supposed that M-matrices are similar between replicates because we studied only 10 generations, which is not enough to the emergence of *de novo* mutations and we carefully formed identical replicates from an initial population with a large genetic variance. However, the fact that we imposed different selective regime (and thus different fitness landscape), we expected to identify a difference (see Box 6.1). A possible explanation could be that we did not run the experiment for a sufficient long time to observe significant modifications of the shape of G-matrices between selective regime and results we obtained might be essentially due to experimental protocol adaptation. A second explanation could be that it is not "possible" to select for individual with very low fecundity and low juvenile survival in only 10 generations. Indeed, a strong canalization might be working to minimize the emergence of phenotypes with such counter selective trait values and thus, 10 generations were not enough to impact a robustness mechanism.

6.4.4 Conclusion

la sélection sur des composants de la fitness c'est chiant et compliqué finalement on pourrait dire qu'on pourrait retenter l'expérience avec des paysages de fitness plus farfelues et surtout moins contradictoires avec un vrai contrôle ou on appliquerait une sélection aléatoire pour être sûr que le protocole n'est pas responsable de l'intégralité de ce qu'on a détecté ici.

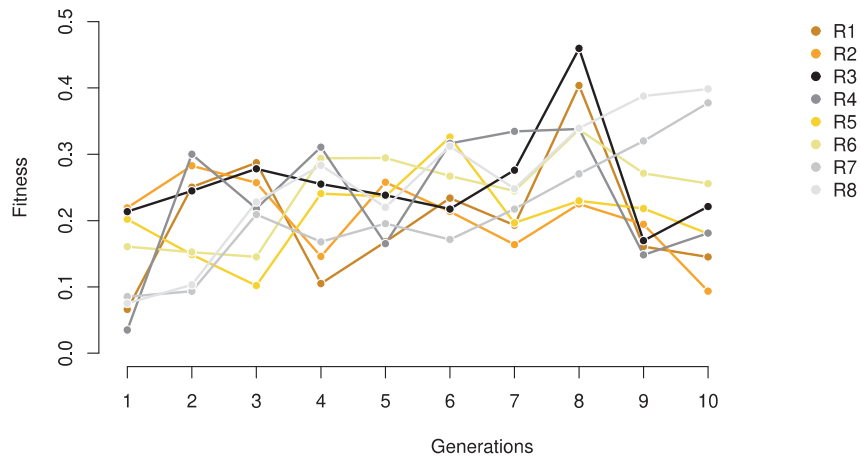


Figure SI2-1: Evolution of fitness across generations. Dots correspond to averaged fitness at one generation for one replicate. Lines between dot symbolize the evolution of fitness between generations. Replicates under stabilizing selection are represented in orange declination whereas grey declination depicts replicates under directional selection.

6.5 Appendices

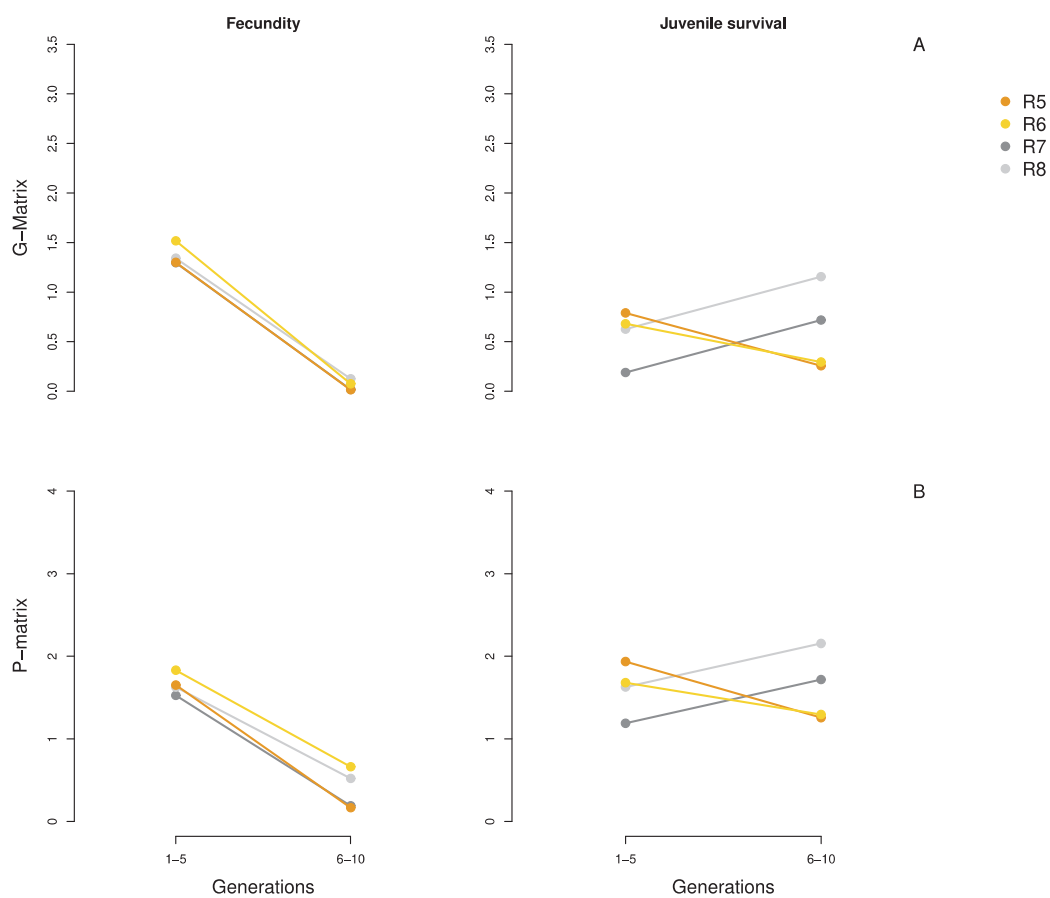


Figure SI2-2: Comparison of genetic and phenotypic variances between generations 1-5 and 6-10. Dots correspond to genetic variances (A) and phenotypic variances (B) for fecundity (first column) and juvenile survival (second column) for each replicates and are linked with their associated values at the next generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection.

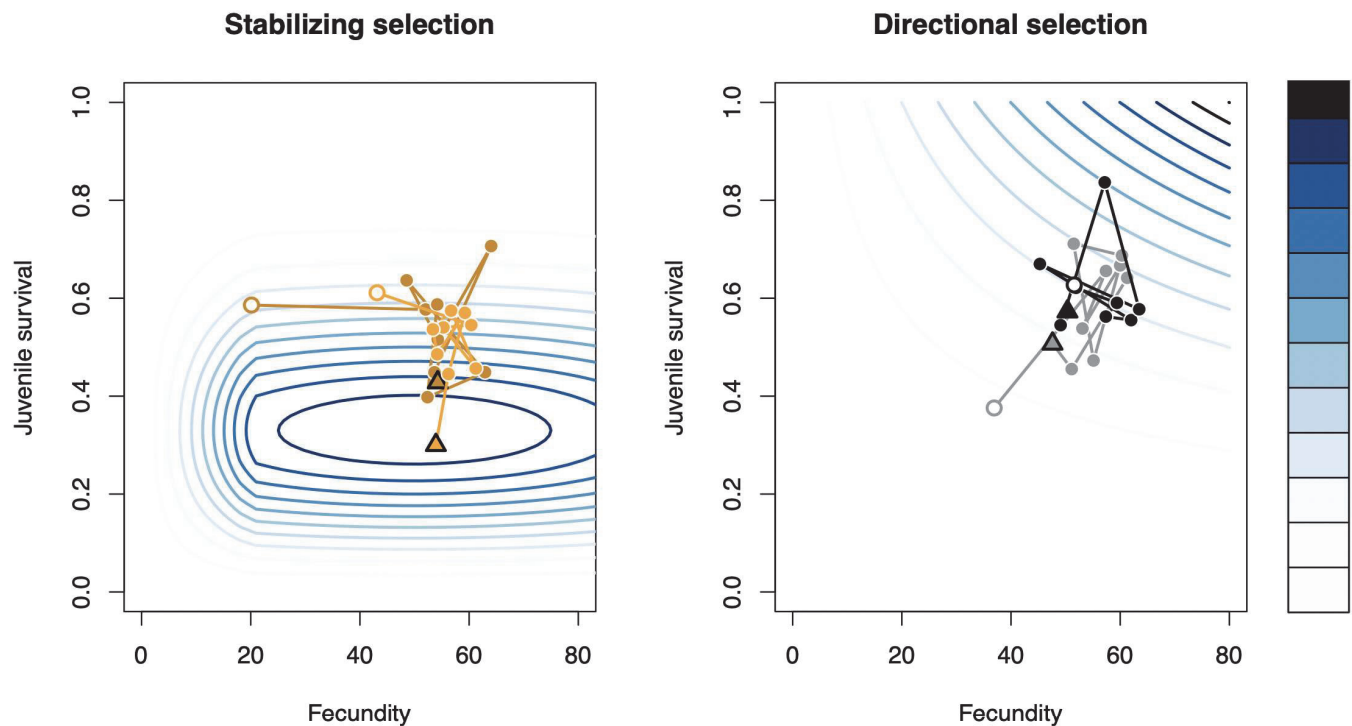


Figure SI2-3: Evolution of fecundity and juvenile survival across generations according to the selection regime applied for the experimental bloc 1 (stabilizing selection in orange and directional selection in grey). Dots represent the mean value for replicates of each selection regime (2 replicates per selection regime) at each generations. First generations are symbolized with white circles and last generations are represented in dark triangles. Lines are trajectories for each replicates across generations. Background colors represent fitness landscape (scale band on the right side : the more dots are closed to dark blue the better fitness values are).

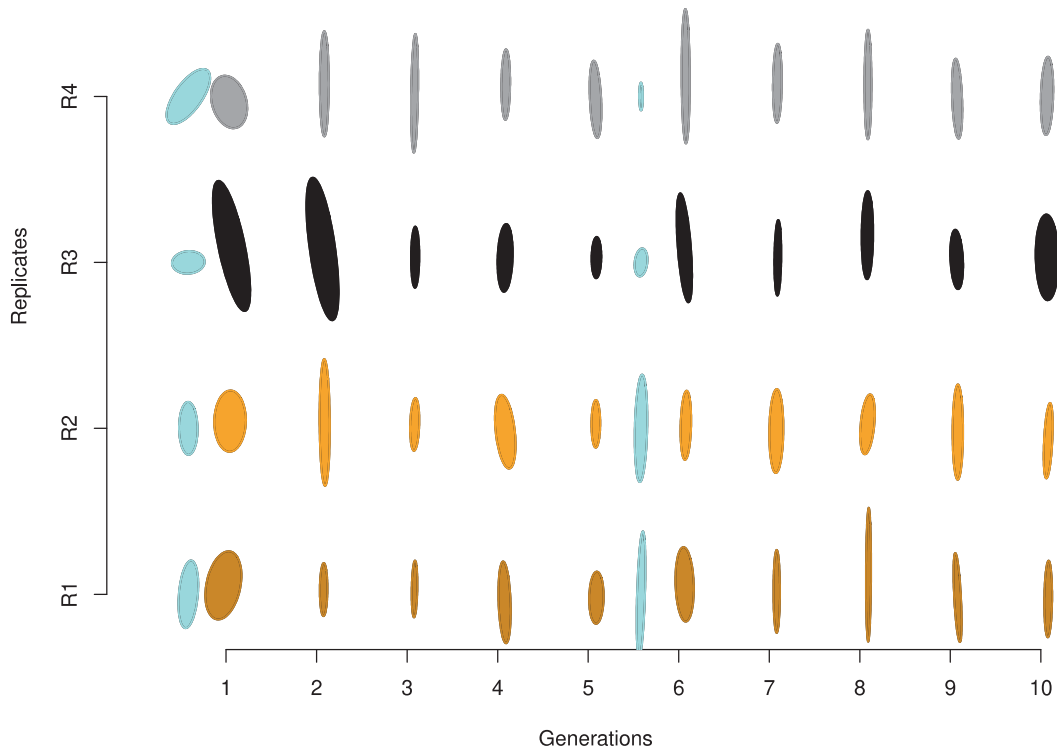


Figure SI2-4: Evolution of the shape of phenotypic variances for the experimental bloc 1. Shapes of the P-matrices for each generations and each replicats are represented at the latent scale. Replicats under stabilizing selection are in orange and replicats under directional selection are in grey. In blue, G-matrices estimated with the animal model.

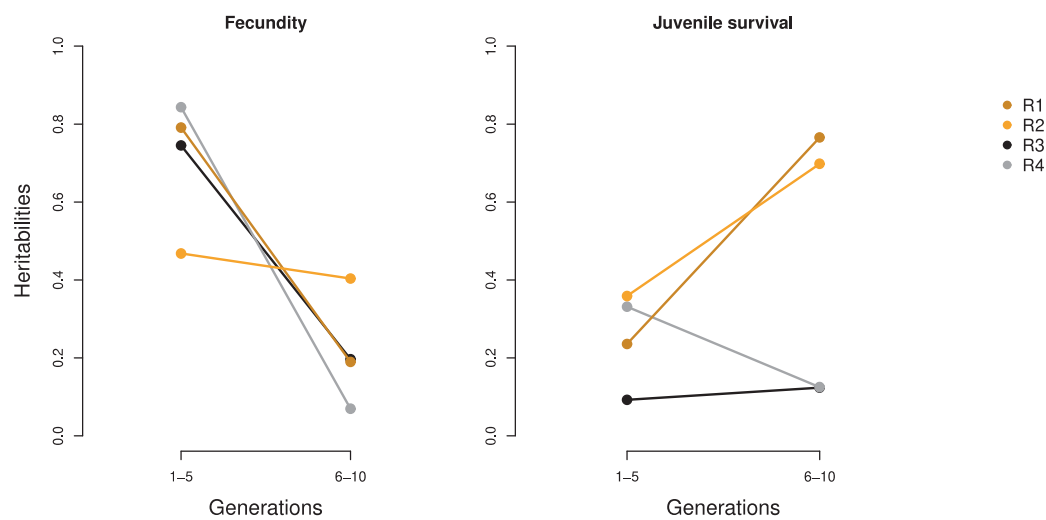


Figure SI2-5: Comparison of heritabilities between generations 1-5 and 6-10 for the experimental bloc 1. Dots correspond heritabilities for fecundity (first column) and juvenile survival (second column) for each replicates and are linked with their associated values at the next generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection.

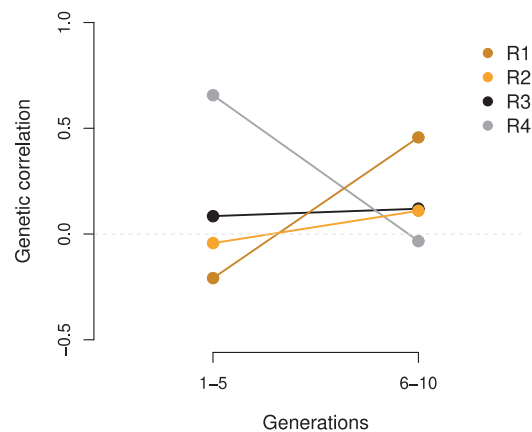


Figure SI2-6: Genetic correlations between fecundity and juvenile survival among generations 1-5 and 6-10 for the experimental bloc 1. Dots correspond to genetic correlation for each replicates and are linked with their associated values at the next generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection. Gray hashed line emphasizes the frontier between positive and negative values.

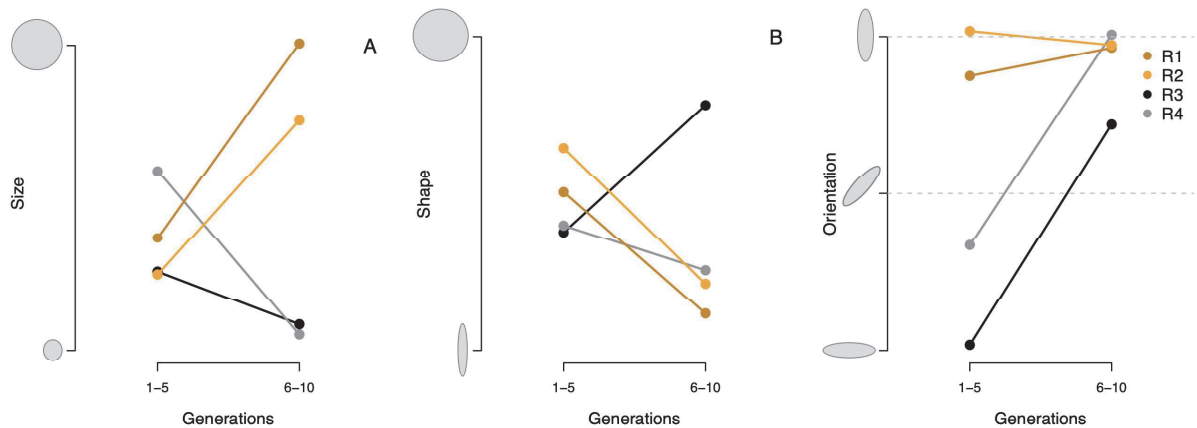


Figure SI2-7: Evolution of the G-matrix form between generations 1-5 and 6-10 for the experimental bloc 1. Dots correspond to size (A), shape (B) or orientation (C) for G-matrices for each replicates and are linked with their associated values at the next bloc generation. Gray dot group represents replicates under directional selection and orange dot group symbolizes replicates under stabilizing selection.

Part IV

GENERAL DISCUSSION & CONCLUSION

General discussion

7.1 Introduction

Each of the previous parts of the thesis contained a specific discussion of the results. Thus, this general discussion provides more of a synthetic overview. Here, I will discuss specific points in more detail and highlight the bridges between the different chapters, in addition to propose idea for future studies as a continuation of the work I conducted during the three years of my PhD. First, I will focus on the notion of the shape of trade-offs and the method developed in the first chapter to measure it. I will also consider possible applications of this method to empirical data and will assess associated limitations. Next, I will discuss the evolution of the shape of trade-offs at both the theoretical and empirical levels, and I will detail the technical obstacles encountered in the empirical study of the underlying mechanisms. Finally, I will highlight the potential of the second model, presented in chapter II, that has not been fully developed. To do so, I will propose improvements of the latter model, in particular, regarding the evolution of underlying hormonal mechanisms.

7.2 How to define and study the shape of trade-offs

As highlighted in the previous chapters, the shape of a trade-off is a parameter rarely investigated. Studies have focused essentially on the detection, or absence, of trade-offs, despite the acknowledgement that the shape of trade-offs constitutes a limiting factor for the evolution of phenotypic traits (Levins, 1962). Indeed, the shape of trade-offs restricts the accessibility to certain genotypes (located far away from the genotypic space occupied by the population) in the short-term.

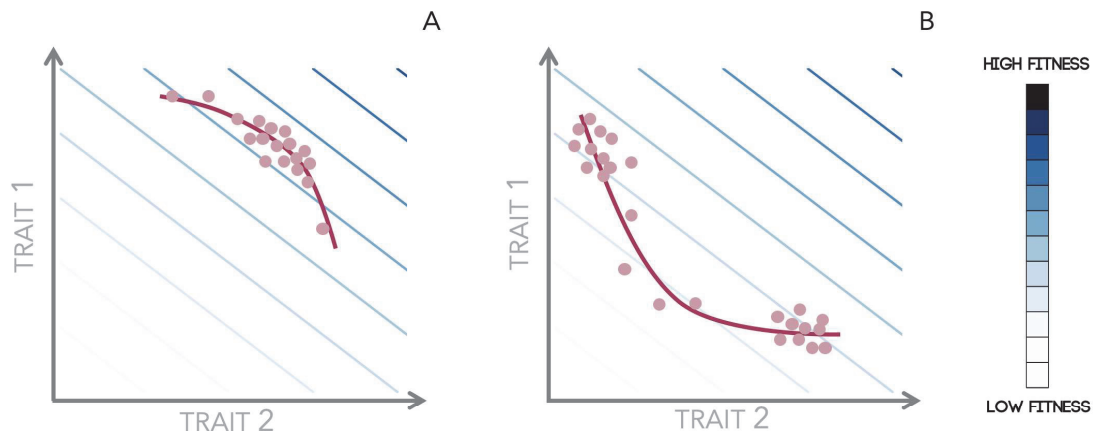


Figure 7.1: Illustration of the role of trade-offs shape in the expected results of evolution. Both situations share the same fitness landscape where the two abstract traits are under directional selection. On the right, a fitness scale is represented : the darker the fitness landscape lines, the highest the fitness values. Hence straight lines are fitness isoclines. Each pink dots represents an individual combination of traits, and the darker pink lines illustrate the trade-offs expressed in the two populations. **A-** Individuals are not spread in an uniform manner on the negative relationship because the central positions have traits values with higher fitness. Hence, individuals at the extremities are at a selective disadvantage. **B-** Individuals tend to position themselves at the edges of the negative relationship because intermediate combinations of traits are related to lower traits values. As a consequence, individuals at the extremities are advantaged.

Under directional selection, when a trade-off is characterized by a curved and concave shape (as represented in figure 7.1-A), there is an optimal position associated with higher fitness values. Individuals expressing extreme trait values have a lower fitness and are removed by selection. Thus, the apparent trade-off is predicted to be short. Otherwise, when the trade-off is characterized by a convex shape (example illustrated in figure 7.1-B), individuals with extreme trait values should be favored. Thus, intermediary individuals have lower fitness values. The population variation can then be divided into two clusters of points and then it might be difficult to detect a trade-off properly. Despite this major role, to our knowledge, no method has been developed to characterize the shape of trade-offs before the one we propose in Chapter 1.

7.2.1 The shape of trade-offs should depend on the ecological environment encountered

In Chapter 1, we presented a method of orthogonal circular regression to determine the circle that passes as close to the data as possible. Using this method, we were able to extract parameters of the circle that provide information about the shape of the trade-off. For instance, the radius of the fitted circle is a proxy for the curvature of the trade-off: if the fitted circle is very large, then the trade-off is linear; on the other hand, if the fitted circle tends to be small, then the trade-off is curved. Although the impact of the shape of trade-off on the expected results of evolution has been demonstrated, these parameters were never evaluated in the studies determining the presence or absence of trade-offs so far.

The results presented in Chapter 1 highlight the fact that trade-offs can have different forms depending on the environments encountered. (Stearns, 1989) already assumed that trade-off could be environment-dependent, but the explanatory factor involved was at the time exclusively linked with the abundance (or lack) of resources. In our first model, where food resource availability is stable, we clearly identify a change in the shape of trade-offs associated with the storage cost. Under an high storage cost, individuals had to rapidly consume their resources to limit the energetic waste associated with the storage. However, since they do not invest their energy optimally, which corresponds to a perfectly fair division between the two traits, they have reduced in conversion efficiency which leads to lower trait values. Thus, individuals who depart from this optimal position decrease their fitnesses and as a result the trade-off is curved and short. In the case of a weak storage cost, individuals had plenty of time to distribute their energy. They can depart from the optimal position without enduring a cost due to conversion efficiency. As a consequence, the trade-off is linear and thus longer (*i.e.*, the distance between the 2 extreme phenotypes, representing the limits of the trade-off, is larger).

7.2.2 Perspectives for the circular regression to determine the shape of trade- offs

Trade-offs are the result of intrinsic properties of organisms and can in principle be population- or species- dependent. Our method developed in Chapter 1 is theoretically applicable to empirical datasets. Thus, it would be possible to test one of the predictions

from the model regarding the link between the cost of storage and the shape of trade-offs. This test could be performed by bringing together sufficiently large datasets from species experiencing different (known or presupposed) storage costs and using our method. Intuitively, we can suppose that migratory birds, traveling long distances each year, would pay a large cost carrying extra weight. Their body morphology, and especially their wings, are optimized to be as light as possible to allow low cost- flying (Pennycuick, 1989). In this context, they are constrained to eat regularly (Weimerskirch *et al.*, 2004, 2017) and thereby most likely endure a high storage cost (related to the energy expended in the transport of extra burden). Conversely, species like cetaceans, which also travel tremendous distances, presumably benefit from fat storage. Fat layers allow them to maintain a stable internal body temperature by providing isolation from the cold (Hashimoto *et al.*, 2015; Young, 1976). They also serve as energetic reserves that can be used during the migrations when cetaceans cross areas deprived of any food resources. For these species (like cetaceans), the storage can represent an important advantage and thus might be optimized. One may even hypothesize that the cost is negative in such a species (they gain a clear benefit from storing that may overcompensate the cost), a situation that I have not considered in the theoretical investigation of the impact of storage cost. An inter-specific comparison of trade-offs would have to study global traits shared by different species, such as longevity or clutch/litter size. It would also be necessary to consider species expressing similar life history strategies (*e.g.*, long-lived or short-lived species for instance) to limit bias associated with life history strategies, since we already assume that differences of trade-offs exist between species exhibiting such different strategies. If we follow our predictions, we could expect that migratory birds, which are constrained by their mode of transport (*i.e.*, flying), express curved and short trade-offs due to the limited number of trait combinations of traits that would maximize fitness (see figure 7.2). For species which are less constrained by the storage cost, linear and long trade-offs resulting from large variations of trait combinations between individuals (without negative impact on their fitness) could emerge.

Differences in the shapes of trade-offs could also be due to phylogenetic reasons. Hence, we could compare the shape of trade-offs in a same species but between natural and captives (or laboratory) populations¹. It is well known that short-lived species placed in captivity benefit from this situation because it significantly decreases factors of extrinsic

¹Roff and colleagues performed such experiments but in a particular species exhibiting only 2 morphs and then strongly constraining the shape of trade-offs. Indeed, we would chose a species able to express a large variety of trait combinations.

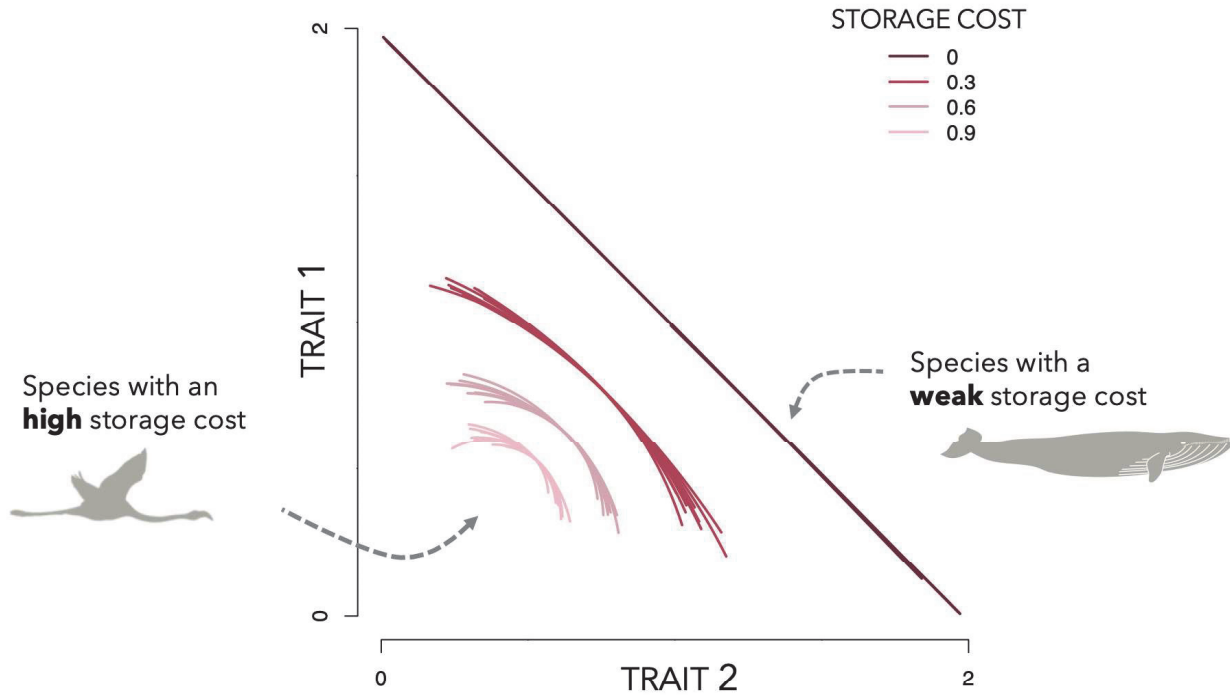


Figure 7.2: Shapes of trade-offs could differ between species with different storage costs. Shapes of trade-offs are different depending on the storage cost applied in simulations. Ten representative simulations per storage cost values are illustrated. The higher the storage cost, the more trade-offs are curved and short. Simulations originate from (Bourg *et al.*, 2019). Species represented as having different storage costs and thus different shapes of trade-offs only serve as an illustration of the idea.

mortality (such as predation) (Lemaître *et al.*, 2013; Tidière *et al.*, 2015, 2016). By removing the cost of the storage and survival found in the field, individuals could accumulate more resources without any cost. The shape of trade-offs is thus probably modified and we could study these variations. Following our predictions, in an environment less constrained, populations should display more linear and longer trade-offs. On the contrary, in a constraining environment, we expect to detect less variation in the population because phenotypes departing from the optimal position would pay a strong cost on fitness and would be removed by selection.

7.2.3 Detection of the shape of trade-offs in closely related species

Thanks to an intern that I co-supervised (with Natacha Kremer and Etienne Rajon), we tried to detect and compare the shape of trade-offs in different species. To do so, we studied the trade-off between longevity and fecundity in three species of *Drosophila* that we sampled from the field (see results in figure 7.3). Because we have not been able to gather enough measurements to effectively use our orthogonal circular regression method,

we chose to analyze our data with classical linear regressions.

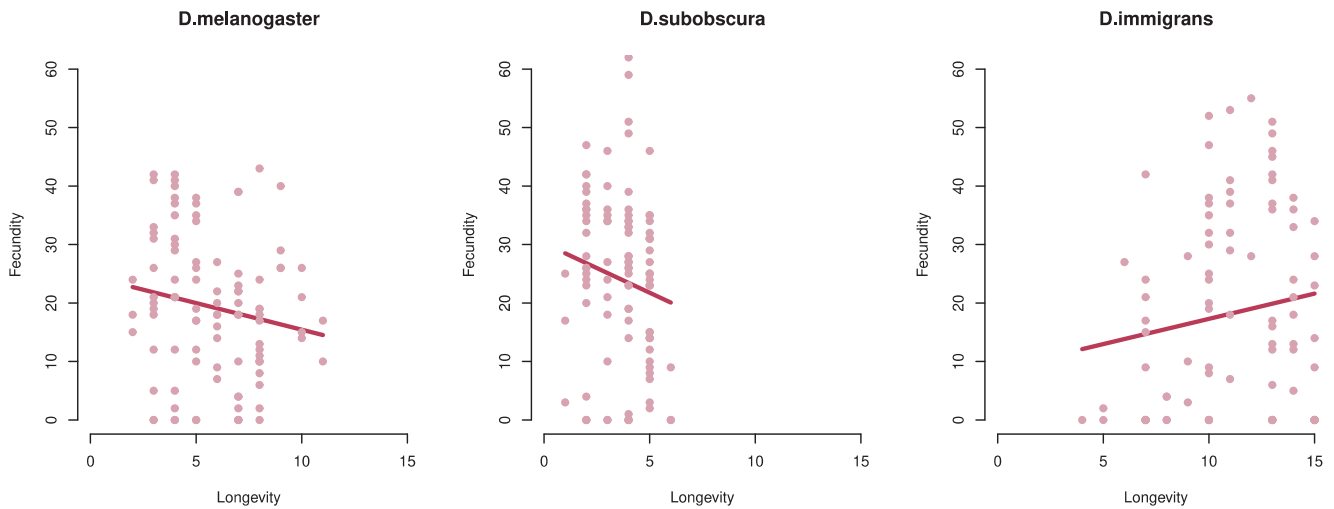


Figure 7.3: Trade-offs between longevity and fecundity compared in three *Drosophila* species. Each pink dot is an individual measure of the two studied traits. Linear regressions are represented in dark pink and are not significant, we only detected tendencies.

Despite a lack of statistical significance, we observed different tendencies between the three species: we did not detect a negative relationship between longevity and reproduction for *D. immigrans*, while we observed such a relationship in the two other species. This result is most likely due to *D. immigrans* being the largest fruitfly in our study and thus was probably able to benefit from the *ad libitum* alimentary regime. Out of the 3 studied species, *D. subobscura* exhibited the shorter trade-off. This species is smaller than the other two, and occurs in the forest. Therefore, *D. subobscura* eventually store food in a lesser amount than the other species, which could impact their storage cost.

In conclusion, the method we developed in the first chapter could be difficult to use on empirical data to effectively compare forms of trade-offs both between species and populations. However, it remains mainly suitable for simulations since large datasets are necessary to efficiently determine the shape of the trade-offs. It would nevertheless be possible to have access to datasets in sufficient quantity by using, for instance, longitudinal monitoring of natural populations or zoo life tables which list a large amount of information (such as birth and death data, sex, litter size, offspring survival) on numerous captive animals (Lemaître *et al.*, 2013; Tidière *et al.*, 2016).

7.3 The evolution of the shape of trade-offs

We have just seen that empirically studying the shape of a trade-off is likely to be complicated, and the same goes for the study of its evolution. For instance, if studies compare trade-offs between populations living in contrasted environments and show that the shapes are different, then a trade-off's shape evolution could be implied but not actually observed. Nevertheless, a powerful way to study the evolution of trade-offs is by using artificial selection. In this section, I will tackle the experimental chapter of my thesis and discuss the role of the acquisition of resources in these results. I will also provide an explanation of the reasons that led us to not empirically investigate the physiological mechanisms regulating trade-offs.

7.3.1 Theoretically, trade-offs can evolve

Before proceeding to the discussion at the empirical level, I wanted to take a few steps back by providing short theoretical reminder. It is assumed that trade-offs should evolve as they result from regulatory mechanisms that are genetically coded, and therefore are subject to evolution. Thus, organisms can express hormonal mechanisms in accordance with the environment they encounter through mutation-selection processes. As a consequence, the trade-offs generated from these interactions can also evolve. Based on these assumptions, we built the first model and showed that trade-offs are indeed capable of evolving. The organism increases its trait values thanks to mutations on the hormonal system, thereby distributing individuals around a same allocation of resources and forming a cluster. After this step, another hormonal mechanism evolved and the individuals can be distributed along a relationship (more or less curved depending on the cost of storage, as we mentioned earlier).

7.3.2 Empirically, few studies observed such an evolution

In our artificial selection experiment, we placed *Drosophila* populations in controlled fitness landscapes. Under these particular conditions, the trade-off shape could (theoretically) change because it was strongly favored by the fitness landscape. Nevertheless, it remains difficult to qualify what we observed as a change in the form of trade-off. We identified an average population displacement in the fitness landscape over generations, but there was no more covariance between traits. In fact, after the first two generations

of breeding, fecundity values showed very low variance values, while juvenile survival still varied. This pattern was maintained in the next generations. As discussed in the chapter 3, the covariance loss could be partly due to the shape of the fitness landscape we applied (where a large range of juvenile survival values allows high fitness). However we detected an evolution of the G-matrix. Due to the limited number of individual measures, we did not use our circular regression method to determine the shape of trade-off. At this point I need to acknowledge, that it was difficult to derive conclusions from the selection study for other reasons like (i) the way base population was constructed (it is possible that strong linkage disequilibrium was at work), (ii) the reduced statistical power due to elimination of half of the replicates and (iii) the uncontrolled nutrient input.

Therefore, an improved version of the existing protocol could be envisaged if we aim to observe the evolution of a trade-off and to characterize its form. In this protocol, I would advise to compare the evolution of traits (fecundity and juvenile survival) in 3 different contexts: stabilizing selection, directional and neutral. The neutral context would allow to detect more easily the intrinsic protocol artifacts. The stabilizing selection would be much more drastic than the one we have previously applied. I would build a based population in a same manner but I would wait 10 generations of crossing before the beginning of the experiment. In a hypothetical world where time and money are not limiting, the number of individuals per replicate and the number of generations would be doubled. Finally, I would also implement an acquisition control protocol to reduce the inter-individual variance.

7.3.3 The control of resources acquisition: a technical obstacle ?

One of the possible explanations to the results obtained with the experimental evolution could be linked to the variations of inter-individual resource acquisition. Indeed, the females were placed in *ad libitum* feeding conditions, thus leaving the possibility to the individuals to feed differently. The role of variations in resource acquisition linked to the difficulty of detecting trade-offs is well known (see Introduction – section *Invisibility of expected trade-offs*). Controlling individuals feeding is difficult to master (especially in very small species) unless the individuals are voluntarily starved. Nevertheless, rather than controlling what each individual ingests, it might be even more interesting to know how much they ingest and to study this variation once quantified. The ability to acquire resources or to feed more could be heritable. Under this hypothesis, with the total ac-

quisition estimated, we could determine the proportion of resources allocated to the two traits. Indeed, it is not the absolute traits values that are the most interesting but rather how the resources are divided between the two traits. Moreover, it would allow to study the impact of acquisition variations on the allocation as explored by King *et al.* (2011) in *Gryllus firmus*.

During additional manipulations carried out with the intern previously mentioned, we were interested in the quantification of resource acquisition in *Drosophila*. We adapted a protocol that was working on larval stages (but that needed to be sacrificed at larval stage, which made impossible to measure traits on adults). We supplemented the food medium with 5% of a blue colorant (eriolglaucine) and let fruitflies feed on it. Then, fruitflies were mashed in a dilution tamper and the optical density was measured in a spectrophotometer to reveal the amount of food ingested. We assumed that the flies that eat the most would also have absorbed more blue. We are still working on some improvements to fully master this technique since the very high inter-individual variations observed did not allow us to accurately determine the amount of food actually ingested.

7.3.4 Physiological mechanisms measures

The entire chapter on experimental evolution completely ignores the underlying mechanisms regulating trade-offs. Indeed, our main objective was to observe an evolution of the shape of the trade-offs induced by a modification of the fitness landscape. Hence, the experimental evolution was probably not the ideal method to realize this type of analysis. Indeed, we had too much uncertainty about the individual position on the trade-off in terms of resource acquisition which prevented us from determining whether the observed physiological mechanism was the result of the allocation, the acquisition or an interaction between the two.

Moreover, the methods used to quantify certain parameters of physiological mechanisms are mainly focused on the measurement of hormonal concentrations. Indeed, these methods are mastered in a large number of species (such as non-invasive measurements using fecal samples (Schwarzenberger *et al.*, 1996; Harper & Austad, 2000; Touma *et al.*, 2004) or more invasive measurements using blood samples (Wingfield & Farner, 1976; Reburn & Wynne-Edwards, 2000)) and are achievable. However, as noted by many authors (Ketterson & Nolan Jr, 1992; Burns *et al.*, 2014; Rosvall *et al.*, 2016; Cox *et al.*, 2016), hormonal levels do not constitute the best measures if we aim to characterize an endocrine

mechanism. Indeed, their concentrations fluctuate during the course of both the day and the life of an individual. On the other hand, it would be very interesting to quantify the density in receptors or the affinities between hormones and receptors. These methods are much more complicated to implement and imply organ biopsy or individual sacrifices (Latouche *et al.*, 1989). Nevertheless, it is possible to quantify the gene expression of the genes linked to the production of these proteins, which should represent a good proxy for the quantity of receptors produced. Another solution could arise from bioinformatics to determine affinities between hormones and receptors through 3D modeling of proteins such as it is realized in biophysics.

To empirically study the link between endocrine mechanisms and trade-offs, one could consider performing individual trait measures (thus providing information about the individual's position on the trade-off) and associating them with gene quantification measures (conducted on genes related to hormone production and receptors) or hormone-receptor affinity measurements. With these data, it would be possible to observe a link between the individual allocation compromise and endocrine variables. In a second step, it would be interesting to apply artificial selection and to compare the co-evolution of the shape of trade-offs and endocrine measures.

7.4 Evolution of underlying mechanisms linked with the regulation of trade-offs

While many studies have focused on the role of hormones in development (Riddiford, 2012; Flatt *et al.*, 2005; Björnsson *et al.*, 2002; Itoh, 2010) and their impacts on various life history traits (Ketterson & Nolan, 1999; Zera & Harshman, 2001; Ricklefs & Wikelski, 2002; Flatt & Heyland, 2011), very few theoretical studies have looked at the evolution of endocrine mechanisms and trade-offs. A recent discipline known as evolutionary endocrinology (Zera *et al.*, 2007) is concerned in particular with the evolution of hormonal systems in relation with their impacts on life history traits, but the conducted so far are mainly empirical (only few theoretical studies such as (Dantzer & Swanson, 2017)). In this thesis, I sought to develop a theoretical framework which can relate into evolutionary endocrinology and is mainly concerned with the impact of hormones on trade-offs. In this part I come back to the results of the first model where we could not identify physiological mechanisms specific to a form of trade-offs. Later, I will discuss the results of the second model and the evolution of the parameter of dependence on the environment. Finally, I will suggest ways to complete the second model and increase its realism.

7.4.1 Multiple genotype spaces for a single shape of trade-offs

In Chapter 1, we observed very different forms of trade-offs depending on the cost of storage, and we assumed that this result originated from a particular endocrine mechanism was at the origin of this result. After several multivariate analyzes (PCA and CCA: results not shown) performed on both genetic and physiological parameters, we could not identify a specific pattern related to a specific shape of trade-offs. On the other hand, we have noticed that the cost of storage influenced the speed of conversion of energy into trait values (as shown in figure SI-8). In this model, we have placed a saturation function between the amount of energy allocated to a structure at each time step and its conversion into trait values. Indeed, too fast consumption leads to a waste of this energy (a part of it is not metabolized and therefore lost). Thus individuals subject to low storage cost could slowly consume their resources without paying any costs and thereby maximize the conversion of energy into trait value. Conversely, individuals subject to high storage costs had to be faster in conversion without losing too much energy and therefore optimized their speed of conversion. However, probably due to the many combinations

of concentrations and affinities and therefore sub-units of genotype space that allow the establishment of a similar system, we were unable to identify how endocrine mechanisms (hormone-receptor affinity or receptor density) had adjusted the absorption of energy.

7.4.2 GP-map of endocrine systems

The first model was developed with the aim to build realistic relationships between genotypes and phenotypes. The second model contains a supplementary level of complexity as it integrates the organisms' response to a fluctuating environment. We know that many others factors could play a major role in the evolution of the shape of trade-offs but we chose to focus on these specific ones as a first step. However, we recognize it would be particularly interesting to add parameters modeling, for instance, differences between sexes or noise in gene expression. In the actual version of the second model, individuals described have a sexual reproduction where offspring inherited their genotype from parent gametes (randomly formed by independent sampling of alleles from one of the two chromosomes), but there is no difference between sexes. Thereby, a differential cost in gamete production or variations in parental care (modeled such as an energetic gift to offspring which can differ for the two parents) could be introduced. The addition of noise in gene expression can inform on the environmental robustness of the physiological mechanism and would perhaps lead to highlight mechanisms capable of buffering this random variation, for example by minimizing the affinity between hormones and receptors.

As a second step, future models could consider a third trait which seems to play a major role in trade-offs: the ability to acquire resources. Thus, the energy allocation between individuals would not remain equitable as some of them would be able to acquire more resources than others. The body size of individuals is often correlated with their ability to gather food and to invest it in their growth during their development (Wilson & Nussey, 2010). Hence, in a new model, endocrine mechanism would integrate a third trait as the investment in growth that would favor individuals able to obtain more energetic resources than others.

7.4.3 Modeling the role of hormones in development

In our models, we consider an immediate role of hormones in response to feeding. However, we know that hormones are implied in the coordination of development (Jia *et al.*, 2004). A better way to study the development would be to model hormonal mechanisms

involving many hormones that could influence the production of other hormones (such as feedback loops). Hormones are well known for their implications in very complex interaction networks (see figure 7.4).

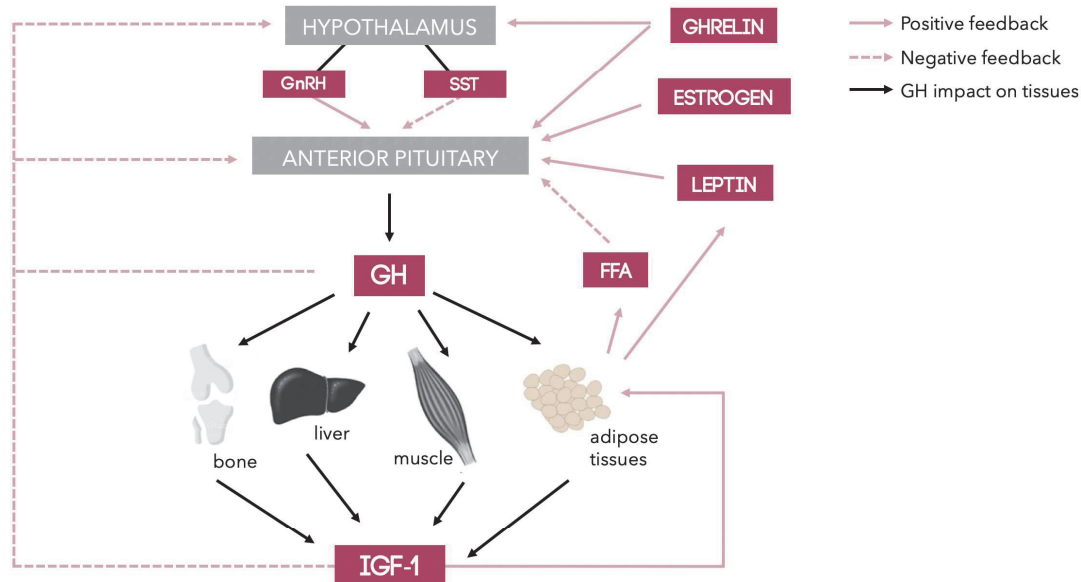


Figure 7.4: An example of hormone regulation (GH and IGF-1) by feedback loops and interaction with other hormones. The Growth Hormone (GH) secretion is regulated by the hypothalamus producing GnRH and Somatostatin (SST), that increase and decrease GH production respectively. GH activates IGF-1 production through its action on several tissues (e.g., bones, liver, muscles, adipose tissues). GH production is down-regulated by negative feedback loops when IGF-1 and GH levels are high and by serum fatty acids (FFAs). GH secretion is also activated by other hormones: ghrelin, estrogen, and leptin. Pink hashed arrows represent negative regulatory mechanisms on GH production, while pink plain arrows symbolize positive ones. Black arrows designate GH action on target tissues that lead to IGF-1 production. This figure is adapted from (Soendergaard et al., 2017)

Surprisingly, this complexity is very much conserved among species (Claeys *et al.*, 2002). A model allowing the establishment of such a complexity of interactions could allow to study the establishment of these hormonal regulation mechanisms. And so, it would allow to study their impacts on the development dynamics and the links with the trait values expressed. These dynamics could also evolve within the lifespan of individuals and thus allow energy allocation shifts at crucial moments in the life of the organisms. For instance, reaching sexual maturity stage, which could result from the moment when individuals acquired a sufficient size to escape predators or acquire enough resources to be able to invest in the reproduction and no more in the growth.

Conclusion

This thesis work has demonstrated the interest of explicitly considering the underlying mechanisms that regulate the allocation of resources to life history traits. This work also stands as a bridge between disciplines that are rarely connected through theoretical approaches, such as evolutionary ecology and endocrinology.

More specifically, my work showed that trade-off's shape can evolve and thus are not necessarily linear. Furthermore, their shapes may also depend on the environment encountered by organisms. Here, I was able to study the evolution of endocrine mechanisms in response to variable elements and to detect their differences. This thesis work also showed the complexity of the experimental reality. Indeed, empirically testing the hypothesis of the evolution of the shape of trade-offs has proven to be a very challenging (in particular due to issues related to the inter-individual variability in the acquisition of resources).

Lastly but not least, the work I conducted during those three years was interdisciplinary both by the fields it connected and by the it answered the questions we addressed (empirically and theoretically). They allowed me to confront experimental problems rarely taken into account by theoreticians and *vice versa*. To conclude, this thesis work has confirmed my interest to adopt an integrative scientific approach to study the evolution of complex traits.

Part V

APPENDICES 1 - INTRODUCTION

(TRADUITE EN FRANÇAIS)

Introduction générale

Au cours des temps géologiques, l'apparence de la Terre a changé de nombreuses fois. Elle est passé d'une roche froide et hostile bombardée de dangereux UV à un habitat riche, fourmillant de vie. Un nombre impressionnant d'espèces se sont succédées aux rythmes des extinctions massives. Malgré l'anthropocène (nouvelle ère géologique caractérisée par l'influence majeure de l'humain sur l'environnement), les cinq premières extinctions sont le résultat d'importantes variations environnementales (Hallam, 1994; Veron, 2008). La plupart des espèces vivantes durant cette période n'ont pas pu survivre à ces conditions particulières et se sont éteintes. Après chaque diminution extrême de la diversité spécifique, une explosion de nouvelles espèces a émergé à partir des espèces restantes. Les successions de ces crises, suivies d'augmentations de la biodiversité, illustrent ce qu'il se passe à des échelles de temps beaucoup plus courtes. En effet, l'évolution agit en permanence. Dans cette introduction, je commencerai par détailler les concepts nécessaires à la compréhension des processus évolutifs. Ensuite, je présenterai les questions qui m'ont particulièrement intéressées pendant ma thèse et qui portent sur l'évolution des mécanismes régulateurs des trade-offs.

Chaque organisme vivant porte des molécules d'ADN (ou d'ARN pour certains virus) contenant son patrimoine génétique; appelé le génome. Pour faciliter l'étude de ces organismes (mais aussi parce que nous ne maîtrisons pas parfaitement les techniques de séquençage jusqu'à récemment), nous résumons ces informations en fonction de leur génotype - *i.e.*, la composition allélique de tous les gènes d'un individu. Cette diversité allélique présente chez chaque individu n'est cependant pas entièrement visible à l'œil nu. En effet, le phénotype constitue l'ensemble des traits observables et résulte de l'interaction entre le génotype et l'environnement (*e.g.*, processus de développement, comportement, disponibilité, ressource alimentaire). La relation entre le génotype et le phénotype est complexe: certaines variations génétiques ont une influence sur les variations de phénotype tandis que d'autres n'ont pas d'impact. Un courant d'étude en biologie de l'évolution vise à

déchiffrer le lien entre génotype et phénotype et à établir une carte de ces relations appelées carte Génotype-Phénotype ². Lors de la transmission d'informations génétiques des parents à la descendance, des réplifications successives d'ADN ont lieu. Si ces réplifications étaient parfaites, alors l'ADN répliqué serait toujours identique. Malgré de nombreux mécanismes censés assurer une copie uniforme, les cellules germinales accumulent de rares erreurs. Ces modifications aléatoires du génome sont appelées mutations et représentent un mécanisme important dans l'émergence de la variation. Une certaine partie des mutations n'a pas ou peu d'impact sur le phénotype (Eyre-Walker & Keightley, 2007) et est dite neutre (même s'il existe un débat autour de cette qualification). A contrario d'autres mutations peuvent avoir un impact important et la majorité d'entre elles sont délétères représentant un désavantage pour leurs hôtes. Mais parfois, une mutation peut améliorer le phénotype (*e.g.* en lui permettant d'exploiter une nouvelle ressource). Ainsi, l'individu porteur de cet avantage (un accès à plus de ressources), pourra être capable de mieux se reproduire et/ou de mieux survivre. Par conséquent, sa descendance portera ce caractère héréditaire, qui se propagera très probablement dans la population. Ce principe représente l'idée de base de la sélection naturelle énoncée par Charles Darwin dans '*L'origine des espèces*'(1859). À partir de modifications aléatoires du patrimoine génétique, de nouveaux variants peuvent s'établir dans une population et remplacer les anciens si ils sont plus performants. Dans un contexte de faible diversité spécifique, beaucoup de niches écologiques ne sont pas habitées. Les mutants peuvent alors coloniser celles qui sont disponibles. Ainsi, génération après génération, une diversité spécifique peut à nouveau faire son apparition (bien entendu, la diversité peut également apparaître dans d'autres situations, comme par exemple dans un environnement fortement compétitif). La description de cette diversité ainsi que l'étude de son émergence et de son maintien constituent un questionnement central pour les biologistes de l'évolution. Pendant des décennies, ils ont essayé de déchiffrer les interactions entre les espèces et leur évolution. Pour les comprendre, ils se sont concentrés sur un paramètre central: la valeur sélective. La valeur sélective peut être décrite comme la capacité d'un génotype à transmettre ses informations génétiques aux générations futures. Pour que cette transmission se produise, il est nécessaire que l'individu focal survive jusqu'à un événement de reproduction. Par conséquent, la valeur sélective est souvent considérée comme une combinaison de la survie et de la reproduction des individus.

Les biologistes de l'évolution ont tenté de décrire puis de comprendre les variations

²Je présente une description de ce courant dans la deuxième partie de cette introduction.

de reproduction et de survie entre les organismes. Afin de maximiser la propagation des gènes à travers les générations, une solution simpliste pourrait consister à augmenter progressivement la capacité de production des descendants. Une relation positive entre la taille de la couvée/portée (souvent utilisée en tant que variable de substitution de la reproduction) et le temps pourrait être attendue. L'étude des variations de la taille de la couvée/portée a particulièrement occupée les biologistes de l'évolution du 19ème et 20ème siècles. Cette question a été notamment étudiée chez les oiseaux. En 1947, Lack publia '*The significance of Clutch-Size*', une analyse détaillée des variations observées de la taille de la couvée chez les oiseaux européens où la majorité des espèces d'oiseaux décrites sont des pondeurs indéterminés, ce qui signifie qu'ils peuvent remplacer les oeufs perdus et ne pondent pas un nombre fixe d'œufs. Des expériences consistant à retirer de manière répétitive les œufs du nid ont été menées. Les expérimentateurs ont ensuite observé une nouvelle ponte jusqu'à ce que l'oiseau atteigne une nouvelle fois le nombre "préféré" de sa population d'origine (Leopold, 1987; Phillips, 1887). Cette observation était très déconcertante pour les chercheurs du 20ème siècle. En effet, pourquoi un oiseau capable de pondre deux fois plus d'œufs qu'il ne produit naturellement se limiterait-il à une quantité spécifique inférieure (Rensch, 1938; Moreau, 1944). Une des hypothèses proposée à l'époque était d'impliquer une raison démographique (Moreau, 1944). Une population d'oiseaux dans un environnement donné a une densité optimale, ce qui lui permet d'exploiter au mieux les ressources et de maintenir une taille de population stable. Deux processus démographiques peuvent agir simultanément sur la taille de la population: les naissances et les décès. La mortalité globale est le résultat de la mortalité intrinsèque (*i.e.*, principalement due à la senescence) et de la mortalité extrinsèque (*i.e.*, de la prédation, des agents pathogènes et des mauvaises conditions environnementales). Par conséquent, la mortalité globale élimine une partie de la population. La population ne peut être stable que si le nombre d'individus éliminés par décès est remplacé par un nombre égal de naissances. Ainsi, pour permettre cet équilibre, et suivant le raisonnement de Moreau (1944), la taille de la couvée correspondrait au nombre d'oiseaux morts divisé par le nombre de couples sexuellement matures, sans tenir compte de la survie des juvéniles. Cependant, ce raisonnement est incompatible avec le processus de sélection naturelle (Lack, 1947). En effet, cela pourrait fonctionner si la sélection naturelle agissait à l'échelle d'une population (ou d'un groupe) or la sélection naturelle agit à l'échelle des individus. Si un individu produit plus de descendants et si ce trait est génétiquement codé, ce caractère se répandra dans la population même s'il est nuisible à l'ensemble de la population. À partir de

cette démonstration verbale, Lack a établi une nouvelle théorie (et précise que la plupart des espèces d'oiseaux ont une taille de couvée constante, quelles que soient les conditions environnementales). Il propose de considérer cette limitation comme une action positive (*i.e.*, la cessation de la ponte) contrairement à une action contrainte (*i.e.*, l'incapacité de produire plus d'œufs). En particulier, il insiste sur le fait que produire trop d'œufs pourrait être désavantageux pour les parents. Par conséquent, la taille de la couvée refléterait le nombre maximal de poussins que les parents peuvent élever sans risquer de perdre un ou plusieurs enfants, et serait dépendante de la saison et de la région. L'action de la sélection naturelle dans cette hypothèse est beaucoup plus claire: les oiseaux ayant une couvée importante seraient favorisés (les gènes codant pour une grande taille de couvée devraient augmenter rapidement dans la population), mais les oiseaux ayant une couvée trop grande seraient contre-sélectionnés (pour éviter de perdre la totalité de la couvée). Dans '*The significance of the clutch size*', Lack ne propose pas de mécanisme explicatif pour ce phénomène, mais a des intuitions différentes.

Premièrement, la taille de couvée ne peut pas croître indéfiniment, car les parents ne seraient pas en mesure de nourrir correctement les poussins. Cet argument précis est central. Lack introduit l'idée principale selon laquelle un trait (ici, la fécondité) ne peut pas varier sans conséquences sur d'autres traits (ici, la survie juvénile). Cette effet balancier est plus connu actuellement sous le nom de trade-off (ce terme n'a pas été employé par Lack en 1947) et constitue le sujet principal de ma thèse. Les variations des traits phénotypiques ne doivent pas être étudiées de manière indépendante (Houle *et al.*, 1996) car des relations génétiques ou physiologiques (comme les trade-offs) relient ces traits entre eux. Les trade-offs sont définis par Stearns en 1989 comme "*le coût payé en valeur sélective lorsqu'un changement bénéfique sur un trait est associé à un changement négatif sur un autre trait*". Un des mécanismes les plus intuitifs pour illustrer les trade-offs est l'allocation différentielle des ressources. Ce mécanisme est celui qu'avait prévu Lack en 1947. L'idée principale est que chaque organisme a accès à une quantité finie d'énergie et qu'il doit l'allouer de manière à maximiser sa survie et sa reproduction. La division de l'énergie entre deux traits en compétition illustre le compromis.

Deuxièmement, la taille de portée (ou de couvée) peut dépendre de l'état physique de la mère (Plard *et al.*, 2015). En effet, une mère en bonne santé est capable d'élever plus de descendants pour plusieurs raisons : elle peut produire des gamètes de meilleure qualité ou leur fournir plus de soins parentaux en les nourrissant plus abondamment par exemple. Ainsi, le poids est souvent utilisé comme indice de qualité des individus et de

leurs capacité à acquérir des ressources. Cette capacité d'acquisition d'énergie représente aussi un paramètre essentiel dans l'étude des trade-offs.

Les travaux de Lack ont suscité un vif intérêt pour l'étude des trade-offs. Certaines études se sont intéressées à la détection des trade-offs dans diverses espèces (**mammifères**: Derting & McClure (1989); Rutkowska *et al.* (2011); Oksanen *et al.* (2002); Charnov & Ernest (2006); **oiseaux**: Smith *et al.* (1989); Ketterson & Nolan Jr (1992); Ghalambor & Martin (2001); Brzęk & Konarzewski (2007); Soler *et al.* (2003); **insectes**: Cotter *et al.* (2008); Flatt & Kawecki (2007); Desouhant *et al.* (2005); Czesak & Fox (2003); Khokhlova *et al.* (2014); **poissons**: Jonsson & Jonsson (1999); Fleming & Gross (1990); Gunderson (1997); Abrahams (1993); **reptiles et amphibiens**: Uller & Olsson (2005); Massot *et al.* (2011); Rivalan *et al.* (2005); Camargo *et al.* (2008); Sinervo & Licht (1991)). D'autres études ont cherché à identifier des relations négatives entre des traits qui n'avaient jamais été considéré comme reliés jusqu'à alors ((Gustafsson *et al.*, 1995; Siikamäki *et al.*, 1994). La variété d'études portant sur le thème des trade-offs et leurs détections sont traités dans la section *Trade-offs : débats et mécanismes sous-jacent*. Dans un second temps, les biologistes de l'évolution ont essayé de comprendre plus en détails comment les trade-offs émergeaient et ont tenté de vérifier leurs hypothèses (Roff, 1990; Flatt *et al.*, 2005; Zera & Zhao, 2006; Flatt & Heyland, 2011). Mon travail de thèse s'inscrit dans la continuité de ces études. Je me suis particulièrement concentrée sur l'évolution des trade-offs chez les organismes multicellulaires. Cette question a été rarement considérée pour plusieurs raisons. Premièrement, une littérature assez ancienne considérait les trade-offs comme le résultat de contraintes physico-chimiques et donc immuables (Tuomi *et al.*, 1983). De plus, le terme trade-off possède plusieurs définitions ce qui rend la caractérisation de ce concept nébuleuse (ce sujet est traité dans la partie 2 de cette introduction). Enfin, certains auteurs ont essayé de catégoriser les trade-offs en fonction de l'échelle à laquelle ils émergeaient. À mon sens, il est nécessaire d'étudier les trade-offs de manière intégrative (en considérant les mécanismes sous-jacents qui les génèrent), je détaille cette opinion dans la 3ème section de cette introduction. Dans la partie suivante, je décrirais le fonctionnement des processus évolutifs essentiels ainsi que les différentes échelles dans lesquels ces processus interviennent et parfois interagissent. Dans la seconde partie, je détaillerai les mécanismes responsables de l'émergence des trade-offs à partir d'exemples théoriques et empiriques. La troisième et dernière partie est consacrée aux peu d'études qui ont envisagé l'évolution des trade-offs ainsi qu'au développement d'un cadre théorique portant sur cette question.

La GP-map: description et conséquences évolutives

Pour prédire l'évolution, il est nécessaire de connaître quels individus sont favorisés dans un environnement donné. Cependant, l'émergence de nouvelles caractéristiques et leurs sélections n'ont pas lieu au même niveau. En effet, l'évolution est le résultat de deux processus : les **mutations** (*i.e.*, formation de nouveaux génotypes – niveau génétique) et la **sélection** (*i.e.*, les phénotypes sont sélectionnés en fonction de l'environnement rencontré et de la pression de sélection – niveau phénotypique) (McCandlish & Stoltzfus, 2014). Une des solutions développées pour étudier l'évolution a été de s'intéresser au lien entre le génotype et la valeur sélective. Sewall Wright dans les années 1930 avait compris le caractère multidimensionnel que représentait un tel travail pour prédire l'adaptation. En effet, il paraît très ambitieux d'imaginer un espace contenant toutes les combinaisons alléliques possibles pour chaque gène associé à une valeur sélective. Afin de surmonter cette complexité, Wright proposa une représentation abstraite continue des variations de valeur sélective sous la forme d'un paysage. La première illustration en deux dimensions 8.1 se lit comme une topographie de carte de randonnée. Les lignes de variations d'altitude sont représentées en pointillées et nous laisse imaginer des surfaces comme des montagnes. En réalité, ces variations d'altitude symbolisent les variations de valeur sélective. Les pics sont associés aux valeurs sélectives élevées et aux changements brutaux d'altitude comme des pentes escarpées. A contrario, les vallées sont des zones planes (avec peu de variation d'altitudes) associées à des valeurs sélectives faibles. Dans la représentation des paysages adaptatifs de Wright en 1932, les axes des abscisses et ordonnées ne sont pas labellisés. Probablement, car il est pratiquement impossible d'élaborer une unité suffisamment précise pour décrire un objet aussi multidimensionnel en seulement 2 dimensions (Dietrich & Skipper Jr, 2012). Bien que les axes soient des représentations symboliques, les positions au sein du graphe ont un sens précis. Chaque point correspondrait à un génotype et la distance euclidienne entre 2 points serait proportionnelle aux nombres de mutations séparant ces deux génotypes. Par des processus de mutation et de fixation,

les génotypes peuvent se déplacer dans le paysage et grâce à la sélection ces mouvements sont "guidés" vers les pics de valeur sélective.

Les variants associés à des valeurs sélectives faibles et représentés dans les vallées sont nettoyés par la sélection. À la suite de cette contribution majeure, de nombreux auteurs ont ré-utilisé (et parfois modifié) cet outil représentatif (Dobzhansky *et al.*, 1937; Simpson, 1944; Lewontin & Kojima, 1960; Lewontin & White, 1960) qui fait encore aujourd'hui l'objet de nombreuses études (Tenaillon *et al.*, 1999; Martin & Lenormand, 2006; Mills & Watson, 2006; Manna *et al.*, 2011; Ferretti *et al.*, 2016; Bajić *et al.*, 2018; Fragata *et al.*, 2018). L'étude des paysages adaptatifs a donc largement contribué à une meilleure compréhension de l'évolution. Néanmoins, ces études omettent une part importante de la réalité biologique : les phénotypes. En effet, dans certains cas il peut être pertinent de s'intéresser aux changements de valeurs sélectives associées à des mutations mais dans des environnements fluctuants par exemple, une même mutation pourra avoir des effets différents sur la valeur sélective en relation avec la performance du phénotype dans l'environnement en question. Ainsi, d'autres approches s'intéressent au lien qui existe entre génotype et phénotype et essayent d'établir une carte de ces relations, plus connue sous le nom de Genotype-Phenotype map (résumé GP-map ci-après). Dans les paragraphes qui suivent je décrirai ce qu'est la GP-map (les éléments qui la composent) mais aussi les conséquences évolutives associées.

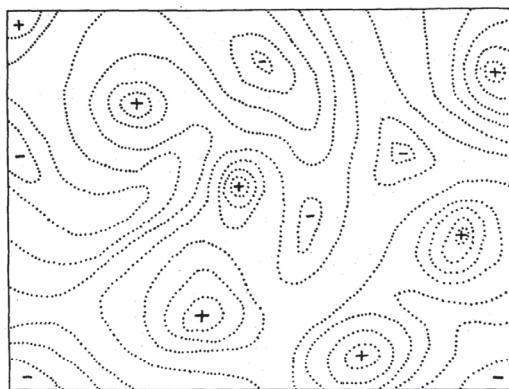


Figure 8.1: Diagramme du paysage adaptatif extrait de Wright (1932). Première représentation de cet outil, où les lignes en pointillées représentent les variations d'altitude (*i.e.*, valeur sélective). Les axes n'ont pas été labellisés précisément par Wright dans son papier fondateur mais ils doivent correspondre à une unité symbolisant un objet multidimensionnel.

8.1 Notions essentielles structurant les études sur la GP-map

Schlosser & Wagner (2004) définissent la GP map comme "*la relation entre la variation génétique et la variation phénotypique tel que seraient spécifiées les différences génétiques donnant lieu à des différences phénotypiques*". L'objectif principal est de construire une carte contenant tous les effets possibles de chaque mutation sur chaque trait. Dans la suite de cette partie, je décrirai d'abord le rôle des mutations et les relations entre les génotypes et un trait puis je présenterai les processus impliqués dans des échelles multitraits. Enfin, je listerai les différentes méthodes qui ont permis de construire différentes parties de la GP-map.

8.1.1 Espace des génotypes en relation avec le phénotype

La production de nouveaux génotypes dans une population à la génération $t + 1$ dépend des génotypes présents dans la population à la génération t , n'importe quel génotype ne peut pas émerger n'importe quand. En effet, un génotype se trouvant très éloigné de ceux présents dans la population n'a aucune chance d'être généré à la génération suivante. Inversement, un génotype très proche de ceux présents dans la population a une forte probabilité d'apparaître à la génération $t + 1$. Un outil très utilisé pour appréhender ces variations qui émergent, est le concept d'**espace des génotypes** qui relie les génotypes entre eux. L'espace des génotypes est un outil représentatif utilisé pour symboliser les réseaux de génotypes 8.2. Il se compose de noeuds et connections les reliant. Chaque noeud représente un génotype et chaque connection est une mutation ou un évènement de recombinaison (Smith, 1970; Draghi *et al.*, 2010; Rajon & Masel, 2013). L'espace des génotypes contient tous les génotypes possibles ainsi que toutes les combinaisons de connections entre eux. Ainsi, la totalité d'un espace des génotypes d'une population représente un énorme réseau complexe multidimensionnel. La taille d'un tel objet peut être très compliquée à définir mais étudier l'évolution d'une sous unité de cet objet représente un challenge plus accessible. Jörg *et al.* (2008) ont déterminé la taille d'un espace neutre (*i.e.*, un sous-ensemble de l'espace des génotypes contenant tous les génotypes qui codent pour un même phénotype, Masel & Trotter (2010)). Ils ont estimé que le nombre de génotypes exprimant un même phénotype était d'environ 1021 (Jörg *et al.*, 2008), ce qui, si on l'extrapole à l'échelle entier d'un organisme, représente un

nombre vertigineux de génotypes.

À chaque instant, une population occupe un sous-ensemble de l'intégralité de l'espace des génotypes. L'évolution agit au travers de déplacements dans cet espace, du aux mutations et aux événements de recombinaison, ainsi, la population peut atteindre de nouvelles régions de l'espace des génotypes. En contact direct avec le nuage de point formé par la population, se trouvent d'autres génotypes accessibles par un unique événement. Cette sous-unité proche constitue le voisinage mutationnel d'une population (voir 8.2)

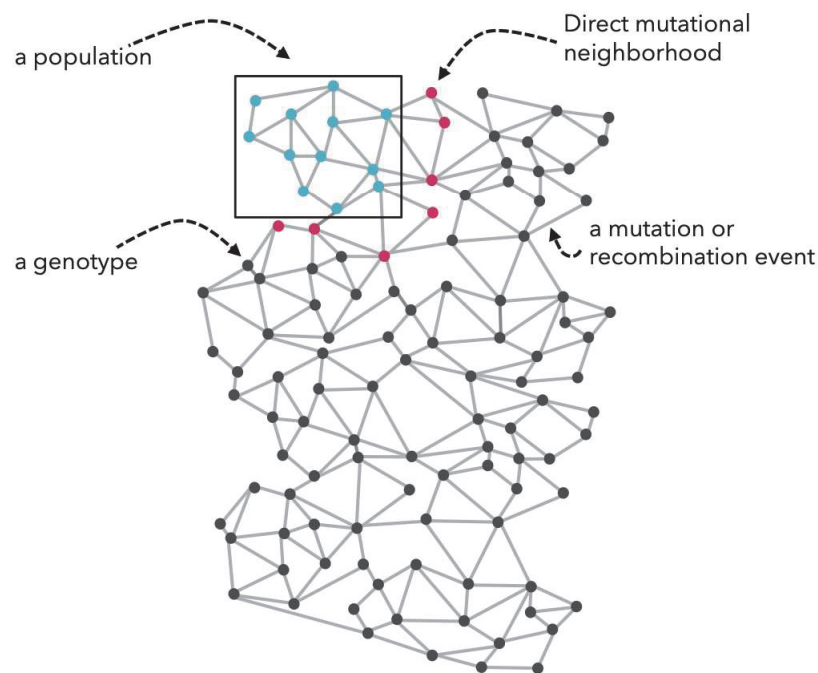


Figure 8.2: Représentation d'un espace des génotypes. Chaque noeud correspond à un génotype et les connexions entre eux sont des mutations ou des événements de recombinaison. Les points bleus représentent une population occupant un sous-ensemble de l'espace des génotypes total.

En fonction de différents paramètres (tel que la variation génétique, la recombinaison ou le taux de mutation), une population a accès à un voisinage mutationnel plus ou moins grand (Draghi *et al.*, 2010). L'accès rapide (*i.e.*, peu de mutations ou d'événements de recombinaison) à de nouveaux génotypes détermine en partie l'**évolabilité** d'une population. L'évolabilité peut être définie comme "*la capacité d'une population à produire de la variation phénotypique héritable de telle manière qu'elle ne soit pas unconditionnellement délétère*" (Masel & Bergman, 2003; Masel, 2006; Masel & Trotter, 2010). Dans une population exprimant une évolutivité élevée, l'évolution peut agir rapidement et l'adaptation est favorisée (Wichman *et al.*, 1999). Cependant, répondre rapidement au niveau phénotypique à des événements de mutations peut, en théorie, être désavantageux.

Dans un environnement stable, un individu produisant des descendants avec des mutations n'aurait probablement pas une bonne valeur sélective (une grande partie des mutations étant délétères; Houle *et al.* (1994)). De plus, il existe un mécanisme évolutif qui permet de résister à ces perturbations, cette capacité est appelée la **robustesse**. Schlosser & Wagner (2004) qualifient un système de robuste si il continue à fonctionner malgré les perturbations (qui peuvent être de différentes natures : changement génétique, bruit d'expression ou changements environnementaux ; Schlosser & Wagner (2004)).

La robustesse et l'évolvabilité semblent ainsi être deux forces contraires: l'une limitant les impacts des mutations sur les phénotypes tandis que l'autre les maximisent. Paradoxalement ces mécanismes agissent ensemble : la robustesse peut favoriser l'évolvabilité (Wagner *et al.*, 2007). Pour comprendre ce paradoxe, il est nécessaire de considérer séparément la robustesse mutationnelle et la robustesse environnementale. Un organisme peut être robuste si il exprime le même phénotype malgré des mutations – *i.e.*, robustesse mutationnelle (Montville *et al.*, 2005) – mais il peut aussi être robuste si le phénotype reste stable malgré des variations de son environnement – *i.e.*, robustesse environnementale (De Visser *et al.*, 2003)–. Dans notre cas, nous sommes intéressés par la robustesse mutationnelle.

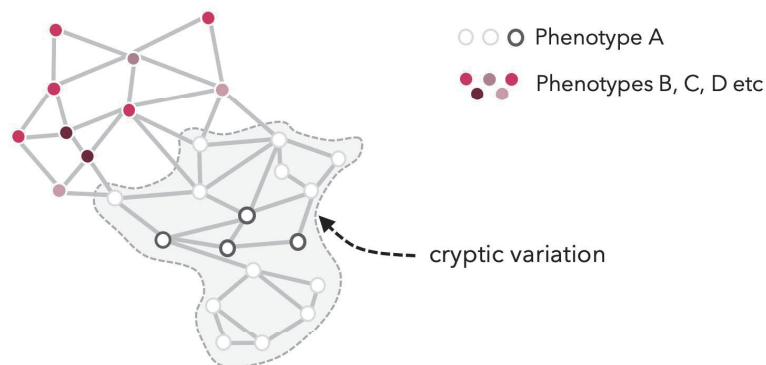


Figure 8.3: Sous-ensemble d'un espace des génotypes cachant de la variation génétique. Les noeuds blancs correspondent aux génotypes exprimant le même phénotype. Les noeuds blancs entourés de noir sont des génotypes formant une population. Les noeuds roses sont des génotypes produisant de nouveaux phénotypes, qui pourraient présenter un avantage sélectif (ou non). Les lignes grises sont des mutations entre les génotypes. La population peut se déplacer dans un espace neutre sans modifier le phénotype exprimé. Ainsi, la population est robuste aux mutations jusqu'à ce que la population atteigne les génotypes roses, qui conduisent à de nouveaux phénotypes. La robustesse cachait de la variation génétique qui est appelée dans ce cas particulier "variation cryptique". Toutes ces mutations et espaces des génotypes sont hypothétiques.

Les espaces neutres mentionnés précédemment sont liés à la robustesse mutationnelle car par définition, une population sous l'effet des mutations se déplace dans un espace

neutre si elle est mutationnellement robuste. Ces mouvements n'ont pas de conséquence sur les phénotypes exprimés mais permettent à la population d'atteindre de nouveaux sous-ensemble de l'espace des génotypes avec de futurs impact potentiels sur le phénotype. Dans ce contexte, la variation génétique est présente bien que cachée (*i.e.*, cette variation non visible est appelée variation cryptique), elle permet à l'évolution neutre d'agir. Un exemple de variation cryptique est illustré dans la figure 8.3, où une (très petite) population de 4 différents génotypes produisant le même phénotype peut se déplacer dans un espace neutre sans engendrer de changement dans le phénotype exprimé (– points blancs dans la figure 8.3, la population est robuste aux mutations) mais après ces mutations la population se déplace et peut se rapprocher de génotypes pouvant exprimer d'autres phénotypes (– points roses sur la figure 8.3, la population est évolvable). Cependant, la relation entre l'évolvabilité et la robustesse peut se révéler bien plus complexe, comme démontré par Draghi *et al.* (2010). On pourrait s'attendre à une relation monotone entre l'évolvabilité et la robustesse mais il apparaît qu'une robustesse intermédiaire est optimale et maximise l'évolvabilité. En effet, dans une population fortement robuste, presque aucune mutation n'a de conséquence sur le phénotype et l'adaptation est donc extrêmement lente. À l'inverse, dans une population très peu robuste, la plupart des mutations sont délétères et la population ne contient pas d'individus potentiellement "adaptable" ce qui ralentit aussi l'adaptation (Draghi *et al.*, 2010).

Un autre processus peut agir à l'échelle du génotype et affecter un trait : l'**épistasie**. L'épistasie peut décrire une situation dans laquelle les actions d'un locus masque les effets allélique d'un autre locus, de la même façon que les allèles dominants masquent les effets des allèles récessifs sur un même locus (Carlborg & Haley, 2004). Un exemple classique d'un phénomène est trouvé chez le cochon (illustré dans la figure 8.4) entre les loci *KIT* et *MC1R*. Si l'individu est hétérozygote ou homozygote avec l'allèle dominant sur le locus *KIT* alors les variations alléliques sur le locus *MC1R* n'ont aucun impact, tous les cochons sont blancs. À l'inverse, si le génotype sur le locus *KIT* est homozygote récessif, alors deux autres phénotypes peuvent émerger : les cochons peuvent être marrons ou noirs.

8.1.2 Processus multitraits impliqués dans la GP-map: pléiotropie et modularité

Certains gènes ont des effets simples sur le phénotype : ils régulent seulement un trait. En revanche, d'autres gènes peuvent contrôler plusieurs traits. Comme par exemple le gène

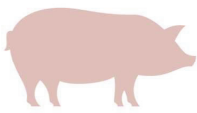
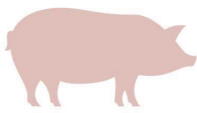
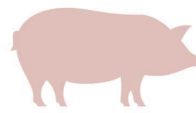
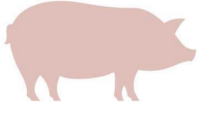
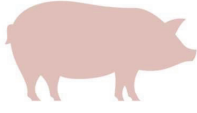
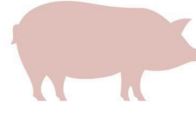
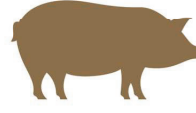
Locus KIT	II			
	li			
	ii			
		EE	Ee	ee
		Locus MC1R		

Figure 8.4: Exemple empirique d'épistasie chez le cochon.. Le gène *KIT* dissimule l'effet du gène *MC1R* dans l'expression de la couleur du cochon. Quand l'allèle dominant I du gène *KIT* est exprimé, le cochon est blanc-rose, peu importe l'allèle du gène *MC1R* l'exprimé. À l'inverse, quand le cochon est homozygote récessif (ii), l'effet de *MC1R* est révélé et le cochon peut être noir (EE, Ee) ou marron (ee). Ainsi, le gène *KIT* est dit épistatique. Cet exemple est extrait de Carlborg & Haley (2004).

Pax6, qui est retrouvé chez *Drosophila melanogaster* et d'autres invertébrés, produit un facteur de transcription essentiel pour la prolifération cellulaire, la migration et l'adhésion. Ainsi, plusieurs développements organiques en dépendent comme les yeux, le système nerveux central et les glandes endocrines (Simpson & Price, 2002). Un autre exemple est le gène albinos chez *Mus musculus*, les souris mutantes ne produisent pas de tyrosinase (*i.e.*, enzyme nécessaire à la biosynthèse de mélanine) et donc expriment un phénotype blanc. Cette mutation impacte aussi la production de catécholamines (*i.e.*, composé organique jouant le rôle de neurotransmetteur) et provoque des modifications comportementales. Les souris mutantes sont moins actives, s'échappent moins rapidement de l'eau et discriminent moins bien le noir du blanc que leurs congénères pigmentées (Fuller, 1967).

Ces gènes affectant de multiples traits sont appelés pléiotropiques (Zhang & Wagner, 2013). Différentes méthodes existent pour détecter la présence de gènes pléiotropiques empiriquement, comme la méthode d'analyse en QTL (*i.e.*, méthode utilisant des marqueurs aléatoires pour identifier les portions de génome affectant le trait d'intérêt) ou les méthodes d'association pangénomiques (*i.e.*, plus connues sous l'acronyme GWAS pour Genome Wide Association studies qui sont utilisées de manière préliminaire aux études QTL) (Schlosser & Wagner, 2004; Paaby & Rockman, 2013). La pléiotropie est phénomène très

répandu et particulièrement étudié en biologie évolutive de manière théorique (Williams, 1957; Cheverud, 1996) et empirique (Dudley *et al.*, 2005; Ohya *et al.*, 2005; Wagner *et al.*, 2008; Albert *et al.*, 2008; Wagner & Zhang, 2011). Le concept de pléiotropie a une définition très large et est parfois utilisé pour désigner des notions différentes. Il semble que cette définition dépende principalement de ce que l'on considère comme trait et nécessite d'être explicitement spécifié (Paaby & Rockman, 2013). Le caractère général de ce concept a d'ailleurs été questionné, en particulier, après le développement de la théorie de la pléiotropie universelle. La pléiotropie universelle est définie comme le fait que chaque gène peut avoir un impact (via mutation) sur chaque trait de l'organisme de manière directe ou indirecte (Stearns, 2010; Orr, 2000; Fisher, 1930b). L'hypothèse de la pléiotropie universelle aurait pour conséquence une adaptation très lente des organismes. En effet, cela nécessiterait de trouver la mutation parfaite permettant de modifier l'intégralité du phénotype afin qu'il soit idéalement adapté à l'environnement rencontré tout en sachant que chaque nouvelle mutation arrivant sur ce génotype viendrait bouleverser potentiellement l'intégralité des traits de ce dernier. Comme Stearns (2010) souligne, le débat autour de la pléiotropie universelle ne devrait se résoudre avec une réponse binaire : oui, la pléiotropie est universelle versus non elle ne l'est pas. Les études devraient plutôt identifier dans quels contextes et à quel degré la pléiotropie est universelle. En effet, des études récentes montrent que les gènes pléiotropiques agissent rarement sur un très grand nombre de trait (Wagner & Zhang, 2011) et que les gènes pléiotropiques représentent uniquement une petite proportion de la totalité des gènes (Wang *et al.*, 2010).

Une conséquence directe de la pléiotropie sur l'architecture génétique des traits est appelée la **modularité**. Cette propriété signifie qu'un set de traits est coordonné par un set de gènes formant un module (comme illustré dans la figure 8.5). Les modules sont détecté comme des groupes de gènes ayant des interactions plus fréquentes avec un groupe de trait. Ainsi, les modules peuvent avoir différents niveaux d'indépendance : certaines gènes peuvent réguler des traits non-intégrés dans le module, et certains traits du module peuvent être contrôlés par des gènes extérieurs au module. Cependant, la différenciation entre modules dépend d'une intégration maximale entre les gènes et les traits au sein du modèle et une différence maximale entre les traits et les gènes impliqués dans d'autres modules (laissant supposer que les interactions inter-modules doivent rester rares). Le caractère modulaire de la GP-map est essentiel car il joue un rôle dans l'évolution. En effet, les traits régulés par des gènes au sein d'un même module ne peuvent pas évoluer indépendamment. Ils réagissent à la sélection comme une même unité (Magwene, 2001).

À l'inverse, l'indépendance maintenue entre les modules implique une indépendance évolutive entre les traits qu'ils régulent (Schlosser & Wagner, 2004). Comme démontré par Fisher (1930b), les petites mutations constituent la base génétique de l'adaptation (Orr, 2005) et une pléiotropie restreinte combinée à une forte modularité devraient émerger car elles augmentent la probabilité de trouver des mutations optimales maximisant la valeur sélective. En effet, si l'on suit la métaphore de Fisher à propos du microscope (où l'objectif est de régler parfaitement le microscope avec les différentes molettes), la pléiotropie universelle signifierait de tourner toutes les molettes aléatoirement à chaque essai ce qui rallongerait considérablement le nombre d'essai avant de trouver le réglage optimal. Alors que si les molettes (gènes pléiotropiques) étaient regroupées en petit sets (modules) reliés à une fonction particulière du microscope (traits) tel que l'oculaire, les objectifs ou le diaphragme alors après quelques (en tout cas moins que sous l'hypothèse de la pléiotropie universelle) tests le réglage optimal pourrait être trouvé.

La nature modulaire de la GP-map contredit donc la théorie de la pléiotropie universelle proposée des décennies plus tôt. En effet, sous l'hypothèse de la pléiotropie universelle, comment expliquer la détection de module ? Différents auteurs soutiennent d'ailleurs que la modularité prime sur la pléiotropie universelle (Wagner *et al.*, 2008; Su *et al.*, 2010) tandis que d'autres essayent de démontrer empiriquement la plausibilité d'une telle hypothèse (Knight *et al.*, 2006). Néanmoins, la pléiotropie modulaire permet aux organismes de répondre rapidement et de manière moins ciblée aux mutations. Ainsi, malgré les contraintes que peuvent générer de telles structures, il est fortement probable qu'elles permettent une adaptation plus rapide. Quoi qu'il en soit, pour étudier ces questions, il est nécessaire de prendre en compte les effets des mutations pour comprendre correctement les mouvements dans l'espace des génotypes qu'elles provoquent. Pour ces raisons, ma thèse a été basée sur des développements permettant de prendre en compte de manière réaliste les mouvements dans l'espace des génotypes ainsi que les conséquences générées sur le phénotype.

8.1.3 Exemples de constructions de GP-map

La prise en compte explicite de l'espace des génotypes permet d'identifier les principaux processus à l'origine de l'évolution, mais ne permet pas de déterminer comment la variation génétique devient de la variance phénotypique. La quantification précise des effets des gènes sur les traits à l'échelle du génome entier est, tout au moins, très complexe. En

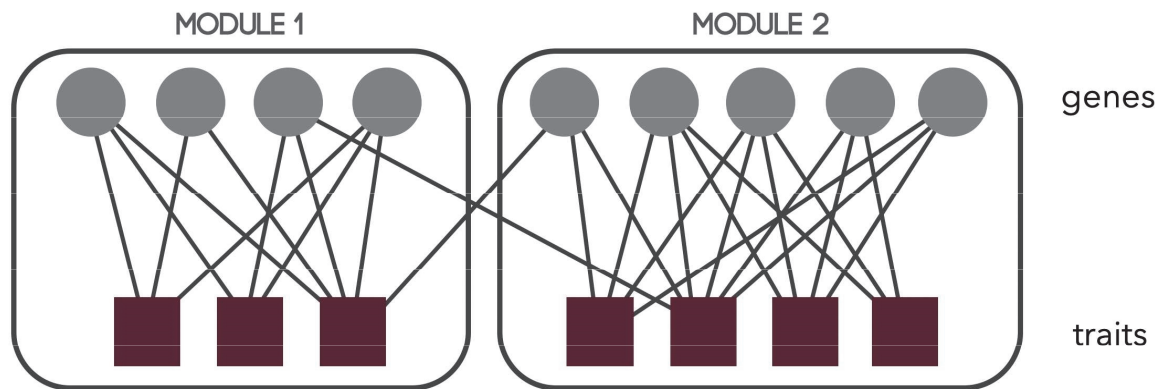


Figure 8.5: La modularité de la GP-map. Un cercle gris est un gène et un carré violet est un trait. Si un cercle et un carré sont reliés par une ligne, alors le gène est impliqué dans l'expression du trait. Un groupe de gènes peut être impliqué dans l'expression d'un groupe de trait. Cette combinaison particulière est appelée pléiotropie modulaire et est représenté avec deux modules séparés (1 et 2). Cette illustration est reproduite à partir de (Wagner & Zhang, 2011).

effet, la GP-map est difficile à déchiffrer chez les organismes vivants, mais des études empiriques sur cette question révèlent des informations intéressantes sur les agencements possibles de la GP-map.

La GP-map peut être étudiée à différents niveaux : de la structure protéique aux traits morphologiques. Des études computationnelles se sont intéressées à des échelles très fines en étudiant les conséquences de mutations de séquences d'ARN sur la structure des protéines produites. En particulier, (Stadler & Stadler, 2006) montraient que la variation génétique ne menait pas systématiquement à des variations dans les structures protéiques exprimées. En effet, il y a toujours plus de séquences nucléotidiques que de formes de protéines, ce qui constitue un résultat attendu puisque que la majorité des mutations sont neutres. Bloom *et al.* (2005) a démontré qu'avec l'augmentation du nombre de substitution, la probabilité que les protéines préservent leurs structures augmentaient exponentiellement. Ces résultats théoriques confirment des résultats empiriques sur la protéase *subtilisin* (Shafikhani *et al.*, 1997). Bloom *et al.* (2005) a aussi montré que les protéines avaient une robustesse mutationnelle plus élevée si leur stabilité thermo-dynamique augmentait (cette stabilité peut être dépendant de la température, de la pression ou du pH).

Les études réalisées en utilisant les méthodes d'analyse en QTL ont permis de déterminer l'architecture génétique de traits morphologiques. Schlosser & Wagner (2004) ont comparé différentes études QTL sur les traits morphologiques chez la souris et il apparaît que les traits physiologiques connexes tendent à être régulés par les mêmes groupes de gènes, alors qu'ils restent déconnectés des gènes impactant d'autres traits. Ces ré-

sultats confirment le caractère modulaire de la GP-map (en tout cas pour les traits morphologiques chez la souris). Néanmoins, les auteurs ont souligné le fait que la modularité détectée n'était pas absolue car certains gènes détectés pouvaient aussi avoir un impact sur le développement global de la structure morphologique.

À une échelle encore plus large, des études comparant les phénotypes sauvages de phénotypes mutants ont montré que la pléiotropie puissante de certains comme les gènes *homeobox* et leurs impacts sur le développement chez *D. melanogaster* (Gehring & Hiromi, 1986). Une étude inter-espèce démontrait que la pléiotropie est détectée chez de nombreux organismes et agit de manière similaire (*i.e.*, une gène affectant peu de traits tandis qu'un très petit nombre de gènes contrôlent de nombreux traits (Wang *et al.*, 2010). Cette étude montre aussi que la modularité détectée dans ces espèces est beaucoup plus élevée que celle qui pourrait être détectée par hasard.

8.2 La réalité est encore plus complexe

L'évolution est un processus agissant à des niveaux : les mutations (niveau génotypique) et la sélection (niveau valeur sélective). Cependant entre ces échelles se trouve un niveau intermédiaire : le niveau phénotypique (représenté in figure 8.6). Ainsi, le lien entre le génotype et le phénotype constitue une part importante du processus évolutif global. L'utilisation de la GP-map combinée aux paysages adaptatifs sont des outils complémentaires et participent à une meilleure compréhension générale de l'évolution. Néanmoins, malgré les degrés de complexités considérés, différents facteurs majeurs restent complètement ignorés : comme l'interaction avec l'environnement (biotique ou abiotique) or les échelles intermédiaires (telles que le niveau physiologique). Comme décrit par la génétique quantitative, le phénotype est le résultat des effets du génotype, de l'environnement et de l'interaction entre ces composants (Lynch *et al.*, 1998). Les effets environnementaux ne sont pas pris en compte dans les études classiques de la GP-map alors qu'ils sont connus pour avoir des conséquences majeurs sur l'expression des phénotypes (Gillespie & Turelli, 1989). Par exemple, des changements de température ou de photopériode induisent des modifications importantes des phénotypes chez de nombreuses espèces (**mammifères**: Reilly *et al.* (2006); Zimova *et al.* (2014); **amphibiens**: Denver (1997); **oiseaux**: McKeechne *et al.* (2006); **insectes**: Whitman (2009); **poissons**: Johnston (1993)). Une telle capacité pour un même génotype d'exprimer différents phénotypes est appelée la plasticité (West-Eberhard, 1989). Parfois, la plasticité implique un changement dans le comporte-

ment alimentaire en présence de parasites ou un changement de comportement reproductif dans un contexte de forte pression de prédation (Chivers *et al.*, 1999). Ces modifications ont des conséquences sur la valeur sélective et jouent un rôle important dans l'évolution (Relyea, 2002). Des études récentes ont montré que les espèces sont capables de modifier leur environnement direct en produisant des métabolites utiles pour d'autres membre de leur espèces. Ainsi, une boucle de rétroaction éco-évolutive peut même émerger et influencer le génotype en retour.

L'environnement "interne" peut aussi affecter les phénotypes des organismes. Tous les eu- caryotes vivent en symbiose avec d'autres organismes : le lichen est une association entre une algue unicellulaire et un champignon, *Wolbachia* est un bactérium unicellulaire présents chez de nombreux insectes, les humains vivent avec plus de 1000 espèces de bactéries dans leurs systèmes digestifs comme *Escherichia coli*, etc. La symbiose peut influencer le comportement de l'hôte avec des repercussions possible sur sa reproduction (Johanowicz & Hoy, 1998; Engelstädter & Hurst, 2009), telles que le génotype de l'hôte dépend de son propre génotype mais aussi de ces symbiotes. Ces relations peuvent être considérées comme des phénotypes étendus (*i.e.*, effets des gènes à des niveaux supérieures que celui de la population) (Dawkins *et al.*, 1982).

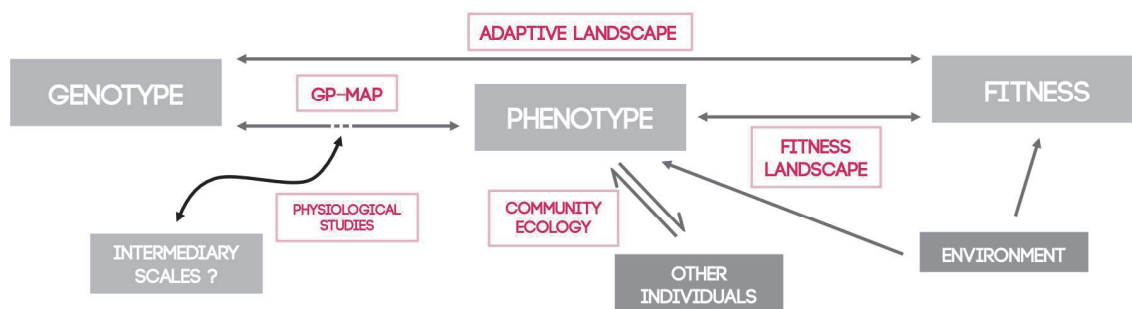


Figure 8.6: Schéma des relations entre différentes unités (boîtes grises) et leurs types d'études associées (boîtes roses). L'étude de l'évolution nécessite une compréhension des interactions entre ces différents niveaux. Ces niveaux représentés ici ainsi que le type d'études associées sont bien sur non-exhaustif. L'objectif est d'illustrer schématiquement la complexité et la diversité des niveaux qui devraient idéalement être pris en compte pour comprendre l'évolution des relations entre traits phénotypiques.

Les domaines d'études (tels que la génétique quantitative, la théorie des traits d'histoire de vie, la démographie etc) sont construits autour d'une idée spécifique et de leurs propres hypothèses. En effet, les concepts provenant d'autres domaines d'études sont rarement intégrés dans le développement de ces théories. Cependant, à l'heure actuelle, nous avons connaissance de ces différentes unités et des interactions qui les unissent, il semble donc

essentiel de multiplier les études transverses et de créer des ponts théoriques entre ces disciplines afin de parvenir plus efficacement à une compréhension globale des mécanismes évolutifs.

Trade-offs : débats et mécanismes sous-jacents

9.1 Universalité et diversité de mesures

Pour Stearns en 1989, les trade-offs représentent le coût payé en terme de valeur sélective lorsqu'un changement bénéfique d'un trait est associé à un changement négatif sur un autre trait. Dans cette définition, il est sous entendu qu'un mécanisme sous jacent provoque un compromis individuel interne. L'expression de ce compromis interne est rendu visible si de la variance inter-individuelle sur l'expression du compromis est présente dans la population (*i.e.*, les individus n'ont pas la même allocation d'énergie). Dans cette situation, une relation négative entre traits peut être observée et la détection de ces relations est l'outil préféré pour mettre en lumière la présence d'un compromis interne. Cependant, en raison d'un abus de langage, le terme de trade-off est utilisé pour désigner deux phénomènes : le compromis interne mais aussi la relation négative qui en émerge au niveau de la population (Roff & Fairbain, 2007). Dans les pages qui suivent, lorsque je ferai référence à la relation négative entre traits j'utiliserai le mot trade-off et j'utiliserai le terme de compromis pour parler du compromis interne au niveau individuel.

Même si les trade-offs ont été principalement détecté dans le contexte du coût de la reproduction (Gadgil & Bossert, 1970; Bell, 1980; Schaffer, 1974), la définition proposée par Stearns offre une large diversité de traits (et de niveaux) possibles. En effet, les trade-offs sont observés à des échelles différentes comme représenté dans la figure 9.1. Ces différentes échelles de mesures sont décrites dans les paragraphes qui suivent. De plus, les trade-offs peuvent être considérés comme ubiquistes et l'existence de concept si généraux est souvent restreinte aux lois physico- chimiques. Ces deux caractéristiques (une définition très large et les différents niveaux auxquels les trade-offs peuvent être détectés) rendent les trade-offs théoriquement universels, ce qui n'est pas le cas, nous le verrons, en pratique.

Un premier niveau où les trade-offs peuvent être observés est entre espèces (échelle

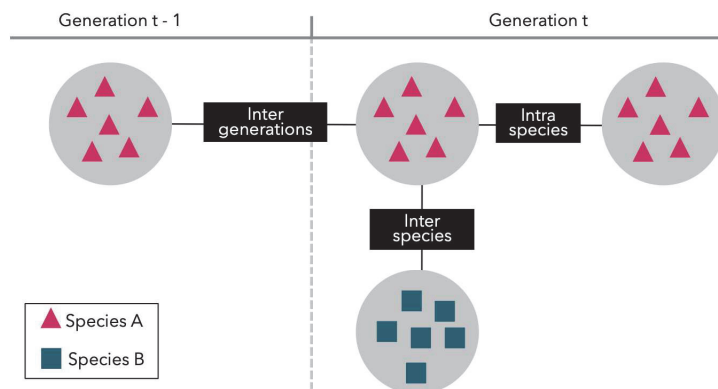


Figure 9.1: Une représentation illustrative des niveaux d'observations auxquels des trade-offs peuvent être observés. Chaque cercle gris représente une population. Ces populations sont composées d'individus (des triangles pour l'espèce A et des carrés pour l'espèce B). Les trade-offs peuvent être observés entre : des générations (inter-générationnelles), des espèces (inter-spécifiques), des populations d'une même espèce (intra-spécifiques).

inter-spécifique). Les études inter-spécifiques sont particulières car elles doivent se restreindre uniquement à l'étude de traits "globaux" partagés par de nombreuses espèces. Les valeurs de traits phénotypiques sont moyennées et utilisées pour chaque espèce, et une régression linéaire des moindres carrés est calculée pour faire apparaître le trade-off. Dans ces études, la variance intra-spécifique n'est pas prise en compte. Ces méthodes ont permis de mettre en évidence des patrons généraux de stratégies d'histoire de vies comme le continuum lent-rapide (slow-fast continuum) (Gaillard *et al.*, 1989; Dobson & Oli, 2007; Jones *et al.*, 2008; Ricklefs, 2010; Salguero-Gómez *et al.*, 2016). En effet, une relation négative entre la longévité et la fécondité est retrouvée chez de nombreuses espèces (Read & Harvey, 1989) (représenté dans la figure 9.2) même après correction par la taille¹ (Reznick, 1985; Jeschke & Kokko, 2009). Dans ces études, il est supposé que la variation en acquisition intra-spécifique est négligeable comparée aux variations inter-spécifiques. Le facteur explicatif souvent proposé est que la mortalité extrinsèque est un paramètre impactant fortement les stratégies d'histoires de vie. En effet, si un individu est confronté à un grand risque de mourir rapidement (comme une souris), la sélection naturel devrait favoriser celles qui sont capables de se reproduire rapidement illustrant l'adage : "living fast and dying young" – "*vivre vite et mourir jeune*" (Promislow & Harvey, 1990). D'autre part, dans le cas d'une faible mortalité extrinsèque (comme chez les ours), les organismes investissent principalement dans leur survie et ont tendance à produire moins de descen-

¹La taille et le poids sont aussi considérés comme des déterminants principaux des traits d'histoire de vie. Evidemment, être grand ou petit a des conséquences sur la vie des individus (Lebreton, 1981) mais d'autres facteurs jouent un rôle important dans les stratégies d'histoire de vie (Gaillard *et al.*, 1989) comme décrit ici.

dant à chaque période de reproduction (mais ils ont une période de reproduction totale plus longue). Ils produisent des juvéniles plus développés and peuvent concentrer leurs soins parentaux sur leur (unique ou peu nombreuse) progéniture et ainsi maximiser leurs survie juvénile.

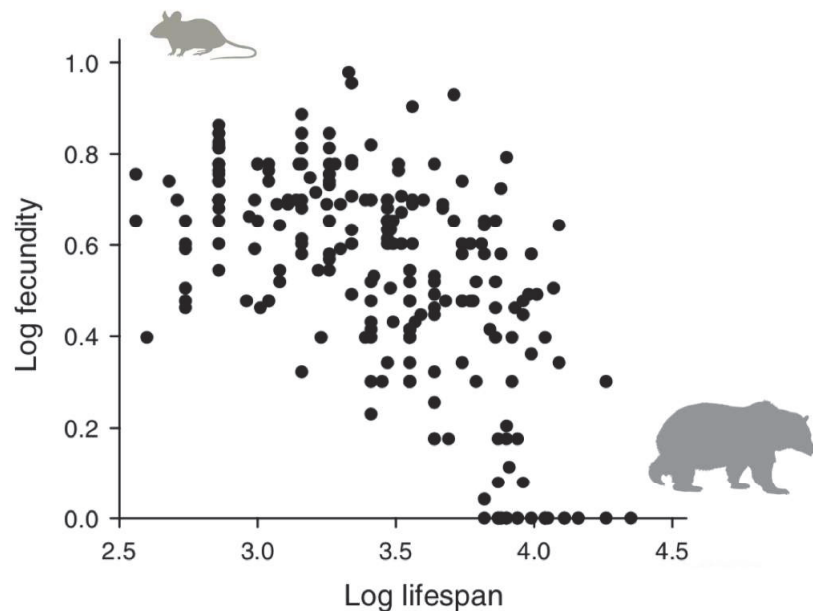


Figure 9.2: Le trade-off entre la fécondité (nombre de descendant par portée) et la durée de vie maximale (en jours) chez des mammifères natifs d'Europe ou de l'Amérique du nord. Cette figure est extraite de Jeschke & Kokko (2009) où les marsupiaux et les chauves-souris ont été retiré de l'étude subsistant 475 espèces de mammifères.

Les trade-offs sont aussi détectés entre individus d'une même espèce (échelle intra-spécifique). Ces trade-offs sont observés en analysant les différences de valeurs de traits phénotypiques chez les individus d'une même population (et parfois entre populations). Comme présenté dans le tableau 9.4 (de manière non-exhaustive), ce niveau de détection est très étudié. Pour reprendre l'exemple de la taille de couvée, un trade-off qui est très souvent mesuré à l'échelle intra-spécifique est celui entre la quantité et la qualité des descendants (Smith *et al.*, 1989; Sinervo & Licht, 1991; Merilä & Wiggins, 1995; Oksanen *et al.*, 2002; Czesak & Fox, 2003; Mappes *et al.*, 2004; Sanchez-Lafuente, 2004; Gibbs *et al.*, 2005; Uller & Olsson, 2005; Charnov & Ernest, 2006; Camargo *et al.*, 2008; Gillespie *et al.*, 2008; Walker *et al.*, 2008; Khokhlova *et al.*, 2014).. Une explication classique suggère que les individus ont une quantité d'énergie limitée à allouer entre deux traits. Ils ne peuvent pas produire un nombre illimité de descendants et en même temps, leur fournir des soins parentaux de bonne qualité (*i.e.*, investissement parental en temps et en énergie fourni pour les descendants). Ainsi, il existe un continuum de combinaisons possibles entre

ces deux traits. Les individus peuvent donner naissance à un seul descendant et lui prodiguer des soins parentaux considérable (point violet sur l'extrémité gauche du trade-off de la figure 9.3). Ou ils peuvent produire un grand nombre de descendant et limiter les soins parentaux apportés à chacun d'eux tout en prenant le risque de perdre une grande proportion de la portée (point bleu sur l'extrémité droite de la figure 9.3). L'étude des trade-offs intra-spécifique est utilisée pour déterminer quels traits sont reliés négativement au sein d'une espèce. Cela peut ensuite permettre de comparer la forme des trade-offs entre différentes espèces et de mettre en évidence différentes contraintes émergeant de la phylogénie ou de l'environnement.

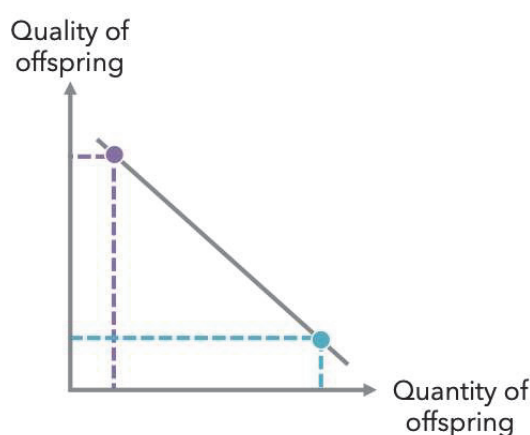


Figure 9.3: Une représentation illustrative d'un trade-off linéaire entre la quantité et la qualité des descendants. Deux positions individuelles sont représentées avec des cercles de couleurs différentes : en violet, un individu exprimant une stratégie extrême (*i.e.*, une grande quantité d'énergie est allouée à la qualité des descendants au détriment de la quantité); en bleu, une stratégie opposée (*i.e.*, un individu va investir fortement dans la quantité et produire de grandes portées probablement de mauvaises qualités).

Un nouveau niveau d'observation de trade-offs a été exploré récemment. L'idée des trade-offs inter-générationnel a émergé pour répondre *a priori* à des observations non-expliquées. Un organisme modèle est *Caenorhabditis elegans*, chez qui certains mutants sont capables de s'échapper d'un trade-off établi entre la fécondité et la longévité (Dillin *et al.*, 2002; Kenyon, 2005)(des résultats similaires ont été trouvés chez *Drosophila* INDY mutant par Rogina *et al.* (2000)). De tels mutants vivent plus longtemps que leurs congénères et ne semblent pas payer de coût sur aucun autre trait (comme la reproduction). Naturellement, on peut se demander pourquoi ce génotype n'est pas fixé dans la population alors qu'il présente manifestement de meilleures valeurs sélectives. Une explication possible de ces observations serait que les descendants de ces individus exprimant de "meilleures" valeurs de traits payent probablement un coût sur leurs propres traits sug-

gérant un trade-off inter-générationnel. Cependant, cette apparente question pourrait être le résultat du choix de trait considéré. Il pourrait être de trouver un coût sur la fécondité si la fécondité réalisée était mesurée (Murua *et al.*, 2003). Néanmoins, ces études cherchent à trouver un trade-off au delà de la vie de l'individu (en s'intéressant aux impacts des performances individuelles sur les générations suivantes) quand un trade-off n'a pas été "détecté" durant la vie des individus. Ces résultats pourraient aussi être expliqués par le fait que ces mutations possèdent un avantage sélectif qui leur permettrait de s'établir sur un autre trade-off. Après invasion de cette mutation dans la population, il pourrait être possible d'observer une relation négative entre ces traits au sein de la population.

À chaque niveau de mesures différents, un nouveau terme est associé au mot trade-off (comme intra-spécifique ou intergénérationnel), ce qui peut contribuer à produire une confusion autour du terme. La diversité de mesure des trade-offs trouve son origine dans l'ambiguïté du mot trade-off car il désigne à la fois une cause et une conséquence (Coste, 2017). En effet, nous utilisons classiquement le terme de trade-offs pour désigner un compromis interne qu'un individu rencontre lors de l'allocation de sa propre énergie entre différentes fonctions (la cause) mais nous utilisons aussi ce mot pour parler d'une corrélation négative entre traits dans une population émergeant d'allocation différentielles individuelles (la conséquence) (Roff & Fairbairn, 2007). Ce qui peut générer des incompréhension en ce qui concerne l'existence des trade-offs dans certains contextes. De plus, l'observations des trade-offs est souvent complètement décorrélée de l'étude des mécanismes sous-jacent qui les régulent et des mécanismes évolutifs agissant dessus. En effet, la relation négative entre traits émerge si il y a présence de variance génétique et donc dépend des génotypes présents dans la population. Les déplacements sur le trade-off dépendent donc de si la position sur ce dernier est héritable ou non, ce qui dépend des mutations qui sont maintenues dans la population ou de la sensibilité des individus au bruit environnemental. La variance génétique présente dans la population ainsi que l'espace des génotypes qu'elle occupe constituent des éléments clés de la compréhension des trade-offs. Il semble en effet, nécessaire de comprendre comment les relations entre génotypes et phénotypes (*i.e.*, la GP-map) évoluent pour prétendre à une compréhension de l'évolution des trade-offs.

Trade-offs	Taxa	Species	Traits	Level of observation	References
Quantity - quality of offspring	Reptiles and amphibians	<i>Uta stansburiana</i>	Phenotypic	intra	Sinervo & Licht (1991)
		<i>Lacerta vivipara</i>	Phenotypic	inter and intra	Uller & Olsson (2005)
		<i>Physalaemus biligonigerus</i>	Phenotypic	intra	Camargo et al. (2008)
	Birds	<i>Parus major</i>	Phenotypic	intra	Smith et al. (1989)
		<i>Parus caeruleus</i>	Phenotypic	intra	Merilä & Wiggins (1995)
		<i>Porphyrio porphyrio</i>	Phenotypic	intra	Sanchez-Lafuente (2004)
	Insects	<i>Pararge aegeria</i>	Phenotypic	intra between male and female	Gibbs et al. (2005)
		<i>Parapulex cheprenis</i> <i>Xenopsylla ramesis</i>	Phenotypic	intra	Khokhlova et al. (2014)
		<i>Stator limbatus</i>	Phenotypic	Intra	Czesak & Fox (2003)
	Mammals	<i>Homo sapiens (pre industrial)</i>	Phenotypic	inter and intra	Walker et al. (2008) ; Gillespie et al. (2008)
		532 species of eutherians mammals	Phenotypic	inter	Charnov & Ernest (2006)
		<i>Clethrionomys glareolus</i>	Phenotypic	intra	Oksanen et al. (2002);
	Fish	<i>Oncorhynchus kisutch</i>	Phenotypic	intra	Fleming & Gross (1990)
		<i>Salmo trutta</i>	Phenotypic	intra	Jonsson & Jonsson (1999)
Current - future reproduction	Reptiles and amphibians	<i>Dermochelys coriacea</i>	Phenotypic	Intra	Rivalan et al. (2005)
	Birds	<i>Junco hyemalis</i>	Phenotypic	Intra	Ketterson & Nolan Jr (1992)
		<i>Fulica atra</i>	Phenotypic	Intra	Brinkhof et al. (2002)
	Insects	<i>Venturia canescens</i>	Phenotypic	Intra	Desouhant et al. (2005)
	Mammals	<i>Myodes glareolus</i>	Phenotypic	Intra	Rutkowska et al. (2011)

Trade-offs	Taxa	Species	Traits	Level of observation	References
Longevity-reproduction	Reptiles and amphibians	<i>Lacerta vivipara</i> ,	Phenotypic	Intra	Massot et al. (2011)
	Birds	<i>Empidonax occidentalis</i> , <i>Turdus migratorius</i> , <i>Troglodytes aedon</i> , <i>Junco hyemalis</i> , <i>Vermivora celata</i>	Phenotypic	Inter	Ghalambor & Martin (2001)
	Insects	<i>Drosophila melanogaster</i>	Phenotypic	Intra	Flatt & Kawecki (2007)
		<i>Anopheles gambiae</i>	Phenotypic	Intra	Dao et al. (2010)
	Mammals	<i>Eutherian mammals</i>	Phenotypic	Inter	Holliday (1994)
	Fish	<i>Poecilia reticulata</i>	Phenotypic	Intra	Abrahams (1993)
		<i>viviparous and oviparous fishes</i>	Phenotypic	Inter	Gunderson (1997)
Immunity - Growth (maintenance)	Reptiles and amphibians	<i>Ctenophorus fordii</i>	Phenotypic (immune activation)	Intra	Uller & Olsson (2005)
		<i>Nerodia rhombifer</i>	Phenotypic	Intra	Korfel et al. (2015)*
	Birds	<i>Pica pica</i>	Phenotypic (immune activation)	Intra	Soler et al. (2003)
		<i>Oceanodroma leucorhoa</i>	Phenotypic	Intra	van der Most et al. (2011)
		<i>Riparia riparia</i>	Phenotypic (immune activation)	Intra	Brzęk & Konarzewski (2007)
	Insects	<i>Spodoptera littoralis</i>	Phenotypic (immune activation)	Intra	Cotter et al. (2008)
		<i>Gryllus bimaculatus</i>	Phenotypic	Intra	Rantala & Roff (2005)
	Mammals	<i>Sigmodon hispidus</i>	Physiological	Intra	Derting & McClure (1989)
	Plants	<i>many species</i>	Physiological	Inter	Denancé et al. (2013)
		<i>meta analysis</i>	Genetic	Inter	Lozano-Durán et al. (2013)

Figure 9.4: Exemple de la diversité de traits qui peuvent être soumis à compromis, de la diversité d'espèce concernés et de la diversité des niveaux étudiés.

9.2 Origines et régulations des trade-offs

Les trade-offs peuvent émerger de différents mécanismes qui peuvent eux même interagir entre eux et engendrer d'autres trade-offs. Dans cette partie, je me concentrerai sur la description de ces mécanismes et de leurs interactions.

9.2.1 Niveau génétique

Les pléiotropies directes et indirectes ainsi que le déséquilibre de liaison peuvent être en théorie responsables de l'émergence de trade-offs entre traits (Sgrò & Hoffmann, 2004; Bennett & Lenski, 2007).

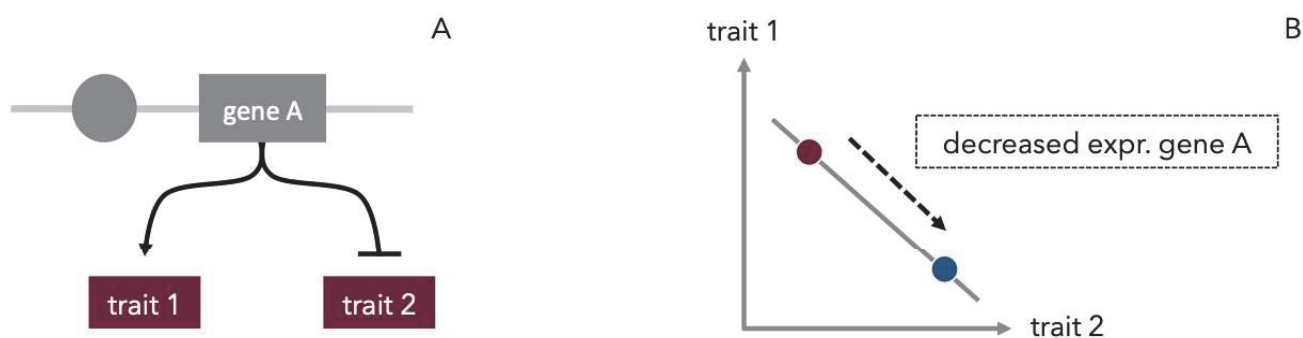


Figure 9.5: Pléiotropie antagoniste directe. A- un gène contrôle deux traits différents, il a un effet antagoniste sur les deux traits. Un individu (point violet) avec une forte expression du gène A qui entraîne une activation du trait 1 et une inhibition du trait 2. Un autre individu (point bleu) a une faible expression du gène A ce qui entraîne une faible inhibition du trait 2 et une légère activation du trait 1. B- Conséquences sur la position des individus sur le trade-off après un changement dans l'expression du gène A.

La pléiotropie directe antagoniste est un mécanisme agissant au niveau génétique. Elle correspond à une situation particulière où un gène code pour deux traits (un tel gène est qualifié de pléiotropique) positivement corrélés à la valeur sélective (Wang *et al.*, 2010). Cependant, son action sur les deux traits est antagoniste. Si on considère l'exemple illustré en figure 9.5-A, l'expression du gène A a un impact positif sur le trait 1 (il augmente sa valeur, comme une augmentation du nombre de descendant si on considère le trait fécondité). D'autre part, l'expression de ce même gène a un impact négatif sur le trait 2 (il diminue sa valeur, comme une diminution de la capacité à échapper à un prédateur dans le cas de la survie). À l'inverse, réduire l'expression de ce gène peut augmenter la valeur du trait 2 et diminuer la valeur du trait 1. Ainsi, chaque changement dans l'expression du gène a un effet antagoniste sur les deux traits étudiés et crée une relation négative entre eux.

La pléiotropie antagoniste est une hypothèse suggérée par Williams en 1957 pour expliquer la sénescence. Il proposait que des gènes à effets pléiotropiques antagonistes (avec des effets positifs dans les premiers stades de vie) soient favorisés par la sélection (quand l'effet de la sélection est faible) même si ces mêmes gènes sont délétères pour

les organismes à des âges plus avancés. Par conséquent, un effet légèrement bénéfique d'un gène en début de vie peut être sélectionné car il dépasse les conséquences négatives associées en fin de vie même si elles sont létales (Kirkwood & Austad, 2000). De plus, la sélection naturelle a un effet plus faible chez les individus âgés si la reproduction est favorisée en début de vie (Haldane *et al.*, 1941; Hamilton, 1966; Charlesworth *et al.*, 1994).

Cependant, ce mécanisme potentiel implique qu'un seul gène serait capable de coder pour 2 traits d'histoire de vie majeurs, alors que la génétique quantitative prédit qu'ils sont potentiellement le résultats de l'action d'une multitude de gènes (Linnen *et al.*, 2013). Les études empiriques à ce sujet ne permettent pas d'établir un consensus. En effet, dans une étude empirique chez *C.elegans*, Estes et collaborateurs n'ont pas trouvé de preuve de trade-offs entre traits d'histoire de vie phénotypiques générés par de la pléiotropie antagoniste (Estes *et al.*, 2005; Roff & Fairbairn, 2007). Néanmoins on retrouve un exemple d'un tel mécanisme chez l'unicellulaire (à ma connaissance il n'existe pas d'exemple équivalent chez les multicellulaires) *Escherichia coli* où un trade-off entre la résistance au stress et la croissance végétative est régulée par le facteur de transcription RpoS (détaillé dans le prochain paragraphe) et est renforcé par une modification structurale de la cellule causée par la concentration en RpoS dans la cellule. La taille des porines (*i.e.*, protéines transmembranaires formant des canaux capables de faire diffuser des molécules à travers la membrane) est déterminé par la concentration en RpoS. Si les porines produites sont larges, alors de grandes molécules peuvent entrer dans la cellule. Par conséquent, plus de nutriment peuvent entrer (augmentant la croissance) mais aussi plus d'antibiotiques (diminuant la résistance au stress). Dans le cas inverse, de petites porines réduisent la croissance et augmentent la résistance au stress (Ferenci, 2016).

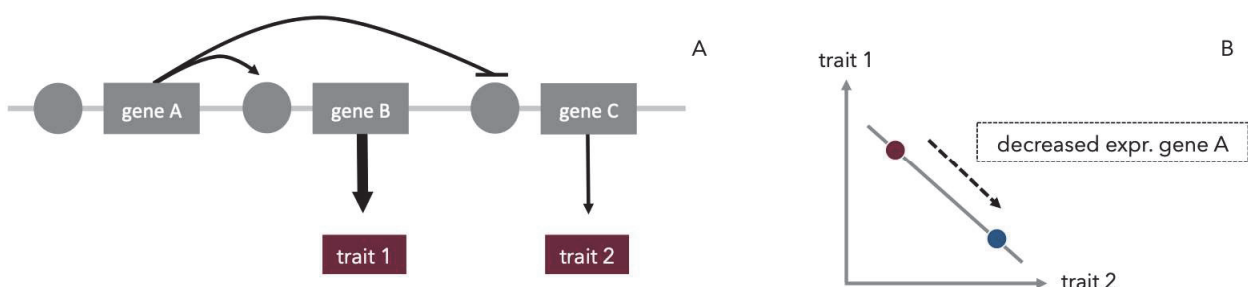


Figure 9.6: Pléiotropie antagoniste indirecte. **A-** Le gène A active le gène B et inhibe le gène C. Les gènes B et C activent respectivement les traits 1 et 2. Un individu (point violet) avec une forte expression du gène A qui entraîne l'activation du trait 1 et une faible activation du trait 2. Un second individu (point bleu) a une faible expression du gène entraînant une activation du trait 2 et une inhibition du gène 1. **B-** Conséquence sur la position de l'individu sur le trade-off quand un changement d'expression du gène A se produit.

La pléiotropie antagoniste indirecte est un mécanisme avec un niveau intermédiaire qui peut permettre l'expression d'un trade-off. Ce cas est illustré dans la figure 9.6-B, où les traits 1 et 2 sont respectivement régulés par les gènes B et C. Ces gènes sont contrôlés antagonistiquement par l'expression du gène A. Si un individu a une forte expression du gène A (point violet sur l'illustration), alors l'expression du gène B est augmentée et la valeur de trait 1 est augmentée aussi. À l'inverse, l'expression du gène C est inhibée et la valeur de trait 2 diminue. Et vice versa si l'individu a une faible expression du gène A (point bleu). Comme pour la pléiotropie antagoniste directe, la modification de l'expression du gène A impacte les valeurs de traits des individus et génère une relation négative. Ce type de mécanisme laisse plus de liberté en terme de nombre de régulations possibles entre gènes et traits phénotypiques et peut permettre l'émergence de trade-offs particuliers même chez les unicellulaires.

Chez ces espèces, la communication intra-cellulaire peut être assurée par des facteurs de transcription (c'est aussi le cas chez les multicellulaires). Par conséquent, le rôle de l'expression génique dans la régulation des trade-offs a été principalement décrite chez les unicellulaires² (Mappes *et al.*, 2004; Jessup & Bohannan, 2008; Bayer *et al.*, 2009; Heine-man & Brown, 2012; Maharjan *et al.*, 2013; Azevedo *et al.*, 2016; Ferenci, 2016). Chez les microorganismes, les deux traits d'histoires de vie principaux sont la croissance végétative (combinant la longévité, le nombre de descendants et la croissance) et la résistance au stress (regroupant toutes les fonctions qui améliorent la survie comme la résistance aux antibiotiques ou à la sécheresse et la maintenance) de part leur mode de vie scissipare. Par exemple chez *Escherichia coli*, un trade-off d'allocation entre la résistance au stress et la croissance végétative est régulée par la concentration en facteur de transcription appelé RpoS (facteur sigma interagissant avec l'ARN polymérase). Dans le contexte d'une forte concentration en RpoS, l'expression des gènes de croissance est inhibée tandis que les gènes de résistance au stress sont stimulés et *vice versa* (Maharjan *et al.*, 2013). Ce mécanisme régulateur peut être considéré comme de la pléiotropie antagoniste indirecte car il fait intervenir de multiples gènes impliqués dans l'expression de deux traits d'histoire de vie différents.

Le déséquilibre de liaison (illustré dans la figure 9.7) est défini comme une association non-aléatoire d'allèles à différents loci (Hartl *et al.*, 1997) et est théoriquement impliqué dans l'émergence des trade-offs. Un gène A code pour un trait 1 et son expression est

²Il existe aussi des exemples chez les vertébrés où un gène spécifique à un régulateur hormonal impacte positivement un gène et négativement un autre (Hau & Wingfield, 2011).

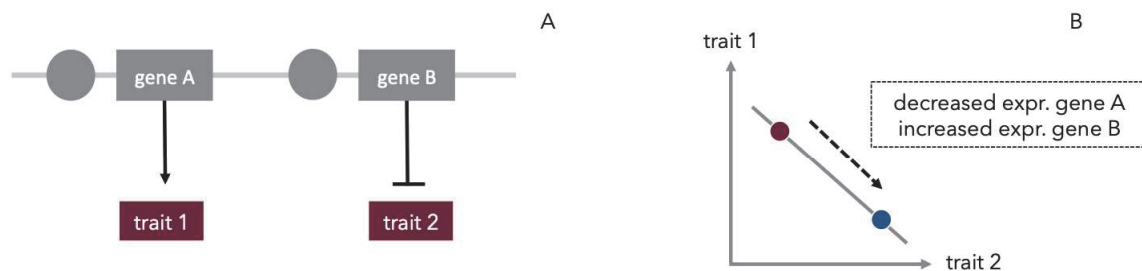


Figure 9.7: Déséquilibre de liaison. Deux gènes sont proches sur un chromosome et par conséquent ont de forte chance d’être exprimés ensemble (peu de chance qu’un crossing over apparaissent entre les 2 gènes). Ces deux gènes codent pour différents traits, l’un active le trait 1 tandis que l’autre inhibe le trait 2.

corrélé positivement à la valeur du trait tandis que l’expression du gène B est associée négativement à la valeur du trait 2. Cependant, il semble peu probable que des trade-offs puissent émerger et se maintenir à partir d’un tel mécanisme parce que cette hypothèse sous entend que les deux traits seraient codés par deux gènes (avec des effets antagonistes) très proches sur une même chromosome. Dans ce contexte, une mutation (chez une bactérie, là où les promoteurs sont partagés) ou plusieurs mutations successives (chez les multicellulaire, une mutation pour chaque gène) devraient avoir un effet similaire pour maintenir le trade-off dans la population. Néanmoins, ce mécanisme est parfois proposé comme une explication de l’émergence de trade-offs (Sinervo & Svensson, 1998). En effet, les corrélations génétiques sont supposées engendrer des trade-offs (Reznick, 1985) et elles peuvent émerger à partir de déséquilibre de liaison entre deux ou plusieurs gènes (ou à partir d’effet pléiotropique) (Lande, 1982). De plus, le déséquilibre de liaison peut être favorisé par la sélection naturelle ou sexuelle mais aussi être décomposé après des changements environnementaux (Lande, 1981).

9.2.2 L’allocation différentielle des ressources

L’allocation différentielle des ressources est le premier mécanisme utilisé pour expliquer la présence de trade-offs. Premièrement énoncé par Levins & MacArthur puis utilisé par Cody en 1966, il reste la façon la plus simple de comprendre comment émergent et se maintiennent les trade-offs. Le principe fondamental est que chaque individu (peu importe l’espèce) a accès à une quantité d’énergie limitée dans sa vie. L’organisme doit diviser (*i.e.*, allouer différenciellement) cette quantité entre toutes les fonctions vitales énergétiquement coûteuses comme la maintenance, la croissance, les fonctions immunitaires, les parades nuptiales (et bien d’autres), qui sont des composants de la valeur sélection :

la survie et la reproduction. Cependant, toute l'énergie investie dans un trait ne peut pas l'être dans un autre. Ainsi, deux traits en compétition pour le même type de ressource sont reliés par une relation négative. Par conséquent, les trade-offs d'allocation peuvent être ressource- et environnement dépendant.

La notion d'allocation différentielle des ressources a été modélisée par Van Noordwijk & de Jong (1986) dans leur article fondateur. Ils considéraient seulement deux traits (la reproduction R et la survie s , boîtes grises dans la figure 9.8) et une quantité A de ressource acquise (étoiles jaunes dans la figure 9.8). Le compromis d'allocation peut être déterminé par l'investissement énergétique dans un trait (par exemple une proportion b (large flèche jaune dans la figure 9.8) de cette énergie est allouée à R) car l'autre investissement est déduit à partir du premier (l'énergie restante pour s est égale à $1 - b$ et sera allouée à cette fonction). À partir de cette équation très simple, toutes les combinaisons de valeurs de traits se répartissent le long d'une relation négative linéaire.

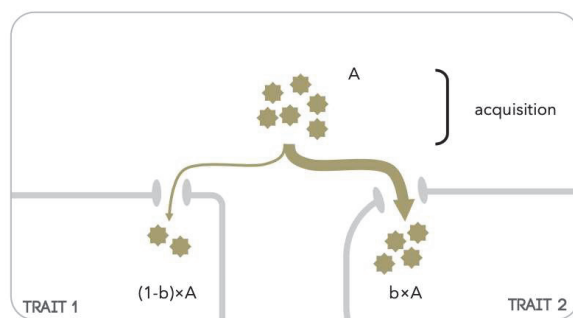


Figure 9.8: L'allocation différentielle des ressources inspirés par le modèle en Y de Van Noordwijk & de Jong (1986)^b. Les étoiles jaunes représentent l'énergie acquise A qui est divisée entre deux traits phénotypiques (boîtes grises). L'énergie investie dans le trait 1 $((1 - b) \times A)$ est l'énergie restante après investissement dans le trait 2 $(b \times A)$.

^bDans cet article fondateur, Van Noordwijk et de Jong décrivaient déjà pourquoi une corrélation positive entre traits pouvait émerger alors qu'une relation négative était attendue (détails dans la partie *L'invisibilité des trade-offs attendus*). Dans l'étude des trade-offs, l'allocation n'est pas l'unique paramètre important. L'acquisition des ressources A représente aussi une variable essentielle à prendre en compte. Même si différents individus allouent dans les mêmes proportions leur énergie, ils peuvent exprimer des valeurs de traits différentes uniquement parce que certains auront acquis plus de ressources que d'autres. Par conséquent, des corrélations positives entre traits peuvent apparaître alors qu'une trade-off relie ces traits.

Ce modèle est classiquement considéré comme le plus mécanistique utilisé dans l'étude des trade-offs. Il reste néanmoins assez superficiel. En effet, il est difficile d'imaginer qu'un "plus gros tube" nourrirait directement un trait plutôt qu'un autre (pour être caricaturale). Bien sûr, on peut se demander comment l'allocation différentielle fonctionne véritablement ? Quels mécanismes (dans le sens réaliste du terme) sont responsables de

cette allocation et/ou comment est-elle régulée ? Chez les organismes multicellulaires, l'allocation différentielle des ressources est souvent régulée par des hormones (comme représenté dans la figure 9.9). Ces molécules particulières jouent le rôle de messagers entre les différents organes et donc coordonnent la majorité des fonctions organiques. Elles sont aussi impliquées dans la réponse au stress, la fécondité, la longévité et les comportements de reproduction. Les hormones sont même retrouvées dans les formes les plus simples de multicellularité (*Volvox carteri*, Starr & Jaenicke (1974)). Elles sont considérées comme les médiateurs clés des trade-offs entre traits d'histoires de vie (Harshman & Zera, 2007; Flatt & Heyland, 2011).

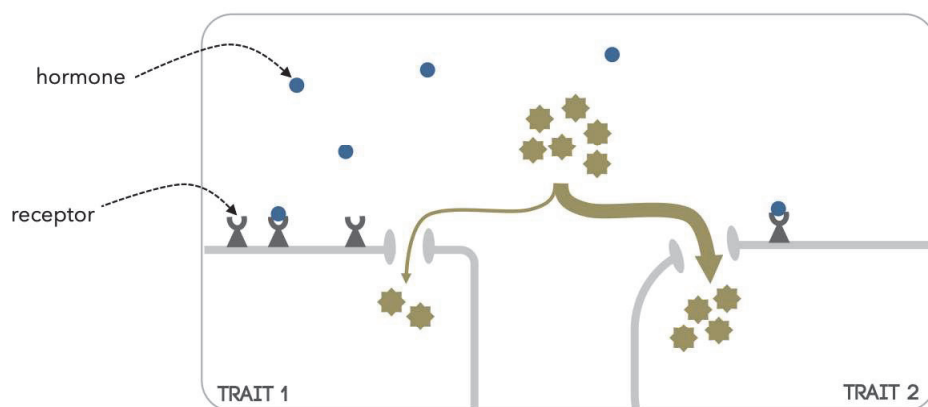


Figure 9.9: L'allocation différentielle des ressources réguler par une dynamique hormonale. Des hormones (points bleus) et des récepteurs (cones gris) sont exprimés dans l'organisme. Ces protéines peuvent se lier entre elles et former des complexes. En fonction des concentrations en hormones et en récepteurs, une certaine quantité d'énergie est allouée entre les deux traits.

Chez *Drosophila melanogaster*, comme chez plusieurs arthropodes, le trade-off entre la survie et la reproduction est directement contrôlé par la concentration de l'hormone juvénile (abrégée, JH ci-après). Une augmentation de la concentration hémolymphatique de JH provoque une diminution de la longévité (et des fonctions immunes) au profit de la fécondité (Tatar *et al.*, 2001). Ce changement de concentration modifie l'allocation différentielle des ressources (Flatt *et al.*, 2005).

Les populations de criquets *Gryllus firmus* sont distribuées sur des dunes de sables temporaires formant un environnement hétérogène (Roff & Fairbairn, 1993). Chaque patch est habitable sur une période de temps limitée puis les criquets doivent se déplacer pour atteindre un nouveau patch (Roff, 1994). Des distances importantes séparent les patches (et donc, séparent les populations de criquets réparties sur ces patches) et les criquets doivent voler pour atteindre de nouveaux patches (Roff, 1994). *G. firmus* exprime naturellement

deux morphes : le morphe "ailes courtes" (abrégé SW pour Short Wings en anglais) avec des muscles alaires atrophiés, qui est incapable de voler et donc de se déplacer sur de longues distances et le morphe "ailes longues" (abrégé LW pour "Long Wings") avec des ailes fonctionnelles, qui est capable de disperser en volant et d'atteindre de nouveaux patches. L'expression de la longueur des ailes est déterminée naturellement par un groupe de gène et ce trait est hautement héritable (Roff, 1986). Les individus SW sont incapables de disperser sur des longues distance mais possèdent plus de tissus ovariens (par conséquent, ils ont une meilleure fécondité). Cette augmentation de la fécondité est due à un compromis énergétique possible grâce au non-développement des muscles alaires. Il semble que l'allocation différentielle d'énergie soit aussi dépendante de la concentration de JH. Durant leurs développements, les individus LW produisent moins de JH que les individus SW. Le rôle causal de JH a été testé en laboratoire. Une injection de JH a été réalisée sur des adultes présentant un morphe LW, ce qui a conduit à une modification du phénotype post-développementale. Après injection, les muscles alaires se sont atrophiés et l'énergie collectée a été réinvestie dans une augmentation des tissus ovariens et donc de la fécondité (Zhao & Zera, 2002). Cependant des résultats plus récents semblent avancer que JH ne serait pas impliqué (Zera, 2016).

9.3 Intéraction entre les niveaux génétiques et physiologiques

L'intégration du mécanisme hormonal permet de comprendre comment l'allocation des ressources est contrôlée au niveau physiologique. Cependant, ce ne sont pas des injections intra-veineuses qui font varier naturellement les concentrations hormonales. Ce mécanisme hormonal est lui-même contrôlé par une régulation génétique. Des gènes codent pour l'expression d'hormones et de récepteurs mais aussi pour leurs conformations tri-dimensionnelles respectives (comme représenté dans la figure 9.10).

Un tel mécanisme est décrit chez la souris *Mus musculus*, où une régulation génétique constitue une étape préliminaire au niveau physiologique. Comme souvent, les études sur les mutants révèlent des mécanismes génétique sous jacent. Dans cette espèce, des souris naines sont mutantes sur le gène *Pit1* (Pituitary factor 1) ce qui implique un dysfonctionnement du facteur de transcription le rendant inactif. Ce facteur de transcription est responsable de l'activation de cascade de gènes cibles. À cause de cette mutation, les

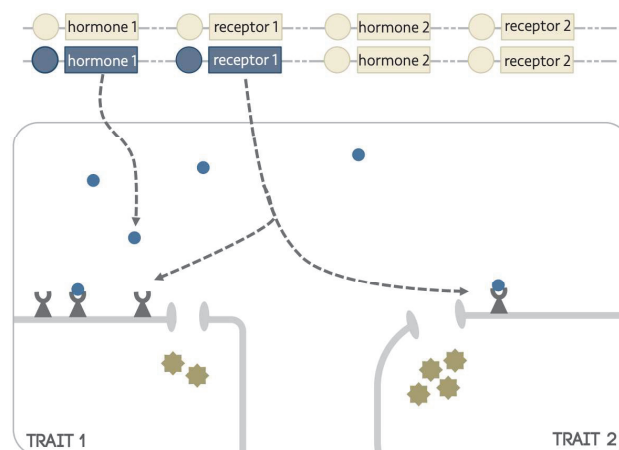


Figure 9.10: Les dynamiques hormonales régulant l'allocation des ressources sont déterminées au niveau génétique. Des gènes codent l'expression et la conformation d'hormone et de récepteurs. Ainsi, des mutations peuvent modifier les relations hormone-récepteur et donc modifier le trade-off.

souris sont incapables de produire trois hormones (l'hormone de croissance GH, la prolactine PRL et l'athyréostimuline TSH). Par conséquent, les souris mutations sont moins fertiles que les phénotypes sauvages d'une même portée mais elles expriment une longévité allongée (Bartke & Brown-Borg, 2004). L'allocation différentielle des ressources entre la reproduction et la longévité, dans cette espèce, est régulée par l'action combinée de ces trois hormones elles-mêmes activées par un seul facteur de transcription.

Si on s'intéresse aux trade-offs entre traits phénotypiques chez des organismes multicellulaires, l'allocation différentielle des ressources ne représente pas un mécanisme suffisamment explicatif de la régulation des trade-offs. Il est évident que l'allocation des ressources peut être étudiée sous la forme d'une simple division énergétique et de variations dans les proportions attribuées aux traits (comme cela est fait avec les modèles DEB pour Dynamic Energy budget (Jager *et al.*, 2013)). Cependant, par définition, l'espace des génotypes considéré est totalement différent s'il est possible de muter directement l'allocation, ou si les mutations ont des conséquences sur les interactions entre hormones et récepteurs (et la dynamique hormonale associée), puis entraînent une modification de l'allocation. Dans le cas d'un compromis exprimé dans une population sous la forme d'une relation négative entre les traits, la variation génétique présente dans la population est centrale et cette variation génétique présente (et future) repose sur l'espace des génotypes. L'émergence de la variation génétique et son maintien peuvent être compris si les modèles considèrent en détail les mécanismes endocriniens. Grâce à cela, un espace des génotypes réaliste peut être généré et, par conséquent, les déplacements dans cet espace (par mutations et/ou

événements de recombinaison) seront également réalistes. Par conséquent, l'allocation de ressource ne représente qu'une étape intermédiaire entre une dynamique hormonale codée génétiquement et le phénotype exprimé, qui peut être sujet à des variations environnementales.

L'étude des trade-offs ne doit pas être divisée entre différentes échelles (génétique, physiologique ou évolutive). Il est évidemment nécessaire de se concentrer sur un niveau précis lorsque l'on cherche à définir dans le détail son fonctionnement. Néanmoins, la description d'un niveau ne devrait pas être accompagné d'une catégorisation du trade-off. En effet, c'est le fruit d'une représentation mentale que de diviser les trade-offs en différents types alors que toutes les échelles sont inter-connectées. Cette vision intégrative avait été anticipée par Ketterson et Nolan en 1992, supposant déjà le rôle crucial des dynamique hormonale dans l'évolution des trade-offs. Par conséquent, en considérant que les trade-offs observés à l'échelle phénotypique sont le résultat d'effet cascade provenant du génotype, il est possible d'envisager l'évolution des trade-offs. Ils avaient alors suggéré que des mutations particulières comme des changements en densité de récepteurs ou l'affinité entre une hormone et son récepteur pourraient avoir des changements majeurs sur les valeurs de traits (Ketterson & Nolan Jr, 1992).

9.4 L'invisibilité de trade-offs attendus

Malgré le solide argument que les trade-offs devraient être théoriquement universels, il est parfois nécessaire de les révéler (Zera & Harshman, 2001). De nombreuses études n'ont pas détecté de trade-offs alors que les auteurs s'attendaient à en trouver. En effet, dans certains cas, les trade-offs sont attendus parce qu'ils avaient déjà été détectés chez une espèce proche ou simplement parce que la théorie prédisait leur présence entre certains traits. Différentes hypothèses peuvent expliquer la non-observation de trade-offs malgré leur existence : premièrement, le trade-off existe mais les individus n'expriment pas de variations sur ces traits (*i.e.*, chaque individu peut diviser son énergie dans la même proportion entre traits conduisant à la même allocation, et sans variance dans la population la relation négative ne peut pas apparaître). Deuxièmement, le trade-off n'est peut être pas mesuré à la bonne échelle pour être révélé. Par exemple, la fécondité devrait parfois être mesurée comme la fécondité réalisée (*i.e.*, le nombre d'oeufs produits sur l'intégralité de la vie d'un individu (Tisdale & Sappington, 2001)) et non pas comme la fécondité potentielle (*i.e.*, approche corrélative basée sur des mesures de taille (Rhainds, 2015)).

Troisièmement, des variations significatives dans l'acquisition de ressources entre individus peut dévoiler une relation positive entre traits. Enfin, il peut exister un lien entre l'acquisition des ressources et l'allocation qui annulerait le trade-off.

Comme démontré par Van Noordwijk & de Jong (1986) et souligné par Metcalf (2016), les trade-offs peuvent être cachés à cause de la variation en acquisition de ressources entre individus. Des corrélations positives entre traits apparaissent fréquemment alors qu'une relation négative est attendue à cause de la variabilité dans l'acquisition. En effet, des individus qui acquièrent une grande quantité d'énergie peuvent allouer plus à chacun de leurs traits et exprimer des meilleures valeurs de traits (Reznick *et al.*, 2000). Ainsi, dans une population, si la variation en acquisition est importante et malgré des variations dans l'allocation (comme décrit dans la figure 9.11-B), la relation entre deux traits peut apparaître positive, même si les traits sont reliés négativement.

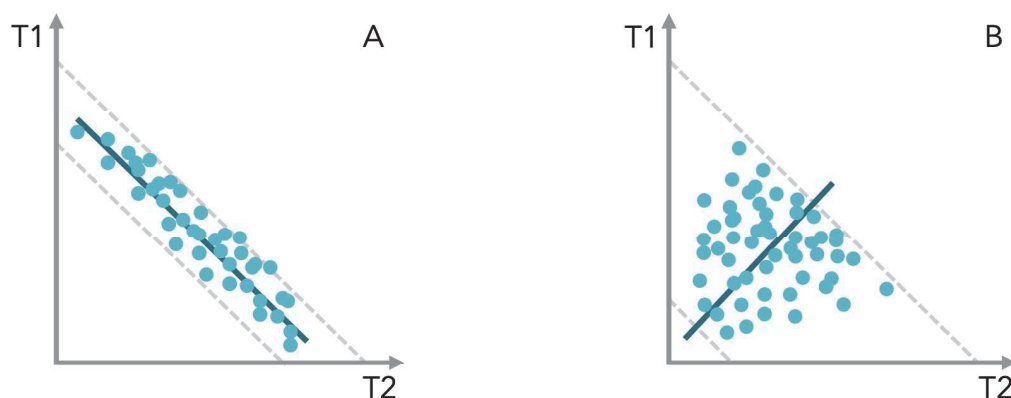


Figure 9.11: Des variations dans l'acquisition peuvent dissimuler des relations négatives entre traits. Dans les deux cas, des combinaisons de valeurs de traits (T1 et T2) individuelles sont représentées avec des points bleus. On considère que la distance entre l'origine du graphe et la combinaison de trait d'un individu représente un proxy de la quantité d'énergie acquise par cet individu. Les lignes grises hachées représentent donc les variations en acquisition. **A-** les individus ont accès à des quantités de ressources similaires ce qui entraîne une faible variation dans l'acquisition entre les individus. Ils diffèrent principalement par leurs allocations différentielles de la ressource. Une relation négative entre T1 et T2 est facilement identifiable. **B-** les individus ont accès à des quantités très variables d'énergie. Certains ingèrent beaucoup d'énergie, d'autres peu, ce qui entraîne des variations importantes dans l'acquisition des ressources entre ces individus. La grande variation en acquisition brouille la relation négative entre T1 et T2. Cette figure est une adaptation de la figure 1 de Van Noordwijk & de Jong (1986).

Une solution pour révéler les trade-offs est de contrôler l'acquisition individuelle afin de minimiser cette variation. Actuellement, le meilleur moyen pour contrôler l'acquisition des ressources par les individus restent de les affamer. Différents protocoles ont été développés pour quantifier l'énergie ingérée mais ils présentent de nombreux désavantages : ils peuvent uniquement être mis en place en laboratoire, ils sont coûteux et très complexes

à appliquer correctement (Deshpande *et al.*, 2014). Pour toutes ces raisons, les trade-offs sont le plus souvent détectés dans des environnements limitant au laboratoire ou sur le terrain dans des conditions de mauvaises années (Roff & Fairbain, 2007; Droney, 1998). Beaucoup d'études ont comparé les relations observées dans une population entre deux régimes alimentaires et ont souvent déduit que le trade-offs disparaissait dans de bonnes conditions (Reznick *et al.*, 2000; Marden *et al.*, 2003). Ces résultats ont été présenté comme une preuve de l'absence de trade-off mais, comme précédemment énoncé, la non-détection d'un trade-off ne signifie pas qu'il n'est pas présent (Roff & Fairbain, 2007). De plus, la comparaison entre deux régimes alimentaires pourrait représenter une question intéressante mais y répondre précisément serait très exigeant. En effet, cela demanderait de contrôler précisément la quantité et la qualité de ressource ingérée par chaque individu.

L'évolution des trade-offs

Même si l'on étudie un trade-off à l'échelle physiologique en examinant la manière dont l'énergie est répartie entre différents traits, des mécanismes génétiques de régulation sont aussi généralement impliqués. Ces mécanismes régulateurs, présentés ci-dessus, sont donc soumis à des processus de mutation-sélection. Ceci n'est pas sans conséquences sur les valeurs des traits exprimés par les individus d'une population. Ainsi, par mutation agissant sur les dynamiques hormonales, des changements cruciaux dans la forme des trade-offs peuvent en résulter (Ketterson & Nolan Jr, 1992). De plus, comme le souligne principalement Roff (Roff & DeRose, 2001; Roff *et al.*, 2003; Roff & Fairbairn, 2007; Roff & Fairbairn, 2012), mais aussi par d'autres auteurs (Houle, 1991; Chippindale *et al.*, 1996; Reznick *et al.*, 2000), la compréhension de l'évolution des trade-offs représente une question essentielle de part leurs implications dans tant de processus biologiques. Dans cette partie, j'explorerai les études empiriques qui ont permis d'identifier des changements sous la forme de compromis. Ensuite, j'expliquerai comment nous pouvons théoriquement envisager d'expliquer cette évolution en présentant mon travail de thèse.

10.1 Preuves empiriques de l'évolution des trade-offs

Une des premières études sur l'évolution du trade-offs a été conduite par Roff *et al.* (2002). Les auteurs ont comparés 4 populations de criquets (*Gryllus firmus*) provenant de différentes conditions d'élevage : 3 venaient directement d'être échantillonnées sur le terrain et la 4ème était élevée en laboratoire depuis 20 ans. Dans cette espèce, 2 morphes sont naturellement exprimés et correspondent aux deux extrêmes du trade-off entre dispersion et reproduction (illustré sur la figure 10.1). Ce cas particulier, oblige la détection de la forme du trade-off à être linéaire et ne peut déterminer que la pente et l'ordonnée à l'origine. Roff et collaborateurs ont observé différents intercept pour les 4 populations et la pente de la population élevée en laboratoire était clairement différentes des autres populations. Même si, la forme est probablement environnement-dépendante,

ce résultat montre que le trade-off peut évoluer rapidement.

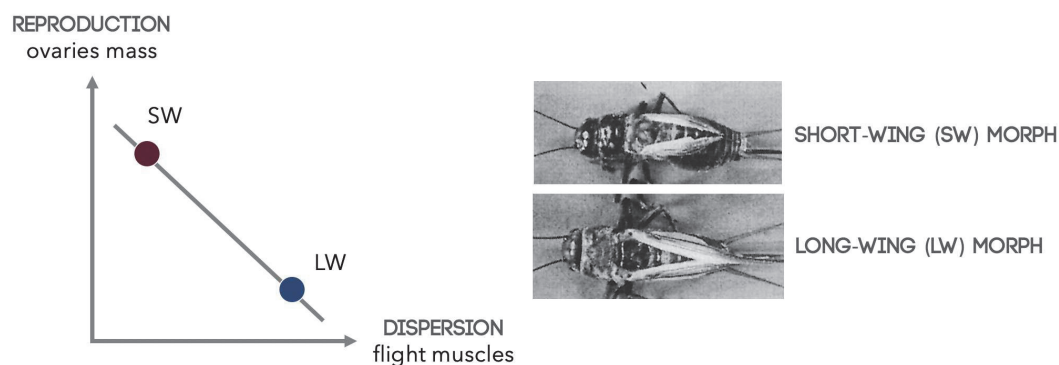


Figure 10.1: Représentation schématique d'un trade-off entre la dispersion et la reproduction chez *Gryllus firmus*. Two morphes sont naturellement exprimés dans cette espèce. Les individus à "ailes courtes" (abrégé SW pour "Short Wings") qui ont une faible capacité de dispersion (ils ne peuvent pas se déplacer en volant) mais ont des ovaires développées. Les individus à "ailes longues" (LW) qui peuvent disperser (ils sont capables de voler) mais ont de petites ovaires. Les photos des deux morphes proviennent de Zera (2005).

Dans une seconde expérimentation, 10 ans plus tard, Roff était toujours intéressé par la même question et a tenté d'y répondre avec le même modèle biologique (Roff & Fairbairn, 2012). Roff et Fairbairn ont conduit une expérience de sélection artificielle chez *Gryllus firmus* pendant 6 générations. Ils ont appliqué trois types de sélection directionnelle : augmentation de la fécondité (masse des ovaires), augmentation des muscles alaires (masse des muscles dorso-longitudinaux), augmentation des deux. Ils ont observé l'évolution des ordonnées à l'origine mais pas des pentes ce qui correspond parfaitement à leurs prédictions faites 10 ans plus tôt. Dans Roff & DeRose (2001)), ils avaient anticipé (en suivant les hypothèses de la génétique quantitative) que le trade-off entre des traits morphologiques (masse des muscles) et un trait d'histoire de vie (fécondité) pouvait évoluer en changeant d'ordonnée à l'origine mais pas de pente. L'étude de la pente et de l'ordonnée à l'origine est particulièrement adaptée pour leur organisme modèle. Cette espèce contraint la forme du trade-off car elle n'exprime que 2 morphes très différents, si bien que même si les deux groupes de points sont plus ou moins éclatés, une régression linéaire restera la meilleure explication des données. Ils précisait aussi que l'évolution des trade-offs entre deux traits d'histoire de vie devrait montrer des "réponses plus complexes". En effet, un trade-off peut avoir différentes formes. De plus, la sélection artificielle appliquée peut être discutée : les mesures de traits nécessitaient le sacrifice des individus et donc ces individus ne pouvaient pas être les parents de la génération suivante (des variants de leurs propres familles étaient utilisés).

D'autres types d'observations soulèvent la question d'un changement potentiel dans la forme des trade-offs. Une partie de la démographie s'intéresse à la question globale de la sénescence: pourquoi vieillissons-nous ? Comment la sénescence fonctionne ? et surtout comment éviter la sénescence ? (Kenyon, 2010). En conséquence, il existe un vif intérêt pour les mutants capables d'exprimer une longévité sensiblement étendue par rapport à leurs congénères. Généralement, lorsqu'une telle mutation est observée, elle est associée à une diminution de la fécondité ou d'autres traits (Walker *et al.*, 2000; Jenkins *et al.*, 2004; Wit *et al.*, 2013). Ces résultats suggèrent seulement un déplacement le long du trade-off. Mais certains mutants semblent être capables d'exprimer une longévité supérieure sans affecter leurs autres traits. Ces mutants ont échappé au trade-off classique pour atteindre peut-être une nouvelle forme de trade-off. Par exemple, des mutants avec une telle capacité sont retrouvés chez *Drosophila melanogaster* quand le gène *INDY* est muté. Marden et collaborateurs ont montré que ces mutants exprimaient une diminution de la courbe de mortalité sans impact sur leurs taux métaboliques, leurs rapidités de vol ou leurs fécondités mais seulement lorsque les drosophiles étaient alimentées avec de la nourriture très calorique (Marden *et al.*, 2003).

Un grand nombre de ces études sont liées à la présence ou à l'absence de nourriture (avec une qualité variable) et révèlent que le comportement alimentaire est déterminé par des voies de signalisation (Greer & Brunet, 2009; Kapahi *et al.*, 2004; Greer *et al.*, 2007). Les biologistes de l'évolution ont donc essayé de modifier ces voies afin de trouver le bon ajustement menant à un mutant immortel et fertile. Lind *et al.* (2019) ont finalement réussi un tel succès. Ils ont montré que le vieillissement peut être régulé par ce qu'ils appellent des trade-offs fonctionnels. Dans leur hypothèse "d'hyper-fonction", ils expliquent qu'un gène sous-optimal en fin de vie provoque le vieillissement via une biosynthèse excessive. Dans ce cas, la biosynthèse serait coûteuse sur le plan énergétique et n'aurait pas d'effet bénéfique en fin de vie. En modifiant les signaux IIS de production en fin de vie, ils réduisent la biosynthèse excessive et augmentent considérablement la longévité sans aucun coût pour la reproduction ou la descendance. Ce travail montre que lorsque des mécanismes intermédiaires (comme une biosynthèse excessive) sont considérés et étudiés en profondeur, l'évolution des trade-offs peut être comprise.

10.2 En théorie

Cependant, les trade-offs ont longtemps été considérés comme fixes (Tuomi *et al.*, 1983). Peut-être parce que la différence entre barrières de design et trade-offs est parfois floue ((Stearns, 1977) dans la littérature. Les barrières de design correspondent à des contraintes structurelles et découlant principalement de lois physico-chimiques absolument statiques et inviolables. Néanmoins, les trade-offs sont le résultat de différents mécanismes (comme montré dans la section *Origines et régulation des trade-offs*) partiellement génétiquement codés et, en tant que tels, ils peuvent évoluer. De plus, les trade-offs peuvent être résumés à la covariance entre des traits tels que la covariance retrouvée dans la matrice G . La stabilité de la matrice G a été très discutée. Apparemment, cette stabilité dépend principalement des traits considérés, de la corrélation mutationnelle et de la sélection corrélationnelle (Jones *et al.*, 2003). Dans le cas particulier des composants de valeurs sélective (tels que les traits impliqués dans les trade-offs), la matrice G devrait être instable à la fois en raison du paysage adaptatif lié à ces traits mais aussi en raison des multiples locus impliqués dans l'expression des composants de la valeur sélective (Jones *et al.*, 2003). Ces travaux théoriques et empiriques (Fong, 1989; Jernigan *et al.*, 1994; Land *et al.*, 1999; Roff *et al.*, 2002, 2004; Cano *et al.*, 2004; McGuigan *et al.*, 2004; Doroszuk *et al.*, 2008) sur la matrice G suggèrent que les trade-offs peuvent évoluer.

Les trade-offs, et en particulier leur forme, limitent l'évolution en restreignant les combinaisons des traits disponibles à court terme. En effet, la forme d'un trade-off provient de la variation génétique maintenue et son évolution est contrôlée par l'architecture du voisinage mutationnel. Par conséquent, à court terme, cette forme limite également la combinaison de traits pouvant être atteints par mutation. Ainsi, la forme d'un trade-off peut déterminer la position optimale dans l'espace des phénotypes (Maharjan *et al.*, 2013; Levins, 1968). Cependant, les études empiriques sur les trade-offs ont, pour la plupart, ignoré ces paramètres d'évolution cruciaux. Les trade-offs sont détectés par des régressions linéaires qui nous renseignent sur la pente mais d'autres paramètres de forme (comme la courbure ou la longueur) sont négligés alors qu'ils peuvent être décisifs (Schaffer, 1974; Stearns, 1989).

Lorsque nous nous sommes demandés (mes superviseurs et moi-même) comment nous pourrions étudier l'évolution de la forme des trade-offs, une solution assez simple nous est venue à l'esprit. Il serait possible de générer une grande variété de formes de trade-offs, puis de voir quelles formes persistent et sont préservées au fil des générations. Le

premier problème avec une telle approche est qu'il est évidemment mathématiquement possible de générer des fonctions avec des formes très différentes, mais qu'il est également hautement probable que nombre de ces formes ne puissent jamais émerger naturellement dans une population. Le deuxième problème réside dans la détermination des relations de voisinage entre ces formes (espace des génotypes) qui sont absolument inconnues et qui auraient dû être déterminées de manière totalement arbitraire.

Pour toutes ces raisons et afin d'étudier et de prédire correctement la forme des trade-offs, il est essentiel de considérer explicitement plusieurs couches de la GP-map. En théorie, l'intégration des trade-offs dans la GP-map pour une meilleure compréhension de l'adaptation, est une manière d'ajouter un degré synthétique d'informations qui manquait auparavant. En effet, les trade-offs dépendent fortement du contexte écologique, car leur forme résume les informations sur la disponibilité des ressources, par exemple.

C'est dans cette vision intégrative que s'inscrit mon travail de thèse. Je voulais examiner les différents niveaux impliqués dans la GP-map ainsi que l'allocation de ressources pour étudier l'évolution de la forme des trade-offs. Toutes ces couches sont interconnectées et jouent un rôle majeur dans l'évolution de ces relations. Comme démontré dans une expérience de Lind *et al.* (2019)(où ils contrôlent une biosynthèse excessive à l'âge adulte chez *C. Elegans* qui conduisent à des individus ayant une durée de vie prolongée sans conséquences négatives sur la production et les performances de leur descendance (Maklakov & Chapman, 2019)), une fois que les mécanismes précis sont compris, nous sommes en mesure de contrôler certains de ces paramètres et de modifier la forme des trade-offs.

Au départ, je me suis intéressée à cette question d'un point de vue théorique. J'ai donc créé un modèle mathématique évolutif qui prend directement en compte les différents niveaux cruciaux:

niveau phénotypique : l'allocation différentielle, au coeur du processus, détermine les valeurs de traits des individus et leurs combinaisons placent les individus dans le paysage de fitness.

niveau génétique : la régulation hormonale est composée d'interaction entre hormones et récepteurs, leurs expressions et conformations sont génétiquement codées. Ainsi, les déplacements dans l'espace des génotypes sont modélisés explicitement.

niveau physiologique : le mécanisme émergent (*i.e.*, l'allocation différentielle des ressources) et le maintien de ce mécanisme (régulation de l'allocation différentielle par des dynamiques hormonales).

Avec ce modèle, nous avons pu étudier l'évolution de la forme des relations entre traits. Grâce à la définition des paramètres de forme, nous avons constaté que le trade-off devient plus court (diminution de la variation génétique maintenue) et plus concave dans un contexte environnemental particulier. En effet, nous avons montré que le coût associé au stockage des ressources représente un paramètre majeur dans l'évolution des trade-offs. Ce coût est rarement pris en compte alors qu'il peut différer selon les espèces ou les ressources ingérées. En outre, ce modèle montre que des changements dans la capacité à utiliser l'énergie pourraient permettre d'échapper au trade-off précédent établi. Par conséquent, grâce à l'action combinée des mutations et de la sélection, les individus peuvent atteindre de meilleures valeurs de trait et ainsi exprimer une meilleure valeur sélective. Ce travail est détaillé dans la partie : *Hormonal pleiotropy and the evolution of allocation trade-offs*.

Dans un deuxième temps, j'ai cherché à intégrer dans ce modèle des variations environnementales de la disponibilité en ressource. Je souhaitais aussi ajouter plus de réalisme dans la dynamique hormonale. En effet, les hormones sont connues pour leur réactivité aux changements environnementaux (Ketterson & Nolan Jr, 1992). Pour ce faire, nous avons modifié le niveau génétique. Ainsi, la production hormonale dépend en partie du génotype codé et en partie de la disponibilité des ressources. En fonction de la variabilité de la disponibilité en ressources, nous observons alors l'évolution de mécanismes endocriniens différents.

Troisièmement, je souhaitais étudier la même question d'un point de vue empirique. Ainsi, j'ai mené une expérience au cours de laquelle des populations de *Drosophila melanogaster* ont évolué dans des paysages de fitness conçus artificiellement pendant 10 générations. J'ai étudié le trade-off classique entre quantité et qualité de la descendance en utilisant respectivement une mesure de fécondité à 24 heures et le taux d'émergence. À cette fin, j'ai développé un dispositif dans lequel n'importe quel paysage de fitness peut être défini de manière arbitraire, en contrôlant la sélection de ces 2 traits d'histoire de vie. J'ai examiné deux paysages de fitness: l'un est censé être universellement rencontré dans la nature, où les deux traits sont sous sélection directionnelle et l'autre qui n'a jamais été rencontré par les individus où les traits sont sous sélection stabilisante. Ce travail est décrit dans la partie: *Experimental modification of a fitness landscape to select for an escape from a classical trade-off in Drosophila melanogaster*.

Part VI

APPENDICES 2 - ARE TRADE-OFFS
REALLY THE KEY DRIVERS OF AGEING
AND LIFE SPAN ? COHEN ETAL 2019

Are trade-offs really the key drivers of ageing and life span?

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Abstract

1. Current thinking in life-history theory and the biology of ageing suggests that ageing rates, and consequently life spans, evolve largely as a function of trade-offs with reproduction. While various evolutionary constraints are generally acknowledged to exist, their potential role in determining ageing rates is rarely considered.
2. This review integrates three types of information to assess the relative importance of constraints and trade-offs in shaping ageing rates: (a) empirical work on the presence of intraspecific trade-offs; (b) theoretical work on factors limiting the force of trade-offs; and (c) consideration of the biological mechanisms of ageing, as currently understood.
3. At the empirical level, evidence for intraspecific trade-offs is mixed, including some surprising failures to observe a trade-off in model organisms. At the theoretical level, the presence of multiple currencies and nonlinearity can weaken the strength and/or generality of trade-offs. Additionally, trade-offs among lower-level functions, such as between sources of mortality, can create constraints at higher organizational levels, for example such that reductions in reproduction are unable to produce decreases in ageing rate. In terms of ageing mechanisms, some mechanisms, such as the regulation of IGF-1 and related pathways, seem to agree quite well with trade-offs as a driving force; however, other mechanisms, such as dysregulation of the vertebrate stress response and stem cell exhaustion, seem more likely to impose constraints than to mediate trade-offs.
4. Taken together, these findings suggest that trade-offs alone are insufficient to understand how ageing rates evolve; instead, both trade-offs and constraints likely play important roles in shaping evolutionary patterns, with their relative importance varying across taxa. Accordingly, it is time to revisit the broad assumption that survival–reproduction trade-offs are the key force structuring much of life-history variation and the evolution of ageing rates.

KEYWORDS

ageing, constraint, cost of reproduction, disposable soma, life history, physiology, senescence, trade-off

1 | INTRODUCTION

Current thinking at the crossroads of life-history theory and ageing biology is that trade-offs between early and late life or between survival and reproduction structure how ageing rates evolve (Kirkwood, 2005; Lemaitre et al., 2015; Rodrigues & Flatt, 2016). Such trade-offs underlie both the antagonistic pleiotropy and the disposable soma theories of ageing (Kirkwood & Holliday, 1979; Williams, 1957) and have formed a cornerstone of our understanding of variation in life-history traits. Several nuances to these general statements are well recognized. For example, trade-offs may appear only under certain severe environmental conditions (Tavecchia et al., 2005), and mutation accumulation unrelated to trade-offs may also contribute to ageing (Everman & Morgan, 2018; Hughes, Alipaz, Drnevich, & Reynolds, 2002). Nonetheless, this overarching framework has continued to be broadly accepted.

Trade-off-based theories of ageing do not suggest a mechanism by which the trade-offs can be escaped. This is problematic in the light of recent work showing that a wide variety of species from across the tree of life, including those with distinct somas, appear not to age (Jones et al., 2014). Empirical work on trade-offs, recent theoretical advances and an increasing understanding of the mechanisms of ageing provide additional reasons to question the traditional framework. The goal of this review was thus to assess the evidence that trade-offs are in fact the primary evolutionary force shaping ageing rates. We use this discussion to propose new empirical and theoretical avenues for the study of how trade-offs shape ageing and life span.

1.1 | Definitions and scope

We consider a **trade-off** to be present when *an increase in fitness or a specific aspect of functioning via one component mechanism/trait inevitably results in a decrement to the fitness/functioning through another component mechanism/trait, producing a limit on the total fitness/function achievable*. We contrast trade-offs, which may be modulated via organismal or evolutionary processes to adjust the balance between the mechanisms/traits in question, with **constraints**, which are *limits on fitness or functioning that are not subject to important modulation*. For example, to the best of our knowledge, rates of wing wear in insects cannot be substantially changed via increased resource allocation; they thus represent a constraint rather than a trade-off. The relationship between trade-offs and constraints is nuanced. For example, trade-offs between mortality components (i.e. causes of death, such as cancer and metabolic dysfunction), discussed in detail below, may create a higher order constraint on the evolution of longer life span. Likewise, some low level of DNA damage accumulation may represent a constraint, but higher levels are likely preventable with sufficient resource allocation and thus reflect trade-offs. Despite these nuances, the distinction between trade-offs (modulable along an axis) and constraints (largely fixed and inescapable at short evolutionary time-scales) will be central to our argument. We also note that our definition of trade-offs is more mechanistic than

some in the literature (e.g. 'costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another', Stearns, 1989). Our emphasis on mechanism rather than pattern is crucial because our core question is to what extent trade-offs drive the evolution of ageing mechanisms leading to variation in ageing rates.

To understand how life span and ageing evolve requires us to understand how trade-offs shape variance in life span and ageing rate between individuals within a species, currently poorly understood. From this perspective, it is useful to segregate trade-offs into genetic and individual variance components. The **genetic component** is caused by allelic polymorphism in genes related to strategies along the trade-off axis (here mainly the slow-fast continuum of life histories, Gaillard, Lemaitre, Berger, Bonenfant, & Devillard, 2016). The **individual component** is caused by individual stochasticity; in other words it is concerned with the outcomes of constraints (mostly physiological) at the individual level that generate the variety of possible realizations of each such strategy (life-history trajectories). Indeed, for a given genetic strategy, an individual's life history may show very different outcomes or trajectories because of individual costs and stochasticity, even in a constant environment.

At the level of individual life courses, the genetic component plays no role beyond defining the range and implications of plastic responses. In contrast, in interspecific (comparative) studies the individual component, though significant evolutionarily (Coste, & Pavard, in press), is statistically overwhelmed by its genetic counterpart (see how this improves drastically the detectability of trade-offs from the intra- to the interspecific level in Bernardo, 1996; Christians, 2000). In between, that is at the population/species level – the workplace of evolution – both components combine to generate a variety of life-history trajectories. At that level, the aggregated effects of these two components can even seemingly offset one another. This is not, however, the main hindrance to the phenotypic emergence of trade-offs, mainly caused by co-occurring non-iso-fitness genetic strategies and by individual stochasticity in resource acquisition. In the first case, the coexistence in a population of different acquisition strategies or of different qualities will generate bias preventing the statistical emergence of a potential genetic trade-off (Houle, 1991). In the second case, the capacity of an individual to acquire more resources (because of a localized and temporary beneficial microenvironment, for instance), than another, will also hinder the detection of the underlying allocation trade-off as famously proved by van Noordwijk and de Jong (1986).

We limit the scope of our discussion in several ways. First, we only consider animal literature, since it is in that kingdom that the trade-off framework in question has mostly been applied to understand ageing (but see Salguero-Gomez et al., 2016). Second, our interest in linking trade-offs to ageing implies that we consider ageing as a *physiological process of inexorable functional decline*, with at least some mechanisms that may be broadly shared across species. We are acutely aware that demographic ageing does not always map perfectly with physiological ageing (Vaupel, Baudisch,

Dolling, Roach, & Gampe, 2004; Wensink, Wrycza, & Baudisch, 2014); nonetheless, we make the simplifying assumption that demographic ageing is a reasonable proxy for physiological ageing. Third, most consideration of trade-offs in the context of ageing has revolved around resource allocation trade-offs, particularly between reproduction and survival functions, which is at the core of the disposable soma theory (Kirkwood, 1977, 2005; Kirkwood & Holliday, 1979). For this reason, our discussion tends to emphasize resource allocation trade-offs, though obviously many other kinds of trade-offs are also possible.

Fourth, our focus is largely on intraspecific trade-offs. There is now substantial evidence for the existence of a fast-slow life-history axis at the interspecific level in many taxa (Gaillard et al., 2016; Lemaitre et al., 2015; Salguero-Gomez et al., 2016), with important implications for how we understand trait structures at macroevolutionary scales. However, these broad patterns are hard to relate back to the mechanistic questions we are asking. For example, population growth rates are normally relatively close to stationary (e.g. between -0.5 and 1.5 across 389 diverse plant species, Salguero-Gomez et al., 2016), implying that if either reproduction or longevity increases, the other trait will decrease due to density dependence, independent of what physiological mechanisms may be involved. Furthermore, intraspecific variation represents the workplace of evolution, and it is here that we observe many of the exciting recent phenomena reshaping our understanding of trade-offs: context dependence, condition dependence, nonlinearity, etc. It is at the intraspecific level that a complex interplay of biology, genetics, allocation, environment, strategic choices and phenotypic plasticity waits to be elucidated.

2 | EMPIRICAL EVIDENCE FOR INTRASPECIFIC TRADE-OFFS

2.1 | Trade-offs in experimental evolution of model organisms

A number of model organisms have been used to study trade-offs, with mixed results. In the fruit fly *Drosophila melanogaster*, classic selection studies gave contrasting results regarding a decrease in reproductive rate following selection for longer life span (but see Luckinbill, Arking, Clare, Cirocco, & Buck, 1984; Rose, 1984). Other selection experiments have shown trade-offs (e.g. Fabian et al., 2018). Recent work on *D. melanogaster* has also failed to confirm key predictions of life-history and the disposable soma theory. Flies on calorically restricted diets showed evolution of both fecundity and survival, but not in the negatively correlated way predicted by theory, and in ways that differed between the sexes (Zajitschek et al., 2018, 2016). Some *D. melanogaster* alleles appear to produce longer life span with earlier costs (Tatar et al., 2001; Yamamoto, Bai, Dolezal, Amdam, & Tatar, 2013), consistent with antagonistic pleiotropy, whereas others produce life span extension without costs that have been detected (Rogina, Reenan, Nilsen, & Helfand, 2000; Wang, Bohmann, & Jasper, 2003). An open question is thus

the extent to which genetic variation related to antagonistic pleiotropy is the genetic variation shaped by selection to produce variation in life span. Additionally, gene-by-environment interactions may make this question even more challenging – and interesting – to explore.

More broadly, a large number of selection experiments have detected 'positive pleiotropy', positive genetic covariation between early- and late-life fitness or condition (Maklakov, Rowe, & Friberg, 2015). This has led to the proposal of a modified version of the mutation accumulation theory of ageing, in which the deleterious effects of mutations are present throughout life, but increase in magnitude with age (Maklakov et al., 2015). Interestingly, this idea is concordant with recent thinking on the evolution of cancer (DeGregori, 2011), as well as with complex systems explanations of ageing as a breakdown in homeostasis (Cohen, 2012, 2016): physiological regulatory networks are highly buffered and redundant, and loss of homeostasis in a given subnetwork thus is most likely to be expressed in the presence of other problems in the same or connected subnetworks (Nijhout, Sadre-Marandi, Best, & Reed, 2017). Early in life, buffering is likely to better mask the effects of mutations than later on when increasing dysregulation exposes the consequences of the mutation.

The positive pleiotropy/modified mutation accumulation theory implies that the declining force of selection with age is still important, but that the trade-offs in the antagonistic pleiotropy theory (between early- and late-life fitness), or in the disposable soma theory (between survival and reproduction) may not be major drivers of the evolution of ageing and life span. In support of such a model, high random mortality in the nematode *Caenorhabditis remanei* induces selection for shorter life span as predicted under classical theories, but high condition-dependent mortality induces selection for longer life span (Chen & Maklakov, 2012). Life span selection in nematodes is, more generally, highly conditional, with sex-specific effects reversible by selecting on complex traits such as learning ability and mate searching proficiency (Ancell & Pires-daSilva, 2017).

Evidence from experimental evolution in model organisms is thus mixed in its support a role for resource allocation trade-offs in determining life spans. While a number of genes in multiple species support antagonistic pleiotropy (Austad & Hoffman, 2018), other genes appear capable of extending life span without particular costs. Selection experiments also produce varied and confusing results. While the experimental approaches generally used with model organisms are considered by many to be a gold standard scientific technique, they do have some drawbacks as well (Austad & Podlutzky, 2005). First, experimental results may not be valid under different conditions, so the generalizability of the conclusions is not necessarily clear. This is particularly relevant in the context of laboratory evolution, where the laboratory environment is far removed from a heterogeneous natural environment. Second, selection experiments are difficult and can be biased by genetic background, breeding protocols and a number of other factors. Third, the organisms that are used in such studies are usually particularly short-lived

even within their clades; it is not clear to what extent this may bias the findings more generally.

2.2 | Trade-offs in the wild: Condition dependence and implications for genetic trade-offs

There is now an extensive literature showing that costs of reproduction (CoR) vary markedly in both presence and strength according to a number of factors. Here, we highlight some key findings relating to survival CoR. We note that this literature needs to be interpreted with some caution, given the substantial differences in a species' measured life span depending on conditions. For example, the Labord's chameleon (*Furcifer labordi*), which normally lives only 4–5 months in the wild and is the record holder for shortest life span among tetrapods, was shown to live up to 16 months if kept under ambient conditions; its life span also varies greatly across habitats and environmental conditions (Eckhardt, Kappeler, & Kraus, 2017).

2.2.1 | Poor environmental conditions can reveal otherwise hidden costs

There is evidence that survival CoR depends on environmental conditions and may be detectable only when resources are limited during specific years and/or for specific sites. For instance, in Soay sheep, survival CoR is only present during severe environmental conditions (wet and stormy winters occurring when population density was high; Tavecchia et al., 2005). A remarkable example in the seed beetle *Callosobruchus maculatus* has shown that environmental conditions (i.e. food availability) can not only change the mean fecundity and life span between environments, but also reverse the signs of phenotypic and genetic correlations from positive to negative under conditions when food is present or absent, respectively (Messina & Fry, 2003). In Alpine Ibex, there was no survival CoR either before or after an epizootic event (pneumonia), but the cost was high during it (Garnier, Gaillard, Gauthier, & Besnard, 2016). By contrast, studies on captive populations, where resources (at least food and water) can be considered as non-limiting, failed to detect any CoR in 18

mammal and 12 bird species kept in zoos (Ricklefs & Cadena, 2007), in Rottweiler pet dogs (Kengeri, Maras, Suckow, Chiang, & Waters, 2013), in laboratory mice (Tarin, Gomez-Piquer, Garcia-Palomares, Garcia-Perez, & Cano, 2014) and captive *Microcebus murinus* (Landes, Henry, Hardy, Perret, & Pavard, 2019). However, dependence of survival CoR on resource abundance is not universal: reproduction was not more costly under unfavourable or favourable environmental conditions in American red squirrels (Descamps, Boutin, McAdam, Berteaux, & Gaillard, 2009).

The condition dependence of CoR means some individuals may appear to escape trade-offs, having both higher reproduction and greater survival. Moreover, variation in acquisition is not independent from variation in allocation as assumed in van Noordwijk and de Jong (1986), which would have implicitly assumed an identical slope of the relationship between survival and reproduction across environment and individual quality (Descamps, Gaillard, Hamel, & Yoccoz, 2016; depicted in Figure 1).

2.2.2 | Is condition dependence detectable at a physiological scale?

Many of the studies examining survival–reproduction trade-offs in the wild are based on short-term survival, which is substantially easier to quantify than impact on ageing rate. Even though ageing is demographically detectable in the wild, for example as increases in mortality rates with age, (Nussey, Coulson, Festa-Bianchet, & Gaillard, 2008; Ricklefs, 1998), the percentage of individuals subject to ageing-related mortality is highly variable but usually low enough to present a measurement challenge. Accordingly, attempts to link CoR to ageing rate have often relied on physiological proxies for ageing, which can be measured short-term and which are not obscured by non-ageing-related mortality (but see Boonekamp, Salomons, Bouwhuis, Dijkstra, & Verhulst, 2014).

The most common proxies have been oxidative stress and telomere attrition (Costantini, 2014; Monaghan & Haussmann, 2006), though the justification for these choices based on the ageing biology literature is now doubtful (Belsky et al., 2018; Hekimi, Lapointe, &

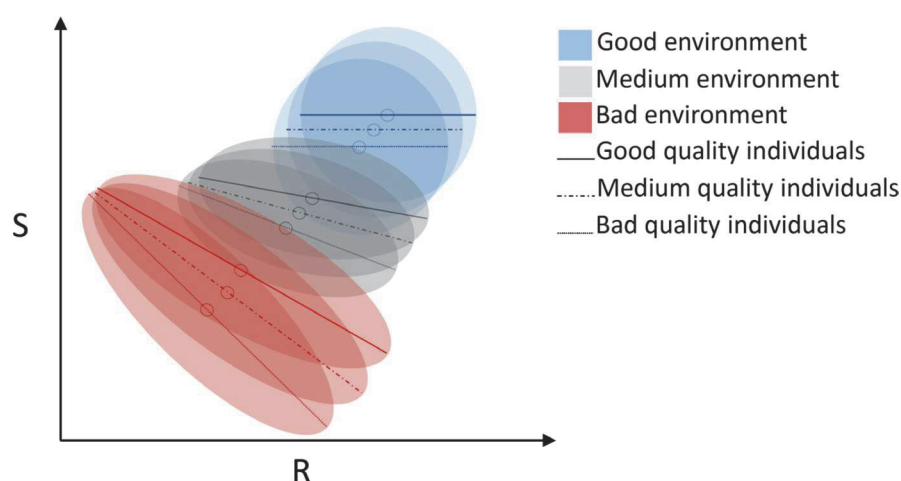


FIGURE 1 Period-individual survival as a function of reproduction (e.g. assuming that survival probability could be measured at the individual level, for instance with a biological marker) depicted in the case where the magnitude of the survival CoR depends on the interaction between environmental condition and individual quality

Wen, 2011; Young, 2018). Furthermore, reproduction is not straightforwardly associated with increased oxidative damage (Metcalf & Monaghan, 2013; Monaghan, Metcalfe, & Torres, 2009; Selman, Blount, Nussey, & Speakman, 2012). While several studies show that reproduction decreases resistance to oxidative stress (e.g. Christe, Glaizot, Strepparava, Devevey, & Fumagalli, 2012), reproduction was found to have no effect on free-ranging female Soay sheep oxidative damage levels (Nussey, Pemberton, Pilkington, & Blount, 2009) and little effect in eastern chipmunks (Bergeron et al., 2011). In birds, several experimental studies demonstrate that reproduction does decrease oxidative resistance (Alonso-Alvarez et al., 2004; Wiersma, Selman, Speakman, & Verhulst, 2004) in a condition-dependent way (Noguera, 2017), leading to short-term mortality rather than a long-term change in longevity prospects (Alonso-Alvarez et al., 2006). This emphasizes the difficulty arising when trying to relate apparent CoR with the disposable soma theory (Monaghan et al., 2009).

2.2.3 | Importance and implications

The integration of all these factors implies that trade-offs are subject to strong stochastic influences caused by multiple driving forces, transforming detectability of survival CoR into a statistical challenge (Descamps et al., 2016) and the evolutionary demography modelling of trade-offs into a population-structured jigsaw (Coste, Austerlitz, & Pavard, 2017). Overall, there is clear evidence for CoR in some species under some conditions, but there is also clearly evidence that CoR is in fact highly contingent on a number of factors.

An important caveat here is that failure to detect CoR is not equivalent to their absence, according to our mechanistic definitions of trade-offs, constraints and CoR. Many studies in the wild have relatively small sample size, and this leads to two contradictory problems: (a) many underpowered studies may fail to detect an effect that is present, and (b) studies that find effects will tend to systematically overestimate them because only studies overestimating effects will be statistically significant (Gelman & Carlin, 2014). As a result, it is hard to know how to interpret the patchwork of evidence presented above: What are the relative roles for statistical artefacts, condition dependence in detection, and contingency in the presence of trade-offs? Likely all play a role, but the overall portrait will require substantial further work.

3 | THEORETICAL REASONS TO EXPECT WEAKER TRADE-OFFS

3.1 | Nonlinearity of trade-offs

Most empirical studies focusing on trade-offs deal with detection at the population level. The main goal for authors is generally to test for a negative relationship between two traits, often survival and reproduction (Charnov & Ernest, 2006; Levitan, 2000; Roff, Mostow, & Fairbairn, 2002; Vøllestad & Quinn, 2003; Walker, Gurven, Burger, & Hamilton, 2008). In this context, the slope estimated through a classic linear regression gives an apparent answer, but without

considering other potential shapes for the trade-off. Levins (1968) showed theoretically that the trade-off shape is an essential parameter for the expected result of evolution. For example, in portions of trait ranges where trade-offs are very steep or very shallow, the trade-off essentially seems to disappear (i.e. optimization is occurring on one trait at a time within those ranges). Despite such considerations, few studies have investigated the question of the trade-off shape (Jessup & Bohannon, 2008; Maharjan et al., 2013). The principal challenge to evaluate the linearity of trade-offs is to collect enough quality data, so theoretical studies are especially useful.

Bourg, Jacob, Menu, and Rajon (2019) studied the evolution of trade-off shape in a large population. They used an evolutionary resource allocation-based model with mutations on the endocrine system involved and demonstrated that trade-offs are not necessarily linear. Depending on the environmental context, the trade-off's shape tends to be shorter and more concave. They revealed that the trade-off shape depends directly on a rarely considered parameter: the cost of resource storage (Figure 2). Thus, depending on the acquired resource and its storage cost, individuals from a single population could evolve along different trade-off shapes. Furthermore, the whole population was able to reach a new trade-off shape only by improving their ability to use the energy thanks to the combined action of mutations and selection. The possibility of escape from a trade-off by mutation or by a diet modification should lead to a decrease of the trade-off's impact. Another interesting implication: for the many resources that cannot be stored, trade-offs should be highly concave and thus should only be strong for narrow ranges of traits.

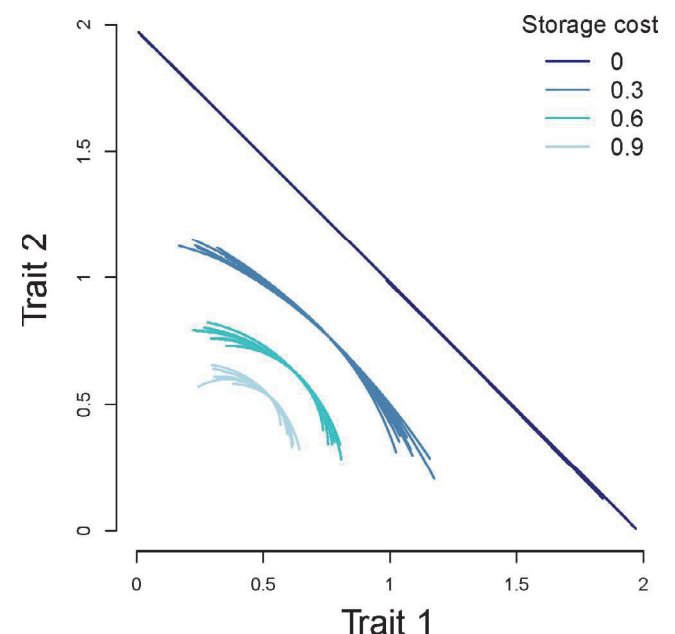


FIGURE 2 Shapes of trade-offs are different depending on the storage cost applied in simulations. Ten representative simulations per storage cost value are illustrated. The higher the storage cost, the more trade-offs are curved and short. Simulations originate from Bourg et al. (2019)

3.2 | The effect of social and sexual interactions

Social interactions within the same sex and/or between sexes can also affect the cost–benefit balances of somatic maintenance and reproduction, often leading to the weakening of trade-offs. For example, cooperation by food transferral from adults to their descendants can promote the evolution of longer life span in populations of overlapping generations (Gurven, Stieglitz, Hooper, Gomes, & Kaplan, 2012; Lee, 2003; Pavard & Branger, 2012); long life and post-reproductive life span can in turn promote the evolution of cooperation (Ross, Rychtar, & Rueppell, 2015) and suppress conflict (Port & Cant, 2013). As soon as the wheels of the positive feedback start to turn, the cost of somatic maintenance can be reduced quickly so that slowed ageing/increased life span does not necessarily cause detrimental effects on reproduction. Interactions between males and females can also strongly influence life span. For example, sexual conflict over mating can directly affect the life span and ageing rate of individuals, often causing reduced life span or even immediate death of females (reviewed in Adler & Bonduriansky, 2014). In social insects, however, mating can be a form of sexual cooperation. For example, mating alone (either with a fertile or a sterilized male) can substantially increase the life span of queens of the ant *Cardiocondyla obscurior*; queens that received viable sperm also have increased fecundity (Schrempf, Heinze, & Cremer, 2005). As we can see, under either sexual conflict or sexual cooperation, fecundity and life span can be shifted in the same direction, weakening/restricting trade-offs between reproduction and maintenance.

Moreover, social and sexual interactions can happen at the same time throughout different life stages of an individual in synergistic or antagonistic ways, further lightening the influence of trade-offs as a determinant of life span. For example, Berger, Lemaître, Allaine, Gaillard, and Cohas (2018) showed recently that in cooperative breeding Alpine marmots, the presence of helpers (subordinate males) on the one hand improves the survival of male pups via thermoregulation during hibernation, but on the other hand can impose strong intrasexual competition pressure on these pups. The opposing influences from social and sexual interactions produced a nonlinear effect of the presence of helpers on the life span of male dominants. For the dominant males who have helpers in adulthood, they lived the longest (max 14 years) when having no helper at all, shortest (max 8 years) when having a single helper, and intermediate (max 11 years) when having two or more helpers at birth (Berger et al., 2018).

3.3 | Multiple resources

A third theoretical argument against the role of trade-offs in determining ageing rate was recently proposed by Cohen, Isaksson, and Salguero-Gomez (2017). They modelled lifetime reproductive success in a hypothetical species where resource allocation decisions were made simultaneously across multiple 'currencies' (i.e. resources that can be differentially allocated to survival or reproduction). Much of the literature primarily discusses energy budgets and energy allocation, but there is increasing evidence that energy alone does not

fully capture the resources that may be allocated. Micronutrients such as carotenoids may be allocated to sexual selection or antioxidant function in some species (Isaksson, Sheldon, & Uller, 2011). Specific dietary nutrients such as proteins might also have differential roles, particularly if reproduction requires certain nutrients more than others (Cotter, Simpson, Raubenheimer, & Wilson, 2011; Raubenheimer, Simpson, Couteur, Solon-Biet, & Coogan, 2016). Non-physical resources such as time and risk may also be differentially allocated (Ketterson, Nolan, Wolf, & Ziegenfuss, 1992). There is thus good reason to believe that multiple currencies do operate simultaneously to mediate any trade-offs that may exist; this model explored the evolutionary consequence of these multiple currencies.

In some runs, the currencies were allowed to have differential 'buying power' for survival and reproduction. For example, one currency might allow a gain of one unit of reproduction for a loss of one unit of survival, but another currency might allow a gain of two units of reproduction for one unit of survival. As long as the buying power differed across currencies, the model found that the trade-off was substantially weakened (i.e. higher lifetime reproductive success could evolve) relative to a single-currency model. Even when buying power was equal (probably unrealistic under real conditions), the evolution of the underlying physiological traits showed substantial stochasticity due to the presence of multiple optima in the state space. These findings imply that, under realistic scenarios of multiple currencies with differential buying power for survival and reproduction, trade-offs are likely to be substantially weaker than generally thought. They do not, however, imply that the trade-offs are completely absent.

3.4 | Trade-offs between mortality components

In the sixth prediction of his seminal article on the evolution of senescence (Williams, 1957), Williams anticipated that '*senescence should always be a generalized deterioration, and never due largely to changes in a single system*'. This idea was later reframed and formalized by Maynard Smith (1962): a synchrony of physiologically independent ageing processes is expected because natural selection will favour any genetic change that makes the physiological system that ages the fastest more durable while it would select less against mutations affecting systems that age more slowly (see Box 1 for an illustration of this principle). Moreover, it has been argued that covariation between risk of different causes of death at the individual level may hinder the effects of selection on mortality components at an evolutionary scale (see Box 1). The idea is that a primary defect of one system has consecutive effects in other systems, leading to inferential difficulties in characterizing causes of death. This has led researchers to envision senescence as the accelerated accumulation of health deficits resulting from deterioration of several physiological functions and leading ultimately to death (Kulminski et al., 2007; Yashin et al., 2007).

These hypotheses have been recently discussed in the light of new empirical evidence (reviewed in Gaillard & Lemaître, 2017) demonstrating that demographic, phenotypic and functional senescence are not synchronous (e.g. in Soay sheep in Hayward et al. (2015) or in

BOX 1 Trade-off between mortality components may explain non-synchronicity of senescence by causes of deaths

Let us assume a theoretical organism, absent of extrinsic mortality, whose adult mortality hazard $\mu(t)$ is shaped by three independent additive Gompertz-shaped (i.e. $\mu(t) = ae^{bt-a}$) causes of death c_1 , c_2 and c_3 , such that $\mu(t) = \mu_1(t) + \mu_2(t) + \mu_3(t)$, with $t > \alpha$, and α the age at maturity. For simplicity, let us further assume that parameter a is constant (all causes have the same level of morbidity at first adult age α) and that cause-specific morbidity differs in the rate b_c at which mortality increases with age (with subscript c standing for causes of death 1, 2 or 3).

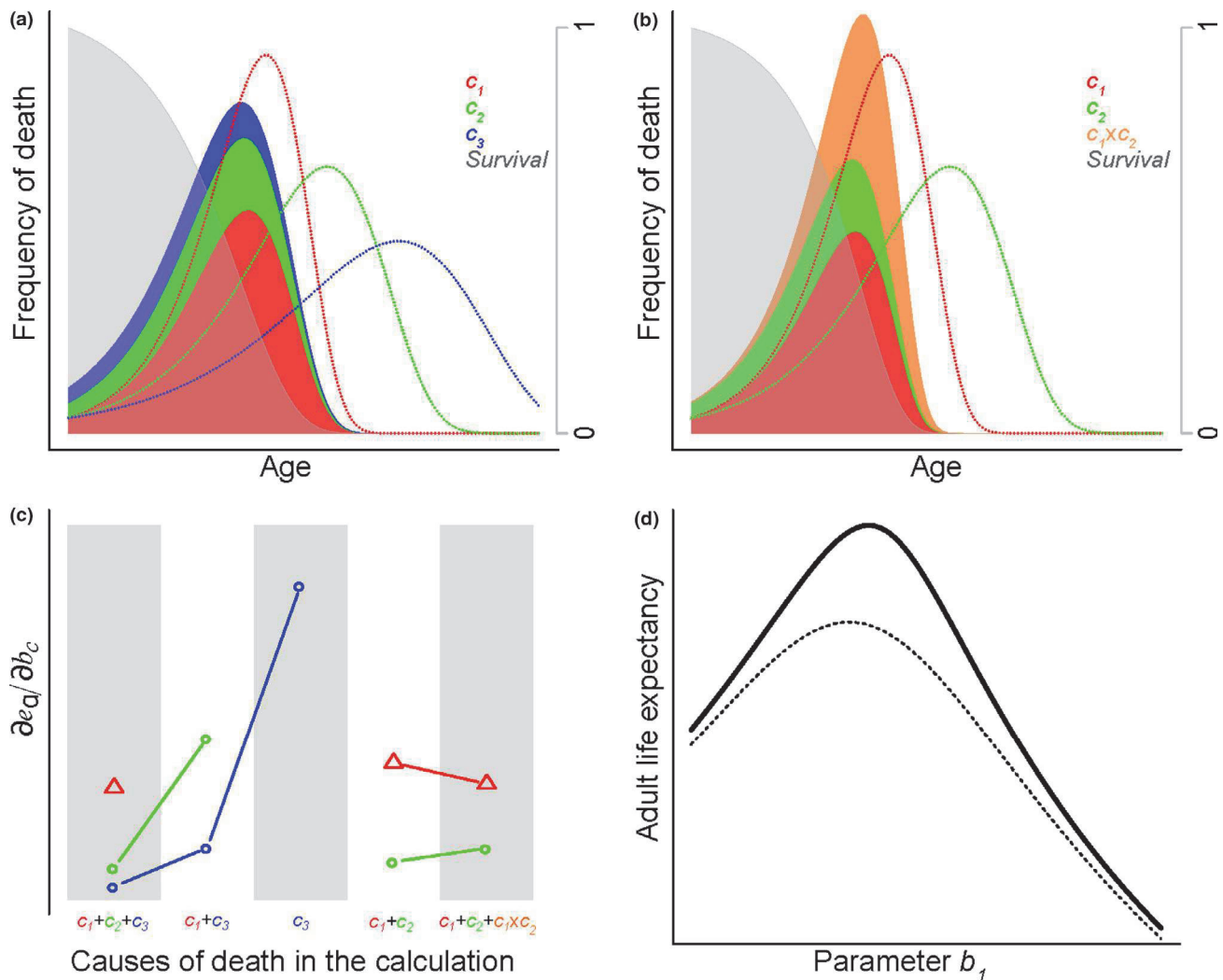


Figure 3a shows the stacked distributions of deaths $f_c(t) = S_1(t)S_2(t)S_3(t)\mu_c(t)$ for the three causes of death c_1 , c_2 and c_3 , the corresponding survival $S(t) = S_1(t)S_2(t)S_3(t)$. It also shows the distribution of death from each cause $f_c(t) = S_c(t)\mu_c(t)$ in the case where individuals die only from this cause (lines). Density of deaths from c_1 is much larger than those from c_2 and c_3 because few individuals survive to ages where c_2 and c_3 are most likely (with $a = 0.0015$, $b_1 = 0.1$, $b_2 = 0.07$ and $b_3 = 0.05$).

Now let us assume a stationary population of a species whose fertility rates are constant over age. In this case, remaining life expectancy e_a is an adequate measure of adult fitness. Thus, sensitivity of e_a with respect to cause-specific parameter b_c , $\partial e_a / \partial b_c$, is a proxy of the strength of natural selection on deleterious alleles increasing the pace at which mortality from cause c increases with age. Figure 3c shows these estimates in three scenarios (on the three left panels) where (i) the three causes c_1 , c_2 and c_3 compete in the population, (ii) only c_2 and c_3 compete, and (iii) individuals die only from cause c_3 . These results show that selection pressure on an allele increasing susceptibility to a specific cause of death depends on the age-specific amount of other deaths in the population. For example, removing c_1 from the population drastically increases the strength of negative selection on c_2 and only a little on c_3 . More generally, gradient of selection occurs not only through age but also through causes of death. For example, assume that alleles for susceptibility to c_2 are at

BOX 1 (Continued)

the mutation–selection balance, with selection just above the threshold at which selection overcomes genetic drift into the population. Negative selection is weak but will eventually purge deleterious mutations. Everything else being equal, alleles for susceptibility to c_1 will be more intensely negatively selected. Purifying selection will decrease the number and the frequency of these alleles, eventually decreasing the amount of deaths from c_1 . By contrast, susceptibility mutations to c_3 are neutral and mutations will accumulate, eventually increasing the amount of death from c_3 . Natural selection will therefore tend to homogenize the rate at which cause-specific mortality increases with age. This was the idea developed by Maynard Smith (1962) and Williams (1957).

But then, do we expect that all primordial functions senesce at the same pace and therefore share the same b parameter? We do not think so, for several reasons. First, cause-specific genetic architecture (i.e. mainly the number, length and expression of genes) may differ between functions, leading to different cause-specific deleterious mutations rates. Second, biological function differs at many molecular and physiological levels. In the case of diseases for example, this leads to disease-specific constraints on its epidemiology (i.e. mainly its age-onset pattern and its penetrance) leading to different levels of selection (Pavard & Metcalf, 2007). We therefore do not expect cause-specific mutation–selection balances to equilibrate at the same allelic spectrum.

Now let us consider that causes of death are not independent and that cause c_3 is now the product, for instance, of a multiplicative interaction $c_1 \times c_2$, between c_1 and c_2 , such that $\mu_{1 \times 2}(t) = za^2e^{(b_1+b_2)(t-a)}$ (where z is the coefficient of this interaction). This models, within an individual life, the fact that factors increasing c_1 may also be prone to increasing c_2 and *vice versa*. Figure 3b shows the distribution of deaths in this case (taking $z = 40$ such that $c_1 \times c_2$ is accounting for more than 25% of observed deaths). Figure 3c (two right panels) shows the $\partial e_a / \partial b_c$ for c_1 and c_2 in this case. Adding interaction between causes of death tends to decrease selection on c_1 and increase selection on c_2 . However, even with a strong interaction these changes are of small magnitude relative to the difference in elasticity between causes of deaths.

Finally, let us assume now a linear negative covariation between b_1 and b_2 . This models a trade-off between causes of death at an evolutionary scale. Figure 3d shows e_a for a range of parameter b_1 (which corresponds here to $b_2 = -7b_1 + 0.14$; plain line). An optimum is then found corresponding to a unique couple but potentially different (b_1, b_2) ; their respective values depend on the magnitude of their covariation ($b_1 = 0.068$ and $b_2 = 0.092$ in this example). Adding positive interaction ($c_1 \times c_2$) to the model tends to flatten the optimum (dotted line), making it less stable, but still evolutionarily relevant.

reptiles in Massot et al. (2011)). Such asynchrony could be due to the relative rates of the decline for different fitness components in relation to their age-specific impacts on fitness (Cohen, 2004). It could also be due to constraint-based mechanisms of ageing (see below), which are not necessarily subject to simple adjustment through selection and may therefore not be easily 'synchronizable'. In particular, trade-offs between mortality components may provide part of the answer. Life-history theory – heavily influenced by the synchronicity theory – has indeed little envisioned the possible importance of trade-offs between mortality components, nor how they might lead to variation in optimal rates of senescence related to specific causes of death (see Box 1 for an illustration of such trade-offs).

In conclusion, trade-offs between mortality components may be an important and underexplored driver of the evolution of ageing. Finding evidence for such trade-offs at a population scale is, however, challenging because individuals die only once and gathering information on the proximal and distal reasons for individual death is difficult in captivity and generally impossible in nature. Investigations of such trade-offs will therefore mostly rely on experimentation or very detailed longitudinal studies, requiring (a) grouping individuals according to factors (genetic or environmental) expected to shape cause-specific mortality outcomes, (b)

measuring biomarkers linked to cause-specific mortality or (c) empirically manipulating, over the course of an individual's life, the functions at the source of the mortality component trade-off. It is likely that mortality component trade-offs are also condition- or environment-dependent; for example, we would predict that the frequency of APOE-ε4 would decrease in sunnier environments as the trade-off between vitamin D absorption and neurodegeneration weakened (Oriá et al. 2007).

4 | WHAT AGEING MECHANISMS CAN TELL US ABOUT THE ROLE OF TRADE-OFFS

Most discussion of the role of trade-offs is divorced from what is known about the mechanisms of ageing. To some extent, this gap is bridged in the literature searching for specific genes and genetic patterns that might underlie antagonistic pleiotropy or mutation accumulation (Austad & Hoffman, 2018; Hughes et al., 2002); nonetheless, the individual genes are not the mechanisms, and there are insights to be gained from asking how known mechanisms could be modulated by trade-offs. A thorough review of ageing mechanisms is beyond the scope of this article; accordingly, we choose

several illustrative examples from the well-known framework of the Hallmarks of Aging (Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013), as well as a few other mechanisms which were not included in that framework but which are broadly accepted (dysregulation after psychological stress; McEwen, 1998, structural damage; Rueppell, 2009 and age-related clonal hematopoiesis; Shlush, 2018).

At a relatively macro level, the role of insulin-like growth factor (IGF)-1 and related pathways supports the role of trade-offs in determining ageing rate. Within vertebrates, increased IGF-1 levels are broadly associated with increases in both somatic growth and reproduction, but also with decreases in life span and accelerated ageing (Dantzer & Swanson, 2012). Crucially, IGF-1 may explain life-history variation at both the intra-individual and interspecific levels: it responds to environmental changes to mediate intra-individual trade-offs, but also appears to explain lineage-specific differences in growth and ageing, such as in dog breeds (Greer, Hughes, & Mesternak, 2011). Furthermore, the IGF-1 receptor gene is a canonical example of a conserved genetic mechanism by which life span can be extended in organisms ranging from yeast to mammals (Tatar, Bartke, & Antebi, 2003). IGF-1 would thus appear to be the ideal mechanistic candidate to explain life-history trade-offs in vertebrates, although more work needs to be done to fully confirm this (Swanson & Dantzer, 2014). One attractive hypothesis is that IGF-1 and related pathways are a 'public' mechanism by which multiple other 'private', or species-specific, mechanisms are regulated (Partridge & Gems, 2002).

Many of the downstream processes likely controlled by IGF-1 and related pathways also lend themselves relatively easily to a trade-off-based understanding of ageing. For example, DNA damage accumulation can likely be modulated, at least to some extent, by investing in mechanisms such as antioxidant protection and repair, which may be resource intensive. Loss of proteostasis as well would seem to be modifiable by allocation of resources to clearance of proteins, an ATP-dependent process (Kaushik & Cuervo, 2015). On the other hand, other ageing mechanisms appear to be more strongly associated with various biological and physiological constraints that would not be subject to modulation via greater allocation of resources. In particular, many known ageing mechanisms are essentially cancer protection mechanisms, notably telomere attrition, cellular senescence and the inflammatory cascades that can result (Schosserer, Grillari, & Breitenbach, 2017; Shay & Wright, 2011). This is a canonical example of the above-mentioned mortality source trade-offs, where a trade-off between ageing and cancer creates a higher-order constraint. Direct allocation of resources (energetic or otherwise) would be unlikely to reduce cellular senescence, since a decrease in cellular senescence would imply an increase in cancer risk, and presumably selection has optimized the balance between the two.

A second example of a constraint-based mechanism is the dysregulatory effects of chronic psychological stress in vertebrates. Organisms appear unable to fully return to a baseline physiological state after prolonged stress (McEwen, 1998), creating a long-term dysregulation which can accelerate other ageing mechanisms

through positive feedback loops (Tomiya et al., 2012). This mechanism is, as far as we know, completely independent of resource allocation strategies or other trade-offs, and reflects an inherent weakness in the structure of the underlying regulatory networks, a constraint.

The distinction between trade-off-based and constraint-based mechanisms is not always clear. For example, while the level of DNA damage might be adjustable via resource allocation, some minimal level is probably unavoidable and might be considered a constraint. Likewise, rates of DNA damage might have impacts on the rates of cellular senescence (d'Adda di Fagagna, 2008), such that even if the mechanism itself represents a constraint, upstream changes in resource allocation could modulate rates of accumulation of senescent cells. Despite these nuances, however, it is clear that many ageing mechanisms are not subject to much modulation by resource allocation or trade-offs. Perhaps the clearest examples are the impacts of chronic stress and structural damage such as wing wear in insects. Another example is age-related clonal hematopoiesis, a process by which natural selection among different clonal stem cell lineages can produce decreases in diversity, with impacts on ageing (Shlush, 2018). This loss of diversity does not appear to be in any way resource related, as far as we know.

Broadly, then, it is useful to consider to what extent ageing mechanisms can be modulated via trade-offs, versus to what extent they are inherent in the physiological nature of the species in question (constraints). The mortality source trade-offs noted above are an interesting case: they are trade-offs at a lower level of organization that produce constraints at a higher level.

5 | DISCUSSION

There can be no doubt that trade-offs are often present in multiple evolutionary contexts, including those structuring ageing rates. Indeed, a wide variety of studies reviewed here confirms this in different contexts: in the lab, in the wild, and based on the known mechanisms of ageing. Nonetheless, there is also now sufficient evidence to say that the trade-off paradigm, while important, is incomplete as a way to understand how ageing rates and life span evolve. Numerous studies both in the laboratory and in the wild have failed to confirm basic predictions, enough to imply that trade-offs are variable in their importance and strength depending on a wide variety of factors. Recent work on hyperfunction theory (Lind et al., 2019), which posits that ageing arises from hyperfunction of reproduction-related genes late in life, also complements our review by suggesting alternative mechanisms for the evolution of ageing beyond trade-offs.

Specifically, we argue that many ageing mechanisms reflect physiological constraints that are largely isolated from modulation by trade-offs, whereas others are subject, more or less directly, to such modulation. The importance of the various ageing mechanisms may depend on both the species/taxon and on environmental conditions, implying that the role of trade-offs is

also likely to vary. Considering numerous other mitigating factors (condition and environment dependence of trade-offs, non-linear trade-off shapes, social and sex-specific factors, multiple resource currencies, etc.), trade-offs should sometimes but not always be a crucial force in structuring life histories generally and ageing rates specifically. Seen another way, an exciting current challenge is to elucidate (a) the variance decomposition of ageing based on covariance with other traits (including links to other mortality components); (b) the variance decomposition of the part of this covariance that is genetic; and finally (c) their translation into variance in fitness in a potentially structured population (e.g. through heterogeneity or sociality) whose vital rates and their covariances fluctuate in time.

To summarize, we are proposing a model of the evolution of ageing in which there are numerous ageing mechanisms, some broadly shared across species, others highly specific to certain taxa (Cohen, 2018). Some of these mechanisms emerge from constraints related to the particular physiology and environment of the species; others reflect trade-offs that are modulable via mechanisms such as resource allocation. The various mechanisms can then interact with each other via feedback effects. The importance of trade-offs in determining the ageing rate of a given species thus depends on the particular combination of mechanisms and their susceptibility to trade-offs. It also depends on a host of factors that influence the strength of trade-offs more generally than in the context of ageing: the functional form of the trade-off and whether the trait values are in a range with a strong negative slope; the number of currencies involved in the trade-off; social and sexual modulating factors; and contingency of the trade-off on environment and condition. Accordingly, we predict that a substantial portion of the variance in ageing rates across species is attributable not only to coherent adjustment via trade-off-based mechanisms, but also to the species-specific set of mechanistic constraints, with the trade-off portion of the variance highly dependent on the various potential mitigating factors listed above. For example, the variance attributable to trade-offs could be substantially weakened in a species where the trade-offs are strong only under highly restricted conditions that do not represent the norm for the species. This theoretical model appears to be supported by the diversity of evidence for trade-offs in the laboratory and in the wild: apparently contradictory studies may be reflecting this complex underlying reality, as much as being the product of methodological and measurement challenges. This is strongly parallel to the recent theoretical work of Baudisch, Vaupel, Wensink and colleagues showing that the classic work on ageing demography by Hamilton (1966) is but a special case, with broader patterns substantially more varied and complex than Hamilton had predicted (Baudisch, 2005; Vaupel et al., 2004; Wensink, Caswell, & Baudisch, 2017). Just as Hamilton's exponential increases in mortality are a special (though important) case rather than a universal phenomenon, trade-offs as key drivers of ageing are also likely a special but important case.

Some readers may find this model unsurprising: Is it not well known that there are both trade-offs and constraints, for example?

We would argue that (a) while the general existence of constraints is acknowledged, they are rarely discussed or incorporated into our paradigmatic way of thinking; and (b) while nuances related to trade-offs are acknowledged, their primacy as a driving force is not generally thought to be called into question, as we are proposing occurs under many circumstances. This current way of thinking has several limits. First, researchers who are not experts in trade-offs and life-history theory are unlikely to consider the presence of constraints in this context, and are likely to overestimate the role of trade-offs. Second, even for those more expert in the field, consideration of a major role for constraints should lead us to ask different questions. For example, research on empirical support for mutation accumulation versus antagonistic pleiotropy has generally supposed that there is a relatively general answer to this question; consideration of the multiplicity of ageing mechanisms, and the constraint- versus trade-off-based nature of these mechanisms, explicitly predicts that there is no universal answer to this question. Third, our model provides a useful framework to understand the heterogeneity of ageing patterns across the tree of life. Some taxa may share physiological traits (and thus ageing mechanisms) that make trade-offs relatively universal within the taxon; others may escape trade-offs to varying degrees for the same reason. Fourth, our model implies that the classical evolutionary theories of ageing (mutation accumulation, antagonistic pleiotropy and the disposable soma) are insufficient to explain the evolution of ageing: ageing can, and probably does, emerge in some taxa largely due to constraint-based mechanisms unrelated to mechanistic trade-offs; in other taxa, all mechanisms may be weak enough for the species to largely escape ageing.

Accordingly, our model also raises a series of new questions/predictions that should be pursued:

1. We predict that there should be taxa in which mechanistic trade-offs play a minimal role in structuring life span. Teleost fishes are a good candidate, with many species ageing very quickly and others very slowly or not at all. Contrasting patterns are clear in well-known examples such as semelparous salmon, killifish, guppies and rockfish.
2. How strongly do mechanistic trade-offs structure interspecific patterns in life-history variation in various taxa? Pattern-level trade-offs are well described in some taxa, and we predict that this will be paralleled by mechanistic trade-offs in some but not all cases. This could be tested, for example, by identification of mechanisms structuring trade-offs (e.g. potentially IGF-1 in mammals), or by finding taxa where no trade-off pattern is apparent.
3. Does the presence of IGF-1 as an upstream control mechanism in mammals imply that trade-offs are universally important in mammals? If so, could we nonetheless detect variation in the strength of trade-offs as a driving force within mammals (e.g. some taxa in which the trade-offs are present but weaker)?
4. What are the relative roles, synergies and antagonisms between genetic and individual trade-offs, and how do these impact the role of trade-offs in determining ageing rates?

5. What is the relationship between constraints and trade-offs in species with negligible/negative senescence? Do these species escape both constraints and trade-offs, and if so in equal measure?
6. How do constraint-based and trade-off-based mechanisms interact with each other? For example, in feedback loops among mechanisms, is the trade-off aspect amplified, or is the constraint aspect amplified, or does it depend?
7. We have focused on ageing as a potential product of trade-offs. Beyond ageing, how important are trade-offs in generating observed evolutionary patterns? Obviously, this is an age-old question, but one that takes on a new light given the various limitations to trade-offs discussed here.

These questions illustrate the ways that an understanding of the limits of trade-offs and the potential role of constraints can reorient our science. At the broadest level, evolutionary processes can be conceived of as a dance between trade-offs and constraints, with one or the other taking the lead at various points but with both always present. Until now, we have focused primarily on one partner, even while the presence of the other was acknowledged; it is time to focus on the interplay. In the context of ageing in particular, this understanding appears poised to lead us towards novel evolutionary theories of ageing with the potential to explain not only how ageing can evolve, but also why it varies as it does across the tree of life.

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AUTHORS' CONTRIBUTIONS

A.A.C. proposed and led the collaboration. All authors wrote, edited, revised and extensively discussed the manuscript.

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