

Playing Darwin. Part A. Experimental Evolution in *Drosophila*

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Abstract In 2009 we celebrate Charles Darwin's second centenary, and 150 years since the publication of '*The Origin of Species*'. After so many years, what has changed in the way we understand Evolution? Obviously we have now a full understanding of the mechanisms underlying heritability. Many molecular tools are available, allowing among other things to reconstruct more accurately the evolutionary history of species and use a comparative approach to infer evolutionary processes. But we can also study evolution in action. Such studies—Experimental Evolution—help us to characterize in detail the evolutionary processes and patterns as a function of environmental challenges, the previous history and present state of populations, and the interactions between such factors. We have now a wide variety of organisms that have been studied with this approach, exploring a diversity of potentialities, in biological characteristics and genetic tools, and covering a variety of evolutionary questions. In this short article I will illustrate the potentialities of Experimental Evolution, focusing in three studies in *Drosophila*. These and other studies of Experimental Evolution illustrate that Evolution is often local, involving complex patterns and processes, which lead both to specific adaptations and to biological diversity, as Darwin already stated clearly in '*The Origin of Species*'.

Keywords Darwin · Experimental Evolution · Adaptation · *Drosophila*

Introduction

The year 2009 was of intense, wide celebration, of both Darwin's 200-year anniversary, and the completion of 150 years after the publication of the book that changed the way we understand Nature (Darwin 1859). The first edition sold out immediately, and the reactions did not wait. After all, till the publication of *The Origin of Species*, most scientists were opposed to the idea of evolution, and commonly referred to God in scientific arguments, concerning living creatures. Fifteen years later, most scientists accepted evolution, though the acceptance of natural selection as an explanation for both the biological diversity and adaptation of the organisms was much more difficult (Avelar et al. 2004; Gayon 1998).

After so many years it is time to reflect on the maturity reached by evolutionary biology. What has changed since Darwin's time, in the way we perceive Evolution, responsible for the intricacies of biological diversity and the complexities of the evolutionary patterns and underlying processes? Several aspects are immediately prominent. We now understand the genetic mechanisms responsible for the similarities between parents and offspring. Darwin emphasized the importance of heritability so that an evolutionary response to selection occurs, but he, as well as the scientific community in general, was ignorant of what caused that similarity. The 're-discovery' of Mendel's laws of heredity caused the first 'revolution after Darwin', which culminated with the integration of Darwinian and genetic principles, giving rise to the 'Modern Synthesis' before the middle of the XX century (e.g. Barton et al. 2007; Futuyma 2006). Later, with the development of several tools, studies at the molecular level became progressively more accessible, giving rise to a new discipline, molecular evolution (e.g. Avise 2004; Li 1997). Some of the assumptions of the

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Modern Synthesis were challenged by this boost of new data, namely the relative importance of natural selection versus neutral processes in evolution. But the ‘Darwinian paradigm’ regained strength, as more data became available at several levels of biological diversity (Barton et al. 2007; Futuyma 2006; Hartl and Clark 2007). At the turn of the XX century, we have entered the post-genomic era, with wide horizons to explore and to deepen our understanding of the mechanisms underlying evolutionary changes. As Rose and Oakley (2007) emphatically put it: ‘The new biology knits together genomics, bioinformatics, evolutionary genetics, and other such general-purpose tools to supply novel explanations for the paradoxes that undermine Modernist biology...’.

This ‘evolution of evolution’ reveals how dynamic Evolution is as a scientific field. We have now an ample array of available tools that can be applied in a variety of organisms, levels of biological organization and processes at each level. We can infer evolutionary patterns and processes, using detailed and ever more accessible molecular analysis (Avisé 2004; Hartl and Clark 2007; Harvey and Pagel 1991). But we can also undertake real-time evolution studies—experimental evolution—using organisms easy to manipulate, maintain and explore with a wide variety of tools (Bell 2008; Garland and Rose 2009).

By experimental evolution is meant research in which populations are studied across multiple generations under defined and reproducible conditions, whether in the laboratory or in nature (Chippindale 2006; Garland and Rose 2009; Rose et al. 1996). This general definition covers various types of experiments that involve evolutionary (crossgenerational, genetically based) changes, whether in natural or laboratorial populations, as long as some control and replication is involved. In the laboratory there are still several types of evolutionary experiments: artificial selection (where selection operates at the individual level, as a function of specific phenotypes), laboratory natural selection (imposition of a novel environment, such as different temperature, nutrients, etc.), or selective culling (stress that eliminates part of the population). In nature both intentional and non intentional environmental changes can be tracked and the evolutionary responses of populations followed periodically. Of course studies in natural populations involve in general more difficulties to satisfy the requisites of experimental design followed in laboratorial studies, but real-time data of temporal changes in natural populations have a strong appeal that may justify being less demanding. Who would question the interest of the long-term evolutionary studies of Darwin finches (Grant and Grant 2002) and their deserved classification as part of Experimental Evolution?

The goal of Experimental Evolution is not to ‘test’ whether Evolution occurs. The goal of Experimental

Evolution is to characterize the ‘details’ of evolution, its dynamics, the evolutionary patterns and processes and their dependence on the history of populations, present genetic variability, environments, as well as determine the limits and degree of predictability of the evolutionary response, etc.(e.g. Chippindale 2006; Rose et al. 1996).

In this paper I will make a brief reference to this area of Evolutionary Biology, after which I will illustrate its potentialities with studies using *Drosophila* as a model organism. After mentioning my own case study of laboratory evolution in *D. subobscura* (that will be more thoroughly reviewed in Santos et al., this volume) I will end with some general considerations about the contribution of Experimental Evolution in *Drosophila* to the field of Evolutionary Biology at present.

Experimental Evolution

Historically, Evolutionary Biology has used chiefly the comparative method to infer evolutionary processes. By ‘comparative method’ we mean the inference of the evolutionary dynamics of a population from comparisons among several contemporaneous populations at different stages of evolution, assuming that each one of them will represent the evolutionary state of a given population at a particular moment (Simões et al. 2009). Darwin used this method widely in his long argument of evolution by natural selection. Advances in the methods used to trace phylogenies, associated with the availability of molecular markers and statistical tools, have rendered comparative evolutionary studies more robust and rigorous (Avisé 2004; Harvey and Pagel 1991). Nevertheless, though the comparative method is essential and sometimes the only method available, its efficacy depends on several assumptions that, when not valid, question the validity of the conclusions drawn, such as errors in the tracing of phylogenies, lack of degrees of freedom, etc. (Lauder et al. 1993). In contrast, the direct study of the evolution of populations characterizing the temporal changes and their relation with environmental factors, either natural or imposed by the experimenter—Experimental Evolution—is free of such assumptions. It allows us to estimate evolutionary rates, trace evolutionary patterns, establish causal relations and distinguish stochastic effects, such as genetic drift, from more ‘deterministic’ mechanisms, such as natural selection or historical constraints (Bell 2008; Chippindale 2006; Simões et al. 2009). Experimental Evolution is also very useful from an applied perspective, such as agriculture (e.g. improvement of methods for animal breeding, studying the evolution of pesticide resistance, etc.), conservation biology (e.g. capacity to adapt to environmental changes, evolution in small populations, the

effect of adaptation to captivity) and medicine (e.g. development of vaccines, evolution of antibiotic resistance, etc.). Finally it can be a very useful tool in generating contrasting phenotypes by divergent selection, which subsequently allows a deeper understanding of underlying biological processes, e.g. physiology, developmental biology, etc. (see Garland and Rose 2009).

Experimental Evolution can thus be considered as Evolutionary Biology in its most empirical sense, being a fundamental method to deepen our understanding of micro-evolutionary processes, that renders even more robust the already solid arguments of Darwin. Curiously Darwin did not accept that evolution was fast enough to be seen at our time scale. Today we have a vast array of records of real-time evolution, either through controlled experiments or accumulation of records in natural populations (Bell 2008; Chippindale 2006). Such studies have allowed us to test with rigour the expectations of specific evolutionary theories, obliging us to rethink some of them and revealing the complexities of evolutionary dynamics (Rose et al. 2005). Darwin already had the notion that evolution was a complex process, both in the intricacies of the relation between the organisms and their environments, and in the processes of ingenious tinkering involved in the adaptation to novel environments. Several decades of systematic studies of Experimental Evolution confirm this vision.

Many organisms have been used for studies of Experimental Evolution. Studies in natural populations are not as abundant as in the laboratory, since several requisites of the method are difficult to satisfy in nature, e.g. monitoring of environmental changes, genetic characterization of populations, presence of control as well as ‘replicate’ populations, frequently longer generation times than laboratory model organisms, etc. (Rose et al. 1996). In spite of these difficulties there are detailed real-time evolution studies in natural populations, such as the well known long-term evolutionary studies of Darwin’s finches (e.g. Grant and Grant 2002) or the experiments on guppies in their natural environment (Reznick and Ghalambor 2005; Reznick et al. 1997). Some studies involve both the collection of temporal data in natural populations and laboratorial studies, such as the classic studies by Dobzhansky in *D. pseudoobscura* (Lewontin et al. 1981). But the majority of studies of Experimental Evolution are done exclusively in the laboratory, with tight control of the populations and the environment imposed by the experimenter, involving a high number of different organisms (Bell 2008; Chippindale 2006). Of these the most famous in asexual populations are the long-term evolution studies in *Escherichia coli* done by Lenski’s team, covering several dozens of thousands of generations (Lenski 2004). *Drosophila*, in particular *D. melanogaster*, is the most widely used laboratory organism in experimental evolution involving sexual

populations, covering many topics in Evolutionary Biology. A brief overview of such studies will be presented below, after which I will examine two studies, testing sympatric speciation and the evolutionary theory of ageing, before mentioning my own studies of laboratory adaptation in *D. subobscura*.

Experimental Evolution in *Drosophila*

Drosophila melanogaster is a small dipteran of the family Drosophilidae, which was first used as model organism by Thomas Morgan, because of its short life cycle, ease of culturing and high fecundity. After identifying the first of many mutants, a white eyed fly, in 1910, T. H. Morgan was joined by Sturtevant, Muller and Bridges. Working together in the famous ‘fly room’ they formulated the revolutionary chromosome theory of heredity which resulted in Morgan receiving the Nobel prize in 1933 (Rubin and Lewis 2000). Soon the discovery of polytene (giant) chromosomes, patterned in cytological bands, allowed the determination of the chromosomal location of genes, further fostering the use of *Drosophila* in research. Since then the use of *D. melanogaster* in experimental biology in general expanded to a variety of fields, from Developmental Biology to Physiology, Ecology and Evolution. Classical studies of experimental evolution using *D. melanogaster* include the artificial selection experiments done by Alan Robertson with the goal of defining the limits of selective response as a function of population size, strength of selection and heritability (e.g. see Clayton and Robertson 1957). These studies illustrate the relevance of Experimental Evolution not only to test evolutionary theories but also with a more applied purpose, such as the definition of the best procedures in animal breeding. *D. melanogaster* has now a fully sequenced genome and has many molecular tools as well as stocks of mutants available, being thus the most widely used *Drosophila* species. Nevertheless, though other species lack the same amount of tools or genetic knowledge, that does not diminish their importance, by allowing us to test for generalizations studies done in *D. melanogaster*, or because they have other properties, such as the high level of polymorphism of chromosomal inversions. Such is the case of Dobzhansky’s studies on the real-time evolution of chromosomal polymorphisms in natural and laboratory populations of *D. pseudoobscura* (e.g. Dobzhansky 1943, 1947). His works were pioneer in Experimental Evolution at an integrated level, using both temporal collection of data in natural populations and studies in laboratory populations. His studies were among the first to report the action of natural selection in the wild, and to test underlying hypotheses in laboratorial populations. In particular

he detected seasonal changes in the frequency of inversions in natural populations, proposing an adaptive explanation for such patterns. He then tested this hypothesis analysing the evolutionary dynamics of inversions in laboratorial populations. The exact evolutionary mechanisms underlying such response to selection are still under debate (Hoffmann et al. 2004), which shows how much Dobzhanky was ahead of his time.

Experimental laboratory evolution in *Drosophila*, with the requisites necessary for robust conclusions (controls and replication, record of temporal changes, big population sizes except when the purpose is to study inbreeding and drift, standard manipulation of all populations except for the evolutionary factors intended to differ between them, etc.) have appeared more frequently since the eighties of the last century. Such studies now cover many issues, and there is no space here to mention all of them (for a general overview see Prasad and Joshi 2003). I will illustrate its potentialities with two studies, which tackle some of the most intriguing evolutionary issues, speciation mechanisms and ageing, specifically the role of habitat selection in sympatric speciation and the testing of the evolutionary theory of ageing and underlying mechanisms, both using *D. melanogaster* laboratory populations. Finally I will briefly mention my own line of research, laboratory adaptation in *D. subobscura* (see also Santos et al., this volume).

Sympatric speciation can result from disruptive selection on habitat preference

Rice and Salt (1990) did a very nice experiment in *D. melanogaster*, with the aim of testing whether strong habitat selection can lead to genetic divergence due to the evolution of reproductive isolation. The authors built a maze with eight final vials (with culture medium), that differed in position, smell (a small tube before the end with different volatile substances), and presence/absence of light, thus representing eight different habitats. They further separated the flies based on their developmental time, by collecting flies from the habitats three times, corresponding to early, mean or late rate of development. Overall there were thus 24 different spatial–temporal habitats. Of these 24 habitats they chose only two as being successful choices in terms of fitness. In each generation, the flies that emerged in the selected vials (half from each), were placed at the center of the maze and allowed to migrate throughout the apparatus. To distinguish the flies that were developed in one and the other habitat Rice and Salt used a pair of markers that they introduced in the population at the beginning of the experiment, through crosses with specific strains. With such markers flies developed in standard medium had yellow eyes while flies developed in kynorenine supplemented medium had brown

eyes. Females from one habitat were cultured onto standard medium and females from the other habitat were cultured in the kynorenine supplemented medium. Offspring from the first habitat were therefore yellow eyed while offspring from the second habitat were brown eyed. This made it possible to distinguish the flies that developed in one and the other habitat, and to track their behaviour in terms of their own preferences. In other words, the authors could analyse whether the offspring returned to breed in the habitat type selected by their parents (philopatry). And because flies mated locally in their selected habitats, this preference would lead to assortative mating. Habitat specialization was recorded every generation as the percentage of brown eyed flies found in one and the other habitats. This specialization gradually increased during the 30 generations of the experiment, by the end of which no individuals were exchanged between the subpopulations of the two habitats. That is, a complete separation due to habitat selection occurred. This separation occurred because reproductive isolation evolved as a consequence of habitat selection. This experiment illustrates that habitat selection may play a role in sympatric speciation, since it results in the gradual evolution of reproductive isolation. Nevertheless, the flies did not show evolutionary changes in mate preference, and the imposition of the different developmental rate of the two subpopulations may have facilitated their divergence. In spite of these and other criticisms (Rice and Salt 1990), this is a clear evidence of the potentials of habitat choice in contributing to fast divergence and ultimately speciation in presence of potential gene flux.

Experimental Evolution of ageing: testing the general evolutionary theory and underlying genetic mechanisms

Among the many studies of Experimental Evolution in *D. melanogaster* perhaps the most famous are the experiments undertaken by Rose and his team to test the Evolutionary Theory of Ageing in general and the underlying genetic mechanisms in particular (see Rose 1991; Rose et al. 2004). One highly pertinent question is: why do we age? After all natural selection seems very efficient in moulding the organisms so that they are capable of responding to environmental challenges, developing from a small egg into an adult with full performance at a certain age. Why is it that most organisms show at a more advanced age a decay of their biological functions, in a non reversible process that we call ageing or senescence? The evolutionary answer was suggested by Medawar and Williams in the 1950s and 1960s. According to the Evolutionary Theory of Ageing this occurs because the strength of natural selection declines with age, even in a population that initially does not show ageing, as long as there is a

separation between the somatic line and the germ line (Rose 1991; Rose et al. 2007). This decline is due to the fact that, even only by accident, an organism has a lower probability of surviving until a later age than until an earlier age. This being the case, after a certain age, it makes little difference whether an organism has a better or worse performance in terms of reproduction or survival. What does have an impact in terms of differential reproduction (fitness) is how an organism performs at an earlier age. Thus, natural selection pays progressively less attention to an organism's performance, which leads to the intrinsic decay of capacities that affect survival and reproduction. This is the general Evolutionary Theory of Ageing. Moreover, two particular genetic mechanisms have been proposed to cause the evolution of ageing: accumulation of mutations, which may lead to ageing if there are genes with age-specific effects; and antagonistic pleiotropy, if a given gene has opposite effects at different ages, e.g. alleles that increase early fecundity reduce future survival or reproduction (costs of reproduction; Rose 1991; Rose et al. 2007).

The evolutionary theory of ageing leads to the expectation that the rate of ageing will slow down if the 'rules' of natural selection causing ageing are 'inverted', e.g. imposing a selective regime that 'pays more attention' to the performance at a later age than at an earlier age. Moreover, by imposing such a regime different predictions are associated with the two genetic mechanisms involved in ageing. With these aims in mind, Rose had the idea of testing the evolutionary theory of ageing in laboratory populations of *D. melanogaster* (Rose 1984; after this successful experiment several other laboratories imposed similar regimes, in general with similar results, that will not be detailed here). Rose defined two selective regimes, with discrete generation times (i.e. reproduction at a single age), each five-fold replicated. In one of them reproduction occurred at an early age, being in fact the standard maintenance procedure of the long-standing laboratory populations. He called these the B populations (the controls in the experiment). The other selective regime involved the collection of eggs for the next generation at a progressively more advanced age. He called these the O ('old') populations. This meant that the fecundity of the flies at an early age was important for the B's but not the O's, whereas the survival till a later age as well as late fecundity was important for the O's but not the B's. This process led to genetic changes in the O populations that have doubled their lifespan relative to the B's. There was also a decline in early fecundity in the O females relative to the B's whereas late fecundity increased, which is in accordance with the expectations of antagonistic pleiotropy due to costs of reproduction across ages. But accumulation of mutations was also involved in

the differentiation between the O's and the B's in some traits, as was shown by a lack of (or slow) reversal when populations returned to the B environment (Rose et al. 2004).

After these initial observations Rose and collaborators have done many other studies in these populations, characterizing their differences in a wide array of traits, such as resistance to several stresses, differences in activity in an array of enzymes, etc. In particular, the observation that resistance changed between the O's and the B's led to the imposition of new regimes, to further understand the mechanisms, both genetic and physiological, underlying ageing. There is no space here to detail these huge sets of experiments, which gave rise to many publications compiled in a book (see Rose et al. 2004). In fact we may say that the large number of differentiated populations represent a unique laboratorial adaptive diversification, showing how much populations can respond to novel environmental conditions. These differentiated populations have allowed to develop experiments beyond the initial scope to understand ageing. It has allowed to test among other things the effects of history in reverse evolution, by imposing the ancestral, B regime to populations that had evolved in five different selective regimes (Teotónio and Rose 2000). More recently the focus of attention has changed from the evolution of ageing to the evolution of late life plateaus, as data on these differentiated populations have confirmed that at a very advanced age ageing ceases to occur (Rose et al. 2007). Though at first glance contradictory, in fact this is an expectation of the evolutionary theory of ageing, and has important implications of a more applied nature. In balance Experimental Evolution of ageing in *D. melanogaster* has contributed a lot to unravel the 'mysterious' base of ageing and its specific mechanisms. Ageing is no longer a paradox considering the expectations of Darwinian evolution. It is a by-product of natural selection, and it is no wonder that the mechanistic basis is plural, given the complexities of all biological processes kept in variable conditions as a function of how much natural selection pays attention to them (Rose 1991; Rose et al. 2004).

Our case study: laboratory adaptation in *Drosophila subobscura* as a mean to characterize local adaptation in general and adaptation to captivity in particular

Most studies of Experimental Evolution involve divergent selection, in which contrasting selective regimes are imposed to laboratory populations and the goal is to characterize the differentiation involved in the adaptation to different environments (Bell 2008; Chippindale 2006; Garland and Rose 2009). An opposite 'strategy' is the study of the way populations initially differentiated evolve when under a novel, common environment. Such studies

allow the characterization of evolutionary patterns and processes as the populations adapt to the novel environment, which is a general theme of Experimental Evolution, whether it involves disparate or common environments between the experimental populations. Moreover, by estimating the degree of differentiation between populations due to different evolutionary dynamics when adapting to a common, novel environment, such studies allow to estimate the importance of contingency versus predictability of adaptive evolution, a long-standing issue in Evolutionary Biology (Joshi et al. 2003; Travisano et al. 1995). Differences between populations in initial adaptive value, evolutionary rate or pattern of temporal change may be due to the interplay between founder effects, genetic background and selection (Simões et al. 2008a). Studies of this kind have been done by Cohan and collaborators, in the selective response of different populations of *Drosophila melanogaster* to knockdown resistance to ethanol (Cohan and Hoffmann 1986), reporting that uniform selection may lead to divergence due to initial differences between populations. Also of interest are studies of reverse evolution, as the outcomes of selection when returning to an ancestral environment predict convergence between the several differentiated populations to the character states of the ancestral populations. A huge experiment of this kind was done with populations differentiated in several contrasting selective regimes in Rose's laboratory, indicating differential effects of history, both between selective regimes and traits (Teotónio and Rose 2000). My own studies, pursued with the help of my invaluable collaborators, have characterized, for almost two decades now, laboratory adaptation in *D. subobscura*, i.e. the adaptive dynamics of laboratory populations since the moment they are introduced in the laboratory, from several independent collections in natural populations. The goals of these studies are diverse: on one side, the laboratory may be seen as just another environment to which populations adapt, and as such the analysis of the temporal changes of traits relevant to fitness in the laboratory is a way to characterize local adaptation in general. On the other hand, considering that it is increasingly needed to maintain endangered species under captivity, in *ex-situ* conservation programmes, it is essential to characterize the evolutionary dynamics under such controlled environments and their eventual consequences when the populations are reintroduced in their natural habitats. With this conservation purpose in mind, several studies in *D. melanogaster* have appeared, particularly since the early 1990s, concerned with laboratory adaptation (Frankham 2005). Nevertheless, most of these studies applied a comparative approach and not real-time evolution, having thus limitations as has been already stated above (see review in Simões et al. 2009). Finally, the study of laboratory adaptation is also of interest to help

solve controversies in the literature about the limitations—or lack of—using long-standing laboratory populations to test general evolution theories, such as the evolution of ageing (see review in Rose and Matos 2004). In particular it is important to test whether populations tend to deteriorate in several traits that are, at least apparently, less relevant to fitness in the laboratory, due to relaxed selection (e.g. Hoffmann et al. 2001 cf. Matos et al. 2004).

Since the start of our studies several foundations of laboratory populations have been done, derived from collections in the wild, both in different years and from two different locations, Arrábida and Sintra, Portugal (see Matos et al. 2000, 2002, 2004; Simões et al. 2007, 2008a). These studies have given us clear indications of laboratory adaptation, involving several life-history traits, of which the most consistent are related to early fecundity. In spite this general indication of adaptation, with evolutionary convergence of more recent laboratory populations to the values of long-established (control) populations, we also found significant differences between laboratory populations derived from different foundations, both in the initial differentiation from the control populations and the adaptive rate (Simões et al. 2007, 2008a). These evolutionary contingencies differ between traits, some being more sensitive to temporal variation of foundations, others to location (Simões et al. 2008a). One important case is the evolution of starvation resistance, which showed contrasting evolutionary patterns between foundations, increasing in some and decreasing in others. This observation is very pertinent considering the above mentioned criticism about the limitations in using long-established laboratory populations, since it may not be valid to generalize between populations, or use a comparative approach to infer evolutionary patterns (Matos et al. 2004; Simões et al. 2009).

Our studies have been enlarged more recently to involve the characterization of microsatellites (see Simões et al. 2008b) and in an ongoing project chromosomal inversions and gene expression also. The integration of the information obtained at these several levels will hopefully enlighten the genetic basis of adaptation in general and of captivity in particular. A summary of some of our findings are presented in the article by Santos et al., this volume.

Final considerations: an evaluation of several decades of Experimental Evolution in *Drosophila*

How much have decades of experimental evolution in *Drosophila* contributed to our understanding of the evolutionary processes and patterns involved in local adaptation and the evolution of biological diversity that surrounds us, the two 'mysteries' solved by Darwin? It is difficult to give a simple answer. On one side, we have progressed in

the understanding of the general aspects underlying the genetic and evolutionary mechanisms. But the ‘refinement’ of our understanding also leads to questioning general approaches, which are tempting but may be false, as more empirical evidence accumulates showing that evolution is local, being highly dependent on particular factors that shape specific evolutionary scenarios (Rose et al. 2005). This calls for caution in generalizations from one organism to another, one environment to a different one, as well as inferences of evolutionary processes or patterns from comparative studies across species.

The question is: is this a problem? Should we question the robustness of the evolutionary conceptual building because we know that each case study should be seen with care before undertaking generalizations that may be misleading? In my opinion it is quite the opposite. The diversity of solutions found in studies of Experimental Evolution, whether in *Drosophila* or other organisms (Bell 2008; Garland and Rose 2009) illustrates the wealth of microevolutionary patterns and processes, helping to explain both local adaptation and biological diversity...even when the future is difficult to predict, whether in a short or long-term perspective. Darwin was already well aware of the complexity of evolution, involving intricate relationships between the organisms and between these and the surrounding environment. It is from these complex webs of relationships that the extraordinary diversity of beings derives.

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