Research Paper

Regulatory Evolution and Theoretical Arguments in Evolutionary Biology*

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Abstract One of the most important claims of evolutionary developmental biology is the cis-regulatory hypothesis. In this paper I examine the theoretical argument for cis-regulatory evolution and its role within evolutionary theorizing. I show that, although the argument has some weaknesses, it acts as a useful example for the importance of current scientific debates for science education.

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Regulatory Evolution and Theoretical Arguments in Evolutionary Biology

1 Introduction

One of the most exciting recent generalizations in evolutionary theory is the cis-regulatory hypothesis, namely the thesis that morphological evolution proceeds primarily through changes in regulatory regions of genes, rather than in coding regions. This hypothesis has triggered a debate over the most prevalent mechanisms underlying evolutionary change. Apart from pointing at various examples of their favored types of mechanisms, participants in the debate have tried to construct *theoretical* arguments. It is precisely due to these theoretical arguments that the debate is philosophically interesting. I am going to argue that the role of these theoretical arguments teaches us important lessons about the methodology of evolutionary biology, but also gives us an important case study for a philosophically informed teaching of science.

My strategy is as follows: in the first part of the paper (sections 2-4), after a brief introduction to the controversy, I will identify a certain component of the debate, the theoretical argument for cis-regulatory evolution, and examine its plausibility. This will lead us to a discussion of the function of the theoretical argument within the debate, from which in turn I will draw lessons for the methodology of biological science in general, and explain the implications for science education (sections 5 and 6). I argue that the present dispute is a typical instance of debates within evolutionary biology, which are debates about *relative significance* (cf. Beatty 1997). I show what theoretical arguments can contribute to such debates as well as to the methodology of evolutionary theory. One could argue that debates such as this one do not promote scientific thinking; what is needed are more empirical data that will ultimately reveal the true hypothesis (cf. Pennisi 2008: 763). Also, one could argue that 'verbal' arguments must be avoided in favor of more precise formulations, perhaps in terms of mathematical models. I argue that both of these statements are false and that verbal (or theoretical) arguments have an important role to play within evolutionary thinking. This analysis in turn can form the basis for an argument about the importance of current debates for science education.

2 Evo-devo and the problem of variation

Perhaps the most important open problem in evolutionary theory today is what can be called the

problem of variation[1], i.e. to specify the molecular mechanisms that underly phenotypic variation. Given that variation is the basis of evolutionary change, the aim is also to determine the molecular basis of phenotypic evolution. The recent identification of developmental genes that control ontogeny and the emergence of the research program of evolutionary developmental biology (Evo-devo) promise, for the first time, to provide the answers to these questions[2]. So, while the origin of variation has been studied since the beginning of evolutionary theory, it is not until now that mutations at the genetic level can be systematically linked with evolutionarily relevant variations at the level of the phenotype.

Indeed, during the past decades, the molecular basis of many cases of evolution has been investigated in detail. But in addition, the generalization has emerged that the most prevalent types of molecular changes underlying morphological evolution are changes in the *regulatory* regions of genes. Before explaining this cis-regulatory hypothesis, as it is called, in more detail, let us very briefly review some biological background.

In molecular biology, a gene is composed of two regions, the *regulatory* and the *coding* region. The coding region is the DNA sequence that after transcription and post-transcriptional processing (including the removal of base sequences called *introns)* will result in the amino acid sequence that will form the protein. The regulatory region consists of DNA bases (the *cis-regulatory elements*) that are not transcribed to form proteins, but are the sites where other molecules bind (e.g. transcription factors) in order to enable the transcription of the coding region. So, the regulatory region can be seen as a switch that determines whether the gene will be on or off.

We can then distinguish between two kinds of genetic changes: first, changes in coding regions: when such a change occurs, this may lead to a change in the resulting protein. We will call such changes *structural mutations*. Second, regulatory changes: this involves mutations in

[1] See Stern (2000), from where I borrow the title for this section.

[2] It is not the place here to examine Evo-devo in detail. For an excellent introduction, see Carroll (2005b). For more on the history and philosophy of Evo-devo, see Amundson (2005), Sansom and Brandon (2007) and Laubichler and Maienschein (2007). Although I am here discussing the cis-regulatory hypothesis, not all Evo-devo researchers focus primarily on the genetic level (see for example Müller and Newman 2005, Newman and Müller 2000). See also Love (2008) for viewing Evo-devo as based on a non-reductive epistemology.

the cis-regulatory elements, and can therefore be called *regulatory mutations*[3]. Such mutations can affect the amount of protein produced and the time and place that the gene is expressed. Evo-devoists argue in favour of the *cis-regulatory hypothesis,* namely the view that most important evolutionary changes involve mutations that alter the regulation of genes rather than mutations that give rise to new proteins[4].

There have been precursors of current Evo-devo ideas about the molecular mechanisms that underlie evolutionary change (Britten and Davidson 1971, King and Wilson 1975). King and Wilson, for example, presented data that showed a very high degree of similarity between human and chimpanzee proteins. This gives rise to an apparent paradox: how can a very small difference in genes lead to so great anatomical differences? King and Wilson's answer was that the differences between chimps and humans are to be explained by differences in gene regulation, that is, differences concerning whether, when, and where a gene is expressed[5]. So, a small difference in DNA sequences can lead to great differences in morphology.

Although these authors argued that changes in mechanisms controlling the expression of genes rather than changes in proteins are the predominant factor in organismal evolution, not much was known about gene regulation at that time. Three decades later, the knowledge acquired by molecular biologists seems sufficient to transform mere speculation into a theory concerning the mechanisms that underlie evolutionary change.

Indeed, within the Evo-devo community, regulatory evolution is regarded as a central principle in our understanding of evolutionary change. According to Carroll, Grenier, and Weatherbee, for example, 'regulatory evolution is the creative force underlying morphological diversity across the evolutionary spectrum, from variation within species to body plans' (Carroll et al. 2001: 173). Eric Davidson also argues that '*there is in fact no other way* to conceive of the basis of evolutionary change in bilaterian form than by change in the underlying developmental gene regulatory networks. This of course means change in the *cis*-regulatory DNA linkages that

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[3] The distinction between structural and regulatory *mutations* is not identical with the distinction between structural and regulatory *genes*. A mutation is either structural or regulatory, but not all genes are either regulatory or structural. Many proteins (e.g. histones) have both structural and regulatory functions. Note also that there are mutations that are structural (involve aminoacid substitutions) but nevertheless alter regulation (e.g. genes that code for transcription factors).

[4] The idea that morphological evolution proceeds through changes in the timing of developmental processes rather than the creation of *new* developmental pathways -what has been called *heterochrony*- is not new (cf. Gould 1977). Recent discoveries in gene regulation enabled the application of this idea to the level of genes.

[5] However, it now seems that 80% of proteins are different between chimps and humans (Glazko et al. 2005).

determine the functional architecture of all such networks' (Davidson 2001: 201, emphasis added).

3 The theoretical argument for cis-regulatory evolution

In a series of books and papers, Sean Carroll has presented a theoretical argument in favour of the cis-regulatory hypothesis (Carroll et al. 2001, Carroll 2005a, 2005b, 2006, 2008)[6]. The task of this section is to clarify this theoretical argument. In the next section I am going to criticize it.

The argument uses three main premises in order to infer the prevalence of cis-regulatory evolution: first, morphological evolution is a different matter from physiological evolution. Second, regulatory mutations have a lower degree of negative pleiotropy, that is it is more probable to be advantageous than lethal for the organism. Lastly, other means of reducing negative pleiotropy are relatively rare. Let us now examine each premise in more detail.

The first step of the argument involves delimiting the domain of the hypothesis. In their 1975 paper mentioned above, King and Wilson took both morphology and physiology as evolving by regulatory changes. Carroll, on the other hand, distinguishes between the evolution of morphology and the evolution of physiology. As he claims, '[c]hanging the size, shape, number, or color patterns of physical traits is fundamentally different from changing the chemistry of physiological processes' (Carroll 2005: 1159). There is evidence that coding sequences mutations are involved in the evolution of the latter, as in the case of the evolution of opsins (proteins involved in vision) or the evolution of proteins that are part of the immune system, but not the former. Carroll's argument for cis-regulatory evolution is thus only about a part of the evolutionary process, i.e. the evolution of form[7]. He allows that the evolution of physiology and behaviour may involve different underlying molecular mechanisms. This doesn't mean that regulatory mutations cannot lead to physiological evolution. Rather, the argument is that as far as morphological evolution is concerned, regulatory evolution is the *primary* mode.

This first step in the argument generates a number of questions: can we really make a distinction between morphological and physiological evolution? Are there any biological reasons

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[6] For discussion of empirical cases the interested reader should consult, apart from Carroll's writings, Wray (2006) and Carroll et al. (2008), and references therein. Stern and Orgogozo (2008) is another detailed discussion of the debate and relevant case studies.

[7] The use of *form* here should not be taken to imply that research in Evo-devo does not focus on the functions of morphological structures and the evolutionary origin of those functions (cf. Love 2006, 2003). Strictly speaking, we should not equate the distinction between morphology and physiology with the distinction of form vs. function.

for doing so or is it only a pragmatic matter? And, if morphological evolution is primarily regulatory, why is this not the case with physiological evolution?

I will discuss these questions further in the next section. Here is how Carroll proceeds to argue that the evolution of morphology proceeds primarily through changes in regulatory sequences of genes. The main idea involves what we can call *the pleiotropy of mutations.* Most often, a mutation will have many different phenotypic effects; in this case, we say that it has a high degree of pleiotropy. In an idealized case where a mutation is non-pleiotropic it will have a very specific phenotypic effect. So, different mutations will usually differ in their degree of pleiotropy.

A well-known example of pleiotropy in humans is a disorder called phenylketonuria (PKU). This disorder arises due to a mutation in the gene that codes for the enzyme phenylalanine hydroxylase, which leads to various defects, such as mental retardation, organ damage and light pigmentation. This happens because a single protein has typically multiple functions in different cells and tissues, and so a change in protein structure can affect many phenotypic traits, as in the case of PKU.

Different mutations will affect the fitness of the organism in different ways. A beneficial mutation enhances the fitness of the organism; a deleterious mutation, on the other hand, has the opposite effect. Carroll assumes that there is a correlation between the degree of pleiotropy of a mutation and the probability that it will have a positive impact on organismic fitness. That is, the higher the degree of pleiotropy of a mutation, the less likely it is to have positive effects. This is because organisms are integrated systems and a mutation that changes the organism in many different ways is more probable to be deleterious than a mutation which changes the organism only in some respects[8]. So, the degree of pleiotropy of a mutation has direct consequences concerning the contribution of various genetic mechanisms to morphological variation. That is, the higher the degree of pleiotropy of a mutation the less likely it is that it will be a source of variation.

So, now the question is: what is the relative degree of pleiotropy between regulatory and structural mutations -that is between mutations in the cis-regulatory elements and mutations in the coding regions of genes? Carroll claims that structural mutations will generally have a higher

[8] It has been discovered that there exist redundant copies of crucial components of developmental processes. This genetic redundancy means that certain mutations can be tolerated even if the are pleiotropic (cf. Kafri et al. 2009, as well as Brigandt (2011) for more on genetic redundancy and its connection to evolvability, i.e. the capacity of an organism for adaptive evolution).

degree of pleiotropy than regulatory ones. The reason is that proteins have typically many different functions and are expressed at various parts of the organism. So, a changed protein can disrupt all the processes in which it takes part, and can also have wide effects if it influences gene regulation (e.g. changes in transcription factors). On the other hand, a mutation in a cis element can have a very specific result: 'The crucial insight from the evolution of *Pitx1*, *yellow*, and *Hoxc8* [genes that control development] is that regulatory mutations provide a mechanism for change in one trait while preserving the role of pleiotropic genes in other processes. This is perhaps the most important, most fundamental insight from evolutionary developmental biology' (1162). So, '[w]hile functional mutations in a coding region are usually poorly tolerated and eliminated by purifying selection, even complete loss-of-function mutations in regulatory elements are possible because the compartmentation created by the modularity of cis-regulatory elements limits the effects of mutations to individual body parts' (1162). That is, a mutation which alters the function of a protein will usually have harmful effects, but this is not the case with regulatory mutations, which are tissue-specific. For example, a regulatory mutation in a cis-regulatory element of the gene *Yellow* can have as its sole result the appearance of a wing spot on a drosophila wing. This happens because the mutation affects the expression of the gene only on a specific part of the wing (see Carroll 2005b for more on this and other examples of the combinatorial nature of cis-regulation)[9].

However, there are other mechanisms by which pleiotropic effects may be reduced. Take for example gene duplication. Gene duplication is a way new genes originate, and subsequent divergence between the copies may lead to a gene with a novel function. Carroll's general strategy is to argue that, apart from regulatory changes, the other mechanisms of morphological evolution, including gene duplication, are relatively rare. For example, there is evidence that arthropod Hox proteins have been modified and the changes are associated with changes in developmental mechanisms. So, under specific circumstances, even widely conserved proteins can evolve new functions. However, Carroll argues that these events are rare and cannot account for most diversification. Moreover, cis-regulatory evolution is a critical component in function diversification both in cases of coding sequence evolution and gene duplication. So, in contrast to other mechanisms, only cis-regulatory evolution can account for the 'continuous

[9] Gerhart and Kirschner further discuss the properties of the developmental system that result in specific phenotypic variation (cf. Gerhart and Kirschner 2007, Kirschner and Gerhart 2005). The key point is that a small number of regulatory changes is enough to generate phenotypic variation that does not disrupt the organism and can in principle be selected. In this sense, development 'facilitates' the generation of phenotypic variation.

diversification of lineages' (1163). From all this, Carroll infers the truth of the cis-regulatory hypothesis: change in regulatory sequences is the primary factor that affects morphological evolution.

4 Against the theoretical argument

In this section I will argue that there are reasons to reject a simple form of the theoretical argument. I will present three kinds of criticism: first, the distinction between morphology and physiology presupposed by Carroll is difficult to substantiate. Second, negative pleiotropy is not enough to infer a high frequency of evolutionary change based on regulatory mutations. Third, there are alternative ways to escape negative pleiotropy. Let us see each criticism in detail.

As we have seen, cis-regulatory evolution is supposed to be important for morphological evolution (including evolution of body plans), but not for what Carroll calls physiological evolution, which includes behaviour, biochemistry, and metabolism. But why does the argument apply only to morphological traits? Is there some biological justification for treating the cases of morphological and physiological evolution differently? In their critique of Carroll, Coyne and Hoekstra ask exactly this question. As they put it, 'physiological and biochemical changes are tissue- and organ-specific in exactly the same way as are anatomical changes, and both types of change occur within developmental networks' (Coyne and Hoekstra 2007: 997). So, the same rationale that leads Carroll to argue in favor of cis-regulatory evolution in the case of anatomical changes, shows that deleterious pleiotropic effects should prevent protein-coding changes in the case of physiological evolution.

Carroll has an answer to this. He claims that the crucial difference between physiological and morphological evolution is that structural mutations in genes affecting morphological traits have a higher probability of being deleterious than structural mutations in genes affecting physiology, *even if* the degree of pleiotropy of physiological and morphological structural mutations is similar. So, take for example a mutation in an opsin protein, which is a protein involved in vision. Although such a mutation may change the spectrum of light detected by the eye, a structural mutation in a tool-kit protein may have as a result the complete disappearance of the eye, as well as changes in other parts. Such a mutation would surely be fatal to the organism. (Carroll 2006: 221).

However, tool-kit genes, which are the genes that control development and are widely

shared among phyla, are only a subset of the genes that affect morphology. What about other genes that are not responsible for controlling basic developmental processes? Is there any reason why structural mutations in *these* genes should be more deleterious than structural mutations in genes affecting physiology? And even if pleiotropy is more deleterious in the case of genes affecting morphology, this does not show that regulatory mutations, rather than structural ones, should be generally preferred. As Coyne and Hoekstra put it, 'is it so clear that activating a gene in a new part of the body, or making twice as much of an enzyme, is more likely to be adaptive than, say, a single substitution of valine for leucine in an enzyme?' (Coyne and Hoekstra 2007: 1000). It seems then that Carroll's claim that different mechanisms underlie physiological and morphological evolution is not justified[10, 11].

There is a different kind of problem for Carroll: the population genetics of new mutations undermines Carroll's reasoning (cf. Coyne and Hoekstra 2007: 1000). That is, there is no direct inference from how deleterious a mutation is to how slow or rapid its evolution should be. Even if we accept that regulatory mutations are less likely to involve negative pleiotropy, the evolution of structural mutations may still be more rapid (and so underlie most evolutionary change) if, for example, structural mutations occur more frequently than regulatory ones.

The third reason against the theoretical argument is this: according to Carroll's reasoning, if a type of mutation has a low degree of negative pleiotropy, then we should expect it to underly much evolutionary change. But regulatory mutations are not the only kind of mutations that can escape negative pleiotropy. Apart from gene duplications, there are numerous other mechanisms. Examples include whole genome duplications, fusion and fission of genes, and recruitment of old genes to new functions. In all these cases, the old functions of genes are retained, and so deleterious effects due to the disruption of old functions are minimized. Moreover, transcriptions

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[10] Craig (2009) suggests that a biological justification for the distinction between physiological and morphological evolution can be found in the mode of paralog divergence of Hox genes (paralogs are genes that arose by gene duplication and evolved new functions). However, there is no evidence that paralog divergence of Hox genes proceeds primarily by cis-regulatory evolution rather than coding sequence evolution (cf. Fares et al (2003), Chiu et al (2001). So, even if we succeed in making the distinction the way Craig suggests, this is irrelevant for the theoretical argument discussed here.

[11] However, recent findings suggest that Carroll may be right after all. Liao et al. (2010) have compared the evolution of what they called 'morphogenes' and 'physiogenes' (genes, changes in which affect morphology and physiology respectively) and have found that morphogenes are more pleiotropic and less tissue-specific and that coding sequence evolution is faster in physiogenes, whereas gene expression evolves faster in morphogenes. However, there seems to be no difference concerning the respective rates of cis-regulatory evolution. This can be explained if morphogenes require fewer cis-regulatory changes, for a given amount of change in gene expression (cf. Monteiro and Podlaha 2009). If this is true, then there exist a biological reason why morphological evolution proceeds differently from physiological evolution.

factors themselves (proteins that regulate genes by binding to their regulatory regions) exhibit a modular architecture: changes in these proteins can be tissue-specific in the same way that are changes in regulatory regions of genes. So, they can retain many of the old functions while changing only a few. This means that structural mutations in transcription factors is another important way to escape negative pleiotropy (cf. Wagner and Lynch (2008). This plurality of mechanisms underlying evolutionary change shows that, rather than being a simple dichotomy, the debate over regulatory evolution is in reality much more complicated.

The aim of this section was not to argue conclusively in favour or against the thesis that evolution proceeds primarily through changes in regulatory sequences of DNA. Rather, my purpose was to criticize a simple form of the theoretical argument for cis-regulatory evolution proposed by Carroll, by presenting theoretical reasons against it. Also, my aim was to give a flavour of the kind of debates within contemporary evolutionary biology. The main conclusion of this section is that, in spite of the prima facie plausibility of the theoretical argument, a lot of empirical research is required before any general claim about the mechanisms that underly phenotypic variation can be expressed with certainty (see Pennisi 2008 for some relevant empirical studies).

5 Cis-regulatory evolution and relative significance arguments

We are used to think of a scientific argument as a presentation of empirical evidence in favour of a specific hypothesis or theory, or (inclusively) as a construction of a mathematically formulated model that is relevant for understanding the phenomena. However, the theoretical argument presented earlier does neither of these things. It is an argument that uses some known facts about the phenomena under study to proceed to generalizations, without an exhaustive empirical support or a precise mathematical formulation. What then, one may ask, is its role within evolutionary thinking[12]?

To answer this question, we must first note that the current debate about cis-regulatory evolution exemplifies a typical form that evolutionary debates can take: it is a debate about relative significance. In evolutionary theory (and in biology in general), and unlike sciences such as fundamental physics, there usually exist many different mechanisms or processes that can

[12] Of course, Carroll's papers include empirical evidence for cis-regulatory evolution. However, the general claim that morphological evolution proceeds mainly through changes in cis-regulation, is not possible without the theoretical argument. The empirical evidence only results in the claim that cis-regulatory evolution is one of many ways morphological evolution can proceed.

generate a certain phenomenon. This is because of the highly complex and contingent nature of a historical science such as evolutionary biology, characteristics absent in fundamental physics (cf. Beatty 1997). For example, there are many different models of speciation; Lewontin and Gould famously argued that there are many different explanations for a given trait (Gould and Lewontin 1979), natural selection for the specific trait being only one of them (others involve genetic drift or the operation of developmental constraints); there are many different ways the gene frequencies of a population can change; and similarly, there are many types of mutations that underly evolutionary change.

A theory in all these areas is usually an argument of relative significance: the geographical isolation theory of speciation, adaptationist thinking, neo-Darwinism in its 'hardened' version (cf. Gould 1983), and of course, and perhaps most famously, Darwin's own argument for the primacy of natural selection in the *Origin*[13], are all arguments of relative significance. They are all arguments to the effect that there are many different ways to generate the phenomenon under study, but one of them is the most prevalent (cf. Lewontin 2000 for the claim that population genetics is such a theory).

I want to suggest here that theoretical arguments such as the one we discussed above, function within a debate of relative significance in order to pick out one of the alternative mechanisms or processes as the most important one. Theoretical or verbal arguments, of which the argument for cis-regulatory evolution is a good example, give reasons to distinguish one of the possible mechanisms as the main one for producing the phenomenon of interest (or, better, to argue that various mechanisms are *more* or *less* important)[14]. The questions now are: firstly, why is such an argument necessary, instead of merely presenting the evidence and judging on the basis of it? And secondly, why cannot we have a more formal argument, one presented in the language of mathematics, for example by constructing a precise mathematical model?

To begin with the second question first, a mathematical formulation of a theory is a

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[13] Recall Darwin's claim that natural selection is 'the most important, but not the exclusive means of modification' (Darwin 1872: 4). Darwin argues that although there are many other means of modification (e.g. sexual selection, the law of use and disuse, or laws of correlation of growth), natural selection is the most frequent and powerful factor in evolution.

[14] It has been argued that selection and drift cannot be conceptually distinguished, and so it does not make sense to argue which is more significant (cf. Beatty (1984) as well as Millstein (2002) for a different view). Could this be the case with the regulatory vs. structural evolution debate? I think not. The various alternative mechanisms mentioned earlier (regulatory or structural mutations, gene duplications etc.) are all types of mutations; the question then is which type of mutation is the most prevalent. So, apart from any practical difficulties to measure relative significance, there does not seem to be a *conceptual* problem in distinguishing regulatory from other types of evolutionary change.

formal framework, e.g. a collection of models, which shows what can happen under various assumptions built into the models. In other words, and in contrast to relative significance arguments, mathematical formalizations model all the various mechanisms accepted by a theory, and do not act as a reason to distinguish one of them. For example, population genetics contains models for all possible causes of evolutionary change. A theoretical argument in population genetics provides reasons why a certain kind of model is more important than others, concerning the actual evolutionary mechanisms under operation within a lineage.

However, it seems that mathematical considerations *are* relevant when distinguishing between alternatives: some mechanisms can be deemed unimportant in producing a phenomenon, as when we construct a model to prove that drift is unimportant when the population is large enough. But the important point here is that the model only is not enough; if we want to have an explanation we have to show that a particular model is a reliable representation of what actually usually happens in nature (in our example to argue that most natural populations are large enough so that drift is not important), and for this last task theoretical arguments are indispensable.

But why cannot we simply enumerate the well studied cases of a phenomenon and argue on the basis of this that a particular factor is the most prevalent? Sometimes, as in the case of the cis-regulatory hypothesis, the well-studied cases of regulatory evolution are not many, and studies of alternative mechanisms are lacking. But even if we had many well studied cases, simple enumeration is not enough: we have to show that the well studied cases are representative of most of the others (cf. Lewontin 2000). And to do that, we need to provide theoretical arguments of the kind discussed here. Of course, this does not mean that the hypothesis that is the conclusion of a theoretical argument of the sort we have been discussing does not typically have confirmatory and explanatory relations with various observations and facts. To the extent that the list of such facts is large enough, theoretical arguments can be very forceful even in the absence of evidence for alternative hypotheses (Darwin's argument in favour of natural selection mentioned earlier is a case in point).

The observations of the last paragraph brings us to what I think is one of the most important characteristics of such arguments: namely, that they function as a guide for subsequent research in the domain of interest. First, theoretical arguments encourage further research in order to identify more positive instances of the favored mechanism. In the present example, there

have been many new studies of cis-regulatory evolution after the formulation of the theoretical argument in order to describe more cases where regulatory mutations led to evolutionary change (cf. Wray 2007). Second, theoretical arguments stimulate case studies of alternative mechanisms. In the present example, this has also started to happen (cf. Wagner and Lynch 2008). This is because, first, in order to justify a hypothesis, we have to demonstrate that alternative hypotheses are false: in particular, we have to show that alternative mechanisms, although possible, are relatively insignificant. To do this, more empirical research on these alternative mechanisms is required. But moreover, scientists are motivated to search for novel mechanisms that could be in operation and which were overlooked by the initial theoretical argument, thus, as Wagner and Lynch put it, 'expanding the realm of the thinkable'. So, a theoretical argument can motivate the generation of new hypotheses and the formulation of new research questions[15] (again, the study of Wagner and Lynch mentioned earlier (Wagner and Lynch 2008) is a good example).

Of course, the case of the adaptationism debate teaches us that sometimes the second function can be blocked. However, in the present case at least (and in many others) this is not the true. For theoretical arguments to function as useful heuristics in *both* the positive and negative sense, scientific debates are necessary. So, rather than being an ultimately meaningless controversy, the debate surrounding cis-regulatory evolution is methodologically productive. More generally, verbal arguments can orient research, promote hypothesis formation, and suggest novel experiments. This heuristic function of the theoretical argument is the reason why it is important, despite the weaknesses we discussed earlier.

To conclude the discussion in this section, we have seen that debates in evolutionary theory usually take the form of relative significance arguments. These arguments contain what we called verbal or theoretical arguments, which provide plausible reasons for distinguishing one over other alternatives. They are neither formal nor mathematical, nor mere inductive generalizations. They constitute a sound methodology by acting as positive and negative heuristics. The debate surrounding cis-regulatory evolution presents an example of a theoretical argument acting within a relative significance debate concerning the molecular basis of morphological evolution.

[15] See Wimsatt (2007) and Odenbaugh (2005) for similar points about the heuristic use of models in biology.

6 Cis-regulatory evolution and science education

The discussion of the previous sections can be used to show how important current scientific practice is for the teaching of science. In particular, I will argue here that the teaching of evolution should include current debates within evolutionary biology, a nice example of which is the debate over cis-regulatory evolution.

An important aim of science education is to teach students the content of today's scientific theories, i.e. to give them the general framework of the contemporary scientific image of the world. How exactly this should be done is of course a difficult issue and I am not going to examine it here. Rather, I want to focus on one aspect of science education as it is commonly practiced, at least in secondary school, namely the tendency to leave out of the teaching material current debates and hypotheses. As far as the teaching of evolution is concerned, this is unfortunate.

At first sight, it may seem odd why a theory that is currently under discussion, such as the cis-regulatory hypothesis, should be among the things that a secondary student, for example, is taught. Since the theory might be proven wrong, why should we teach it? According to this view, only well-confirmed theories have the right to be part of the scientific image. This view, however, unnecessarily limits the scope of science education. The aim of evolution teaching, for instance, is not only to convey some central facts about our current theory of evolution. Moreover, and perhaps *more* crucially, its aim is to inspire the future evolutionary scientists and create scientifically literate citizens. And this aim is accomplished not only by teaching the content of some central scientific theories, but also, and *more* importantly, by teaching how science is done, and how it differs from others unscientific enterprises.

The relevance of philosophy of science for science education is of course a familiar point to readers of this journal[16]. The point that interests me here, and that I think has not been emphasized enough in the literature, is that scientific debates, and evolutionary debates in particular, can do much in order to familiarize students with the nature of science, rather than only with its content[17]. Also, I am here proposing a specific way that evolutionary teaching can incorporate methodological and epistemological considerations, that has the additional

[16] For example, see the discussion on NOS ('nature of science') in Kampourakis and McComas (2010).

[17] For an exception see Silverman (1992), which contains a useful general discussion with many case studies. See also Kipnis (2001), Paraskevopoulou & Koliopoulos (2010), and Braga et al. (2010), which discuss the importance of specific controversies from the history of science for science teaching.

advantage of being an explicit approach to the instruction of aspects of NOS, where students are directly confronted with issues in NOS rather than merely developing an understanding of them by being engaged in science-based activities (cf. Abd-El-Khalick and Lederman 2000). Moreover, it is an example which is free from the interpretative difficulties that historical cases often present. Let us now examine the educational value of the debate on cis-regulatory evolution in more detail.

The controversy over the genetic basis of morphological evolution nicely highlights something that science education often obscures. That is, the fact that science proceeds by continuously formulating, discussing and testing alternative theories and hypotheses, rather than gradually extending a set of scientific truths. The simultaneous existence of alternative and often incompatible hypotheses within a research field is a salient characteristic of science and what differentiates science from mere dogmatism. Within a scientific community there is continuous debate, with no simple method to determine which hypothesis is correct at any given moment. This is a feature of science not usually emphasized in science education, and ignored by critics of science who confuse the existence of disagreements with the absence of knowledge (as for example some creationist critics of neo-Darwinism tend to do). This nature of scientific practice is particularly clear in current scientific debates. This is a very important reason why current scientific practice should inform science education. And there are other reasons too: the human aspects of science, such as the role of creativity and subjectivity in forming hypotheses, are particular clear in scientific controversies. Lastly, the teaching of scientific debates can succeed in arousing student's interest in scientific issues in a way that a traditional approach often fails to do.

Moreover, the debate on cis-regulatory evolution offers an opportunity for an explicit instruction in NOS, i.e. the identification of epistemological and methodological strategies within evolutionary biology. As we saw earlier, the debate on cis-regulatory evolution instantiates a typical form that evolutionary debates can take, i.e. debates about relative significance. So, the present controversy can serve as an example to introduce students to relative significance arguments. Other controversies can then be introduced that instantiate this kind of argument (e.g. Dawin's argument for the importance of natural selection), and students can be asked to identify other instances of the argument themselves, thereby learning about the general form of debates within evolutionary thinking. Second, students can understand that

evolutionary theory is a science different than physics, where 'all possible things happen sometimes'[18]. So, relative significance arguments are prevalent. Third, one can start to understand how scientific controversies can be overcome, by examining the importance of the theoretical argument for cis-regulatory evolution for motivating new hypotheses and research questions that will at some point resolve the disagreement. Lastly, students can become more sensitive to theoretical presuppositions behind currently popular claims in evolutionary theory, such as the cis-regulatory hypothesis of evolutionary change[19].

Do we perhaps want too much? Is the suggestion proposed here interesting perhaps, but infeasible due to the complexity of the scientific argumentation that is part of current scientific practice? But it is always possible to explain complicated arguments in simple terms so that they can be understood by students or the general public; and, after all, one always simplifies, when for example one is teaching Darwin's theory or basic molecular biology, both of which are usually a central part of secondary education. And after all, we have discussed in detail a current evolutionary debate without making it too hard for a committed student to follow.

In his recent book on Evo-devo, Sean Carroll argues that evolutionary developmental biology provides a more accessible introduction to evolutionary theory than neo-Darwinism (Carroll 2005b). This is because Evo-devo deals directly with the changes in the development of organisms that evolution has produced, and talks about familiar animal forms as well as fossils rather than abstract mathematical models. I agree that Evo-devo is useful in evolution teaching and that as an introduction to evolutionary theory, unlike population genetics, does not force 'the explanation toward mathematics and abstract description of genes, and away from butterflies and zebras, or Australopithecines and Neanderthals' (Carroll 2005b: 294). The possibility to become acquainted with methodological and epistemological issues in evolutionary theory and in science in general provides yet more justification for this view.

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[18] I borrow the phrase from Gould and Lewontin (1979: 585).

[19] What about the intelligent design vs. evolution controversy? Should this be part of science education, as proponents of intelligent design have argued? It is clear from the discussion in this section that the answer is negative. Even if we accept that theories which employ supernatural explanations are in principle comparable with scientific ones, the important point here is that this controversy is not part of current scientific discussions, where ID is not considered as an alternative theory to evolution. So, the main reason in favour of the inclusion of scientific controversies in science education (i.e. that they illustrate the dynamics of science) rules out the ID vs. evolution controversy (see Scott and Branch 2003 for more on this topic).

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