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Expression of CAP2 during early Xenopus embryogenesis

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Evolution and Emergence: A Re-Evaluation of the "New Synthesis"[1]

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Abstract
The modern obsession with methodological reductionism in some areas of biology is arguably a product of the exquisitely precise tools now available to dissect problems. Reductionist approaches assume that an understanding of atomized parts will be sufficient to approximate an understanding of the whole. Ironically, the sheer success of this approach and the consequent volume of data generated, particularly as a result of the genome projects, has made comprehension of the larger picture problematic. Consequently, historical patterns of more phenomenologically oriented analyses are re-emerging. This impulse is not new: Gould and Lewontin (1979) argued for a less reductionist view of evolution. They argue that an intense focus upon individual traits risks confusing evolutionary selection with the indirect consequences of other architectural decisions. They also argued that the "baggage" of ancestral traits constrains future possibilities for profound change. The "New Synthesis", a more recent convergence of paleontology, evolutionary biology, genome science, and embryology provides fertile ground for their critique. New approaches to genome analysis and gene categorization have shown that profound inter-species similarities underlie a generic and robust body plan upon which variant morphologies are built. Moreover, phenomenologically oriented approaches have recently revealed functional and organizational similarities among diverse genomes that are indicative of large and preserved gene regulatory behaviours: genomes appear to be organized into similar regulatory blocks irrespective of species. The implications of these recent discoveries suggest that emergent organizational and functional properties of genomes could impose big constraints upon morphological innovation. They might also explain some of the curious and profound examples of convergent evolution that puzzled Darwin.

Keywords
evolution, emergence, convergence, synteny, gene regulation, New Synthesis, genome regulatory block, highly conserved non-coding element

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I have two little embryos in preservative, for which I have forgotten to note the name, and I'm not quite able to determine which class they belong to. They could be lizards, small birds, or be very young mammals. The head and trunk development in these animals is so alike. The extremities are not yet present in these embryos. Even if they were present at the first stages of development, they would teach nothing since the developing feet of lizards and mammals, the wings and feet of birds, like the hands and feet of people, are the same basic shape.

Karl Ernst von Baer (1828)

Introduction

For centuries, and in the absence of precise tools for experimentation, biologists had to be satisfied with collecting, describing, and cataloging the bounty of nature. Understanding how everything worked presented a larger challenge. Even as experimental biology was born, it nevertheless tended to be oriented to the development of general and phenomenologically oriented hypotheses. With the advent of microscopes, radioisotope or antibody labeling techniques, and genetics, the game began to change. New analytical tools meant that questions could be framed, and hypotheses could be advanced and tested with a resolution and acuity that was previously unimaginable. Eventually, the precision of both questions and answers forced a methodologically reductionist philosophy: biologists took the leap of faith that the sum of atomistic parts would accurately approximate the structure and function of the whole. In genome science and in our understanding of how cells regulate their function, impressive technical and conceptual advances speak to the success of this approach. Paradoxically, in these self same fields, the success has created a nearly unmanageable challenge. With such a vast plethora of atomistic data points, how can a larger picture be assembled into a comprehensible approximation of the whole? I will argue that the size and complexity of data sets is forcing the re-emergence of a phenomenological approach to important biological questions. I will also argue that this more phenomenological approach has already exposed emergent phenomenon in the regulation and organization of genomes, and that these phenomena predispose species both to constraints as well as to a facility to deploy modular accessories in response to evolutionary pressures. This modularity of accessories might explain some of the perplexing structures that have been characterized as examples of convergent evolution (the same morphological response to selective pressure evolving in very different animals).

1. Phenomenology

Karl Ernst von Baer noted that at a specific stage of development, different species of embryos were hard to tell apart (Baer 1828). When he organized his observations, he was able to define certain operational and organizational rules, and these exemplify the historical pattern of hypothesis- and phenomena-oriented biological science. For example, some of the rules he devised included:

1. the general characteristics of embryos develop before the more specialized ones;
2. general structural relationships are formed before more specific and specialized ones;
3. the shape of embryos diverges from a general plan of organization—diverse species do not look somewhat alike because they have converged to a similar endpoint.

A prominent successor and contemporary of Darwin took the theories one step further. Ernst Haeckel proposed the laws of biogenesis, one of which effectively stated that "ontogeny recapitulates phylogeny" (Haeckel 1866). In other words, embryos pass through developmental stages that resemble the species's evolution over the eons. An interesting aspect of this perception of biogenesis was that it was explicitly emergent. While Haeckel's work was and remains contentious, nevertheless there are real life processes that exemplify his thinking. Our face and throat derive from branchial or gill-like arches at early stages, and the modern mammalian kidneys appear to pass through a progression of ancentral morphologies before attaining their mature form.

One of the drawbacks of the phenomenological approach is that although it helps us to conceptualize relationships among constituent parts, the generality of the derived model tends to mitigate against hyopotheses that have predictive value and that are easy to test. For example, emergent properties of a system can be postulated, but they are difficult to prove since experimental perturbation might just as easily reflect unforeseen and indirect effects rather than impaired emergent phenomena.

2. Methodological Reductionism

Signal transduction is a fruitful arena for reductionist approaches. This is the field of research dedicated to identifying the genes, proteins, and metabolites

that transduce a cellular stimulus to an outcome or effect. Within a single cell, individual pathways tend to involve dozens of interacting factors that are arrayed in networks involving cross-talk, and both positive and negative feedback. Investigators can spend an entire career studying a single pathway: a sophisiticated appreciation of a single pathway requires knowledge and understanding of hundreds of permutations and combinations of interactions. The network is dynamic and complex, and it can behave in a non-linear fashion, so the outcome of a stimulus is not necessarily predictable, nor is it possible to be sure that all possible members of a pathway have been indentified (Levine et al. 2007). For example, sometimes investigators perturb factors thought to sit at a vital signaling nexus and the experiments yield little or no effect (Suemori and Noguchi 2000). Conversely, unexpected and prosaic factors are found to play a critical role. To make matters worse, different pathways interact with each other, and several will be at play in any given cell. There are presently between 20 and 30 discrete pathways known. Moreover, neighboring cells can employ different networks, and they can in turn affect ~~others~~, either from nearby or from afar. When one considers the huge number of possible interactions, the number and subtlety of outcomes becomes mind boggling. The success of the reductionist approach lies in its capacity to help us to identify new players and to predict how pairs of partners interact. Its failing is that the data set that is derived for a network is so large and complex that our manipulations frequently produce unexpected results.

These reservations aside, methodological reductionism has been immensely fruitful for other studies such as the genome projects. The technology has advanced so far that a genome that formerly cost $2 billion and took nearly a decade to sequence can now be done in a matter of days for $1,000. The volume of data generated in routine experiments is huge, and genome comparisons require massively parallel computing. The Beijing Genome Institute is building towards petaflop capacity (10^{15} operations per second)—comparable to military and weather prediciting computers. In my own lab, a single experiment that takes a few days to complete can produce gigbytes of data. Although innovative tools have been developed to analyse and to present data in comprehensible form, the computing, processing, and conceptualizing challenges remain immense.

3. The Return of Phenomenology

The reductionist approach has not been without its detractors. In their article "The spandrels of San Marco and the Panglossian paradigm: a critique of the

adaptationist programme" Gould and Lewontin (1979) examined the reductionist view and methodology of gene- and trait-centered approaches to evolution. Organisms, they argued, should be regarded as integrated wholes. Within their paradigm, organisms are viewed as embedded in a historical baggage of ancestrally derived genes, networks, morphologies, and processes. This baggage constrains the latitude for innovation of new structures. The spandrels (or more accurately pendentives) in the title of their article are not themselves initially a primary architectural feature: they are the accidental consequence of other architectural decisions. Like a round peg on top of a square hole, the primary decision to place a dome on top of a squared array of arches leaves a gap that must be filled at the corners. Pendentives embody the "filler," but they eventually end up limiting the options available to decorate the dome. For example, it would look silly to have 3, 5, 6, 7, or 9 angels and disciples arrayed on a dome that is supported by four pendentives decorated at the corners (Fig. 1). In their critique, Gould and Lewontin (1979) allude to spandrels/pendentives to make the case that biologists might sometimes mistake an

Figure 1. On the left, an unadorned series of domes sit on top of arched supports in Fontevraud Abbey, France. The arrow indicates a pendentive. Without decoration, the secondarily required pendentives impose no decorative constraints upon the dome. On the right, both the pendentives and the dome within the Theatinerkirche, Munich are heavily ornamented—the arrangement of decorations and windows must resonate with the pendentives.

indirect consequence for the primary trait that was initially subjected to selective pressure.

Gould and Lewontin's conception of evolutionary baggage was remarkably prescient. Over the past two decades, it has become clear that similar genes, gene families, and cellular signaling networks play a functionally similar role in the development of body plans in diverse species. Moreover, among the vertebrates, the genes are deployed to establish an agenda for implementing this plan at a similar juncture irrespective of species: this is the same embryonic stage at which von Baer noticed anatomical similarities, and it represents a bottleneck through which developmental processes appear to pass. Since both the genes at play as well as the body plans that form at this stage are so similar irrespective of phylum or species, the stage has been labeled the phylotype (Sander 1983). Many embryologists would now define the phylotype not so much as a discrete developmental stage, but as a temporally ordered process somewhat akin to what Haeckel described a century earlier (Alberch and Blanco 1996; Duboule 1994). The picture that is developing is that species diverge from a generic body plan that is built by an evolutionarily conserved toolkit of genes (Crawford 2003). Why has this stage of development remained so stable in evolutionary terms? Although evolution changes body morphologies, really dramatic innovations become progressively unlikely since genes, the body plan, the tissues, cells—all of these become increasingly enmeshed and constrained by their historical and evolutionary context, or "baggage."

4. *Hox* Genes: How a Toolkit Evolved to Produce a Phylotype and Remain Stable

The phylotype is in part produced by a structurally and functionally similar set of shared genes. One of the big surprises of the genome projects was that very little differentiates humans from worms or sponges, at least at the level of DNA. The major differences that separate us seem to reside in how these similar sets of genes are controlled. The head to tail arrangement of the vertebrate body plan derives in large measure from the activity of a cluster of genes called the *Hox* genes. In vertebrates, here are more or less 13 types of *Hox* genes that have duplicated into a varying number of clusters. Mammals have four clusters, fish have six, flies have one. In the vertebrates these genes are contained within relatively small domains stretching roughly 100,000 nucleotides. Indeed an entire vertebrate *Hox* cluster of 12 or 13 genes could fit inside the span of a single one of the comparable fruit fly *Hox* genes. This

reflects big differences in the length of DNA sequences between genes, and also between the operational sub-units of genes. We will return to these inter- and intra gene sequences in a minute.

The *Hox* genes confer an identity to each body segment. In flies, the segments are obvious and subdivide the head, thorax, and abdomen. In humans the segments are hidden beneath our skins as individual vertebrae. In addition, *Hox* genes have secondarily been co-opted to pattern the shoulder through to digit segmentation of our limbs (Zakany and Duboule 2007).

An intriguing attribute of the *Hox* genes is that they are arrayed in their respective clusters in the same order that they are turned on in an embryo. The first gene in the cluster turns on chronologically first and with a spatial domain that is closest to the head. *Hox* genes further along in the cluster turn on later and more posteriorly. Subtle differences in how these genes are controlled (precisely when and where they turn on) defines coordinates of the body plan and controls the agenda of subsequent gene activity that differentiates neck vertebrae from thoracic or lumbar ones (Ruddle et al. 1999). When these genes have been experimentally inactivated in mutant mice, atavistic body plans can arise: the middle ear bones might resemble structures more appropriate to our reptilian ancestors; or extra neck vertebrae grow instead of the boney occipital plates that normally form the back of our skull (the occipital vault was an elaboration necessary to enclose our recently expanded brain) (Hall 1995; Rijli et al. 1994; Rijli et al. 1993; Gendron-Maguire et al. 1993; Kessel, Balling, and Gruss 1990; Horan et al. 1994; Condie and Capecchi 1993).

Even more interesting, the DNA sequences that regulate when and where a particular *Hox* gene turns on are distributed throughout the entire gene cluster. Some reside between *Hox* genes, and some are embedded within a neighbor. To make matters more complicated, when the genes are active, some are capable of regulating both themselves as well as neighbors in the cluster (Duboule 1998). Outside of the clusters, new regulatory sequences appear to be responsible for *Hox* gene re-deployment during limb development (Deschamps 2007). The shared and inter-communicating nature of *Hox* gene regulation no doubt contributes to their preservation as relatively immutable gene clusters and to the preservation of a similar functional role in otherwise quite different animals. This intermixing of regulatory and functional sequences also likely plays a role in constraining the possibilities for development of new and different structures (Duboule 1994, 2007). By analogy to business or politics—administration by large committees (or groups of genes) entails inertia and tends to stifle innovation.

5. Phenomenology and the Genome: the Emergence of Expanding Stability and Entrainment

A particularly impressive example of a phenomenological approach to genomic analysis has recently been published. Investigators categorized the timing and ancestry of genes turned on during early development (Domazet-Loso, Brajkovic, and Tautz 2007; Domazet-Loso and Tautz 2003, 2010). A survey was completed to establish which genes were active in different species and at different times. The genes were then compared against a large database (Genbank) to establish when, over the course of evolution, its nearest ancestor first appeared. In this manner, each active gene was assigned an "age" index that indicated how far back down the ancestral tree (phylogenetic tree for the cognoscenti) one had to go to establish its root. For a given developmental stage, the age index of all the active genes was averaged (termed phylostratigraphy) and this average indicated whether ancient or more recently evolved genes held sway. Different animals tend to have very different reproductive strategies (sexual reproduction versus clonal propagation; insemination by a single sperm or by multiples), environmental requirements (terrestrial or aquatic), egg types and sizes (large shell eggs with yolks in chickens, or invisibly small internally nurtured ones in humans), etc. Animals tend to have very different morphologies later in life as well, and these differences embody the responses that have evolved to permit them to survive in different environments, to meet different challenges, and to capitalize upon different opportunities and niches available for existence. Not surprisingly, the genes that are deployed both very early and during adulthood tend to be relatively recent. Conversely, the phylotypic stage deploys genes that are more ancient. (Fig. 2)

In addition to the *Hox* genes, many other relatively ancient genes that are important to development are clustered or arranged in clumps that have resisted shuffling over millennia of evolutionary time. Surprisingly, although DNA re-arrangements occur quite frequently throughout evolution, big changes involving the repositioning of large stretches of genes tend to be relatively rare, while minute changes within and among genes tend to be comparatively frequent (Kikuta et al. 2007). Something holds genes together in sequence, but what could it be? This clumping of genes, referred to as synteny, probably reflects the presence of evolutionarily conserved sequences formerly thought of as "junk "DNA. This so-called "junk" is proving to be important even though the sequences do not encode proteins. Instead, they reside amidst and between the gene sub-units that encode proteins. These highly conserved sequences are distributed over spans millions of nucleotides long, and they

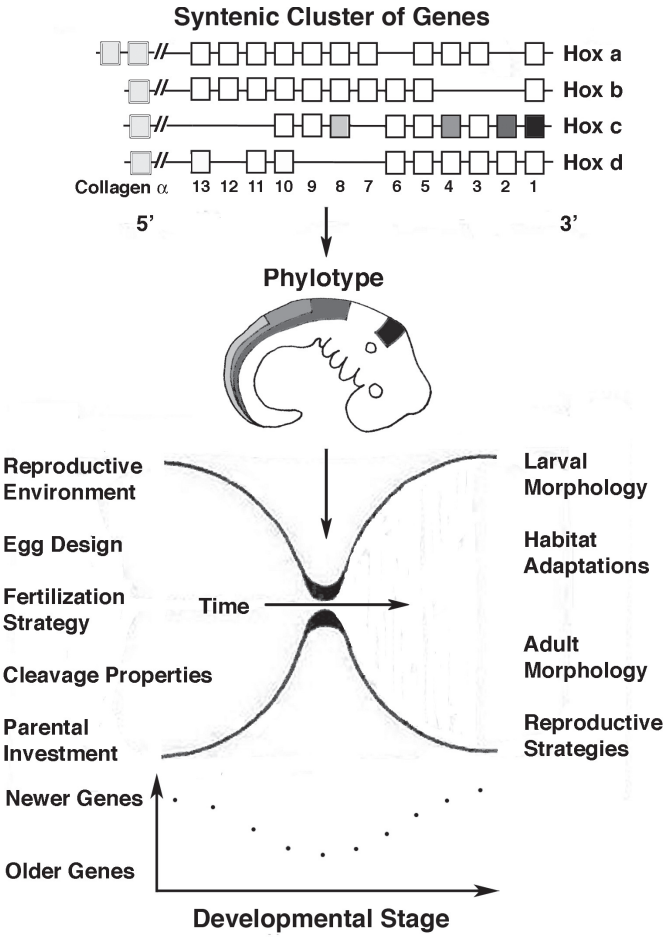

Figure 2. A cluster of roughly 13 varieties of *Hox* genes and their neighboring *Fibrillar Collagen* genes have duplicated into 4 clusters. The *Hox* genes turn on sequentially, and in a temporally and spatially coordinated manner, to set the agenda for development of the phylotype. This embryonic stage is when the body plan has been elaborated and organs are differentiating, but is prior to the period when more specific traits arise to reflect specific larval and adult morphologies. The average age index of genes active at each stage of development varies, as indicated in the graph above.

now appear to play an important role in regulating the genes associated with them. Ancient genes are especially characterized by their proximity to these conserved and repetitive sequences. Genes embedded in a region rich in these conserved elements tend to turn on in roughly the same place and at the same time (Akalin et al. 2009; Dong et al. 2010; Ellingsen et al. 2005). Consequently, regions that are rich in these elements are called genome regulatory blocks because they encompass more than just one gene, and the genes that reside within them tend to be arranged in the same order even in very different organisms.

In these regions, genes are subjected to selective pressure to remain embedded and relatively unchanged because small mutational changes can have amplified consequences—the activities of neighboring genes are altered. This conservative tendency is so marked that even the inactive parts of long-lost genes encompassed within these domains are preserved against the sequence ~~changes~~ that might be expected elsewhere in the genome (Dong et al. 2010). The non-coding elements appear to have proliferated over the millennia, and the genome has consequently acquired a progressively clumpy nature insofar as blocks of genes remain together in functional units to play a coordinated role. Moreover, syntenic gene clusters and gene regulatory blocks have expanded and entrained neighboring genes to a similar behaviour. "Bystander" genes caught in this manner are like pendentives. The fixation of one trait captures and entrains another that can then commission new effects upon the whole. Gene and process modularity ensues. I will argue that bystander genes caught in the expansion of regulatory blocks contribute to an emergent property that influences vertebrate architecture and might explain some of the more spectacular examples of convergent evolution. They could explain, for example, why the Tasmanian tiger/wolf looks more like a dog than its closer cousins, the kangaroos, bandicoots, and possums.

6. Examples of Bystander Genes That Come Along For The Ride—*Fibrillar Collagens*

Specific examples of "bystander genes" caught in a regulatory block might be exemplified by the *fibrillar collagens*. These genes encode proteins essential for bone differentiation: the earliest common ancestor of vertebrate *fibrillar collagens* dawned when the vertebrate skeleton arose (Boot-Handford and Tuckwell 2003). The *Hox* clusters are syntenic with their neighbors (19 to 21 additional genes) in several of the genomes examined. Among the neighbors

sit members of the fibrillar collagen "A" clade (Lee et al. 2006). Sequence analysis confirms that the *fibrillar collagen A* and *Hox* genes share a similar evolutionary history (Bailey et al. 1997). Possibly, in the far distant past, the *Hox* clusters subdivided our worm-like ancestors into discrete segments, and then the adjacent (and entrained) *collagen* genes nucleated the germ of a skeleton via the development of cartilage. These collagen expressing cells presumably arose from the dorsal midline precursor to the spine, namely the notochord, where both *Hox* and *collagen* gene families express (Zhang and Cohn 2008; Prince, Price, and Ho 1998). Eventually, with the passage of generations, segmental identities translated into recognizably discrete bony structures that now comprise our spine.

Elsewhere in the body, two genes, *HHEX* and *Sox4* help to direct pancreas development. They are both situated amidst a genome regulatory block that includes a third gene, *IRX3*. Up until recently, this last gene was not thought to play a role in pancreas development. Investigators impaired *IRX3* activity in zebrafish: the numbers of β-cells in pancreas islets diminished (Ragvin et al. 2010). *IRX3* might exemplify a bystander gene accidentally enmeshed and then re-deployed to perform a new function. If co-regulatory pressures and conserved elements help to consolidate syntenies and behaviors, then modules of genes and processes are made available for more elaborate construction projects. What consequences might this have for the evolution of multiple traits simultaneously?

7. Speculation on the Value of Functional Syntenic Modularity for Evolution and Convergence

Convergent evolution surprised and perplexed Darwin—he was not able to account for why surprisingly divergent plants evolved identical solutions to evolutionary problems (Darwin 1875). A fruitful arena in which to look for the effects of functionally important syntenic modularity might be among examples of convergent evolution. Convergence is simple to grasp, but elusive to quantify and test, and this will present a challenge.

The importance of genetic and process-oriented modularity in evolution has been discussed elsewhere, however pre-programmed sub-assemblies offer economies of scale and resources (Larsen 1997; Simon 1973). The discovery of genome regulatory blocks indicates that genomic modularity can help to canalize sub-assembly and to thereby provide a repertoire of morphological themes for the construction of body architectures. One consequence of the

modularization and clumping of syntenic blocks, is that when a single trait is selected, others with come along for the ride. A tractable example of convergence often discussed is the camera-type structure of eyes among cephalopds (octopus and squid), vertebrates, and cnidaria (jellyfish). Even though these eyes have a similar structure, they arise from profoundly different tissue types, interactions, and mechanics. Despite these differences they deploy similar genes and signaling networks including *Opsin, Crystallins, Pax, Mitf, Six3* and others (Kozmik et al. 2008; Kozmik et al. 2003; Tomarev 1997; Tomarev et al. 1997). Significantly, many of these genes, including *Rx, Pax6*, and *Six3*, are embedded within regions that are rich in highly conserved elements that are the hallmark of genome regulatory blocks (Engstrom, Fredman, and Lenhard 2008). For example, the eye "master gene" *Pax6* is embedded with eight other genes, all of which are expressed in at least eye and brain, and some of which are also linked to eye anomalies (*WT1, ELP4, MPPED2*). Perhaps mutation in one gene in isolation is sufficient to affect a process critical to eye development. On the other hand, perhaps mutation of one transmits behavioral changes throughout the regulatory block to affect neighboring genes. Discriminating between these two possibilities would help to distinguish between the functional importance of co-opted individual bystanders, and the role that they have acquired to moderate activity of the syntenic cluster at large. To what extent are the genetic pendentives informing the decoration and design of the dome?

The Tasmanian tiger/wolf or thylacine (*Thylacinus cynocephalus*) is a spectacular example of convergent evolution that went extinct in 1936. A carnivorous marsupial, it had the body and head of a wolf, and the stripes of a tiger. It also had a pouch for incubating and rearing its young. This marsupial shared the reproductive strategy of its marsupial cousins, and presumably the distinct and complex genetic mechanisms of marsupial gender and germ cell determination. In addition to the consequent and unique pressures this would entail for its breeding and social behaviors, it hunted in an environment and upon prey that differed relative to the canines. Moreover, the thylacine jaw and dental morphologies differ substantially from both canines and felines: they had molars (Dixon 1989). Analysis of preserved specimens suggests that its olfactory bulbs were much smaller than those found in dogs, therefore its brain was implicitly different too (Dixon 1989). How did it evolve such a wolf-like morphology? Does this animal represent a profound example of selective pressure working upon a few traits, but encompassing very many more in the form of the genomic baggage of modular entrainment? One way to assess this would be to compare the sequences, disposition, and the

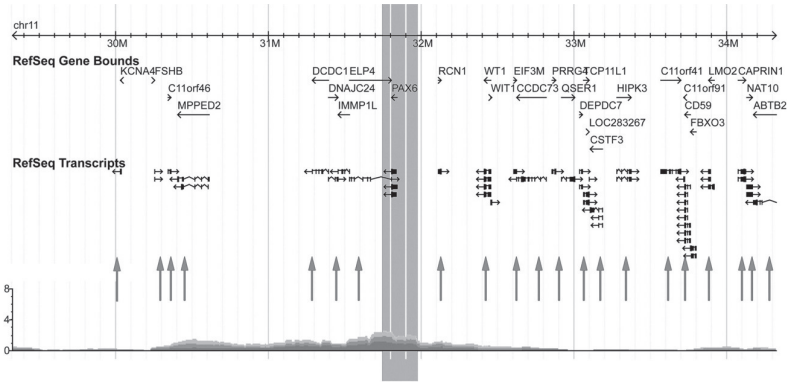

Figure 3. The upper hatched line represents a DNA sequence roughly 5 million nucleotides long. The small black boxes, arrows, and lines below represent the functional sub-units of individual genes, also demarcated by ~~arrows~~. The density and distribution of repetitive sequences that regulate this genomic block are represented as a jagged graph at the bottom. The light grey vertical field encompasses an important and ancient gene for eye development, *Pax6*, which is embedded within a peak of repetitive regulatory sequences. This regulatory block has spread laterally to entrain adjacent genes to a similar pattern of activity.

structure of genome regulatory blocks and gene syntenies. This would require genome sequencing from archived tissues. The technology to accomplish this task is now within reach in terms both of cost as well as mechanical feasibility.

Perhaps as one trait was selected to meet an environmental challenge, it bought a suite of other traits along for the ride and this resulted in the emergence of a wolf-like marsupial. A probe to assess the role of expanding modularity in body plan evolution would be to ask if syntenic blocks surrounding important genes, especially genes that are critical to directing the agenda for development, frequently expand to encompass usefully (re)deployed neighborhood genes. Do the same genes tend to become enmeshed in evolutionarily disparate species that nevertheless share similar traits? Once a selective pressure is brought to bear, do genes come attended by an entourage, a suite, of functionality and structure? My prediction is that emergent phenomena are facilitated in biological systems through the progressive accretion of functions and regulation. At the same time that modularization constrains the breadth

of options available for body plans, it also enhances the ease and richness of the themes remaining via emergence. Emergence in this context represents what Paul Harris described at this conference as a "bottom up" phenomenon. However in biological systems at least, and over large spans of evolutionary time, this bottom-up tendency loses granularity and becomes progressively clumpy. It is this clumpiness, or modularization, that facilitates the rapid emergence of more complex and coherently integrated suites of traits—like snap-on tools from a constrained toolbox of options, marsupials are "transformed" into wolves.

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