Molecular Systematics and Evolution

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Keywords

African Apes
Chimpanzees, bonobos, and gorillas.

Anthropoidea
The so-called higher primates; the subordinal rank that subsumes New and Old World monkeys plus the hominoids.

Clade
A group of related organisms; a group of organisms united by common ancestry.

Derived Feature
A relative character state determined by how restrictively shared a feature under study is.

Hominids
Humans and their fossil relatives.

Hominoids
The group of anthropoid primates that includes gibbons and siamangs, chimpanzees, gorillas, orangutans, and humans.

Homology
Similarity due to common ancestry.

Large-bodied Hominoids
Orangutans, humans, and African apes.

Lesser (Small-bodied) Hominoids
The gibbons and siamangs.

Monophyletic Group
A clade.

Outgroup
A taxon that is outside of (a sister taxon of) the focal group of phylogenetic reconstruction.

Phylogeny Reconstruction
The generation of a theories of evolutionary relationships among taxa.

Primitive Feature
A relative character state determined by how broadly shared a feature under study is.

Primitive Retention
A feature that is shared by the members of a group by inheritance from a common ancestor.

Sister Taxon
A taxon that is the closest relative of another.

Systematics
The study of the pattern and relationships of organisms, including the identification of species and delineation of groups of taxa.

Taxic
Relating to taxa.

Taxon (Plural: Taxa)
Any taxonomic rank, for example, species, genus, family, order, and class.

Studies that rely on genetic and molecular information to address evolutionary questions fall roughly into two different categories: the reconstruction of the evolutionary relationships of organisms (including the times of divergence of groups or lineages), and the formation and emergence of morphological novelties that distinguish or characterize different organisms. The former endeavor is sometimes referred to as *molecular systematics*, and when it is applied to primates and especially the relatedness of humans and apes, "molecular anthropology." The debate over the regularity of molecular change, resulting in a molecular clock, lies within the realm of molecular systematics, and especially that of molecular anthropology. In the past decade, in particular, the primary focus of molecular systematists has been DNA sequences, both nuclear and mitochondrial. Throughout, the assumption has been that the degree of similarity reflects the degree of evolutionary relatedness, because differences accrue once lineages have diverged from a common ancestor. The popularity of molecular systematics in recent years is also predicated on the notion of "a law of large numbers," that is, the thousands of bases that produce DNA sequences. The question is whether these assumptions are sustainable.

The rather newly defined discipline of evolution and development, or "evo-devo" as it has been nicknamed, is less involved in the interpretation of molecular data for purposes of reconstructing evolutionary relationships than it is in trying to identify molecular elements – whether they be transcription factors or other kinds or classes of proteins, as well as "genes" themselves – that are relevant to, or participate in, the processes of development of structure and form. Here, what is important is not a particular RNA, DNA, or amino acid sequence, but the signal transduction pathways or sequences of communication between different molecules in the regulation of development and the origin of structure. Sometimes, hypotheses of when and at what level within groups of organisms specific features emerged are generated as a result of overlaying this information on a presumed theory of relationships of the organisms under consideration. It appears that insights from developmental genetics will prove to be more useful than sequence data alone for systematic and phylogenetic inquiry.

This entry will attempt to summarize the main aspects of these fields of inquiry, including their underlying assumptions, and suggest possible avenues for future research that might bring these disciplines together in light of new ways of thinking about evolution.

1 The Beginning of Molecular Systematics

The first inquiries into systematics and phylogenetic reconstruction by investigation of the "blood relationship" of organisms can be traced to a few individuals, of whom the best known is George H. F. Nuttall. In the research that preceded his monograph of 1904, *Blood Immunity and Blood Relationship: A Demonstration of Certain Blood-Relationships Amongst Animals by Means of the Precipitin Test for Blood*, Nuttall sought to demonstrate that the degree of similarity between animals in their blood serum proteins was a reflection of their evolutionary closeness. The idea was simple: produce an antiserum (antibodies or antigens) to blood serum proteins of one animal, mix the antiserum with blood serum proteins of another animal, and measure the amount of precipitin that settled out. The more profound the precipitation, the greater the similarity (because of a greater number of antibody–antigen binding sites), and, consequently, the closer the evolutionary relationship between the two organisms being compared.

The strength of the antiserum played an important role in determining just how many species might belong to the same group. A weak antiserum would provoke reactivity in the sera of only the most closely related of organisms, while a stronger solution would produce a precipitate when combined with the sera of a greater number of related animals. The more distantly related the groups being compared, the less the reactivity between serum and antiserum. In addition to strength of reactivity, Nuttall thought that the reaction rate was also an indicator of closeness of relatedness. In the end, he decided that "the zoological relationships between animals are best demonstrated by means of powerful antisera." Consequently, he concluded, "if we accept *the degree of blood reaction as an index of the degree of blood relationship* within the Anthropoidea [the so-called higher primates, New and Old World monkeys plus the hominoids, which includes gibbons, chimpanzees, gorillas, orangutans, and humans], then we find that the Old World apes are more closely allied to man than are the New World apes, and this is exactly in accordance with the opinion expressed by Darwin."

Although Nuttall believed that his approach accurately revealed the evolutionary relationships of animals, with the exception of reference in 1922 by the paleontologist, W. K. Gregory, to Nuttall having demonstrated "the anthropoid heritage of man," his work went largely unacknowledged until the 1960s, when various publications claimed to have demonstrated the evolutionary relationships of organisms through study of elements of their biochemistry.

2 The Molecular Assumption

Perhaps the most influential of these publications was by Emile Zuckerkandl and Linus Pauling on fetal and adult hemoglobin, which included a comparison of sequences between human, gorilla, horse, and fish. Although one might question the comparison of gill- with lung-bearing animals, the endeavor demonstrated that the fish was more dissimilar to humans than to the horse, while the gorilla was the most similar to humans in hemoglobin sequence. Since this pattern of taxic distance mirrored the morphologically accepted scheme of evolutionary relatedness of these animals, Zuckerkandl and Pauling suggested that their observations "can be understood at once if it is *assumed* [emphasis added] that in the course of time the hemoglobin-chain genes duplicate, [and] that the descendants of the duplicate genes 'mutate away' from each other." From this assumption, they felt justified in proposing the following: "[O]ver-all similarity must be an expression of evolutionary history," with descendants "mutating away" and becoming "gradually more different from each other." In other words, the more time that elapses after a lineage's divergence, the greater the molecular difference its succession of descendants would accumulate. Consequently, evolutionary closeness became synonymous with molecular difference. Also assumed in this nexus of assumption is the notion of constant and gradual unilinear change.

There is, however, nothing in Zuckerkandl and Pauling's work that justifies the notion that "overall similarity" is "an expression of evolutionary history." They merely assumed such a correspondence, just as they assumed that difference also meant change and the same kind of change, which, in turn, was achieved at a constant and gradual rate. However, the discovery of a hundred nucleotide differences between two taxa does not translate into the rate at which presumed substitutions occurred. One needs additional information, such as a suggestion of the time at which these differences began to accumulate. And then one has to assume that the rate of substitution over this period of time was gradual and that differences did not arise during a few concentrated phases of replacement.

Nevertheless, Zuckerkandl and Pauling's effort resulted in the "molecular assumption" – continual molecular change and its continual accumulation over evolutionary time – which would thereafter become the foundation of molecular systematics. As Adalgisa Caccone and Jeffrey Powell would write in 1989: "Virtually all molecular phylogenetic studies. . .have a major underlying assumption: the genetic similarity or difference among taxa is an indication of phylogenetic relatedness. Lineages that diverged more recently will be genetically more similar to one another than will be lineages with more ancient splits." If one embraces the assumption, the rest may follow logically, but not necessarily because of biological demonstration. One of the major extensions of the "molecular assumption" is the notion of a "molecular clock," the existence of which was promoted initially by Vincent Sarich and Allan Wilson.

Beginning with their first major paper in 1966, Sarich and Wilson sought to elucidate the evolutionary relationships of primates, and especially of humans and the large-bodied apes, using the technique of microcomplement fixation (MC'F), which requires only minute amounts of serum and antiserum for study of immunological

reactivity. Their molecule of choice was the larger blood serum protein, albumin. The degree of reactivity achieved between albumin and anti-albumin was translated into an "index of dissimilarity," which was subsequently referred to as *immunological distance* (ID). The closer an ID value was to 1.0, the greater was the overall molecular similarity and thus, given an assumed equivalence between ID and evolutionary relationship, the presumption of closeness of relatedness between the organisms under study. As an apparent check on the validity of this approach, Sarich and Wilson conceived of the "test of reciprocity." For example, if chimpanzee serum was cross-reacted with antihuman serum (produced by injecting another animal, for instance, a rabbit or chicken, with human serum) the first time around, human serum was cross-reacted with antichimpanzee serum the next time to see if the resultant ID values were reasonably similar. Predictably, the test of reciprocity usually confirmed the initial immunological finding.

The supposed evolutionary arrangement of the primates that Sarich and Wilson achieved was more consistent with the general pattern of primate relationships based on comparative morphology than it was in disagreement. Because of this concordance, they concluded that "the MC'F data are in qualitative agreement with the anatomical evidence, on the basis of which the apes, Old World monkeys, New World monkeys, prosimians, and nonprimates are placed in taxa which form a series of decreasing genetic relationship to man." In 1967, in their second article on this topic, Sarich and Wilson argued that molecular change must have occurred at a constant rate among all major groups of primates. In the third and last article of this series, which was also published in 1967, they concluded that the small amount of difference they detected between hominoids in their albumin reflected little molecular change and that this, in turn, implied that little time had elapsed since the separation of the hominoid lineages. Since, as they claimed, molecular change ticked away at a constant rate, IDs represented a "molecular clock" that could reveal the times at which the various hominoids – actually any species – diverged and went off on their own evolutionary paths.

After calibrating the molecular clock on the basis of paleontologists' interpretation, based on fossils, of when the Old World monkey and hominoid lineages might have separated, Sarich and Wilson calculated divergence dates of about 10 million years ago for the gibbon lineage, eight million years ago for the orangutan lineage, and a mere five million years ago for the human and African ape lineages. The major implication of this calculation was that fossils older than five million years, such as *Ramapithecus* (now referred to the genus *Sivapithecus*) from c. 12- to 14-million-year old deposits in Indo-Pakistan, could not, as paleontologists concluded from study of morphology, be hominid.

However, while molecular systematists were in general agreement on the premise of the molecular assumption – that degrees of similarity reflect degrees of evolutionary closeness – not all of them embraced the notion of a molecular clock that "ticked" at a constant rate. Among the most vocal dissenters was Morris Goodman, who in the early 1960s was the leading proponent of Nuttall's work. Although Goodman went beyond claiming that molecular similarity indicated a very close relationship between humans and the African apes to advocating that these three hominoids should also be placed in the same taxonomic family, Hominidae

(a position that for years caused many comparative primate anatomists and paleontologists to be skeptical of molecular systematics in general), he nonetheless tried to accommodate the paleontologists' identification of pre-five-million-year old fossils as being hominid.

In contrast to Sarich (who in 1971 was the first molecular systematist to reject morphology as being phylogenetically informative), Goodman accepted the paleontologically derived date of at least 14 million years as the time of divergence between hominids and their potential ape relatives. As a consequence, though, Goodman then had to interpret the immunological and biochemical data in a way that would make them compatible with a deep timescale of hominoid diversification. He did so by constructing a selectionist argument to explain the apparent acceleration or deceleration in rates of molecular change that *de facto* must have occurred, given the paleontologically established dates for the earliest appearances of each of the large-bodied hominoids. Thus, for instance, if the various hominoid lineages had indeed originated between 18 and 14 million years ago, the extreme similarity between the various extant hominoids in their albumin had to have resulted from a slowdown in the rate of molecular change of that particular blood serum protein.

Goodman proposed that the inferred deceleration in rate of molecular change in large-bodied hominoid albumin was related to the fact that, although they are similar to other anthropoid primates in developing hemochorial placentation (the most intimate of placental modes in the approximation of maternal and fetal blood systems), large-bodied hominoids differ from other anthropoids in having very long gestation periods. He argued that in animals with hemochorial placentation but fairly short gestation periods, there would be enough slack in the system to allow unimpeded molecular change to occur and accrue. The fetus would be born before the mother's immune system could produce antibodies to it. A large-bodied hominoid's gestation period, however, would be long enough not only for maternal-fetal immunological incompatibility to build up but also for maternally produced antibodies to diffuse through the placenta, with deleterious consequences to the fetus.

The dilemma which then had to be confronted was: How could an animal have both hemochorial placentation and a long gestation period? Goodman's answer was that natural selection must have acted to reduce the possibility of immune responses from the mother toward her fetus's proteins. Consequently, there had to have been a slowdown of molecular change that was commensurate with a prolongation of the gestation period. Although albumin had been the basis of Sarich and Wilson's constant-rate molecular clock, Goodman used the same molecule as the exemplar of how the molecular clock could run at different rates.

Concurrent with the various approaches – immunological reactivity as well as gel-column electrophoretic separation – that were brought to bear during the 1960s and 1970s on the determination of evolutionary relationships was an increased interest in protein sequences. The first major study was Zuckerkandl and Pauling's 1962 analysis of hemoglobin, and, for some years thereafter, hemoglobin was the most intensely studied molecule. Beginning in 1973, however, a research group headed by A. E. Romero-Herrera analyzed myoglobin sequences across a number of primarily mammalian taxa. As more

taxa were added to the study, the resultant, most "parsimonious" arrangement of the hominoids placed the gibbon rather than the orangutan closer to a human-African clade. Only by arguing for a more complicated scheme of myoglobin "evolution" in hominoids – including, for instance, the condition that there had to have been various "back-mutations" to a state similar to the presumed unchanged state – could one arrive at the more commonly accepted arrangement of divergences: gibbon first, then the orangutan, and then a human-African ape group.

But while protein sequencing was seen by some, especially Zuckerkandl as late as 1987, as the molecular level on which to focus for purposes of determining evolutionary relationships (primarily because amino acid sequences are less subject to the problems with DNA, such as insertions, deletions, and back-mutations), there was a growing consensus that the best systematic information lay at an even deeper "genetic" level. Since base differences at the third position of a codon (a nucleotide triplet) did not yield different resultant amino acids, the concern of DNA sequence advocates was that demonstration of similarity in protein sequences could produce "false" phylogenies because the underlying nucleotide sequences themselves may be different. Consequently, it became imperative to get to the level of DNA – nuclear DNA – itself, especially because the general belief or at least expectation (which was based on bacteria and inferred for metazoans) was the existence of a direct correlation between specific DNA sequences and specific genes. And, as genes were conceptualized, there was also supposed to be correspondence between one or perhaps a few genes and a specific feature or structure. In addition, because DNA sequences were composed of thousands upon thousands of bases, the apparent massive scale of the comparison had the appeal of providing an overwhelming amount of phylogenetically relevant information.

3 DNA Hybridization

Although DNA sequencing was possible by the late 1970s, it was an expensive and laborious procedure, which made the endeavor itself, much less the comparison of DNA sequences for phylogenetic purposes, prohibitive. There was, however, another way in which DNA sequence information could be achieved: DNA–DNA hybridization.

The theory behind using DNA–DNA hybridization (or just DNA hybridization) as an approximation of overall similarity in DNA sequences between different taxa lay in the fact that if the two strands that form the helical structure of nuclear DNA were dissociated (which could be accomplished by subjecting it to heat), their complementary bases would rebond and the two strands would reform their original helical organization. However, it is not only two cleaved but also originally helically arranged DNA strands from the same individual that will reassociate (reanneal). Analogous to the geneticist Theodosius Dobzhansky's discovery earlier in the twentieth century with chromosomes of different lengths from different species of fruit fly, any two strands of DNA, from different individuals of even different species, will attempt to anneal.

As in Dobzhansky's fruit-fly experiments, in which the larger chromosome would loop and fold so that whichever of its loci were also present in the shorter one

would match up, single strands of DNA (derived from heat splitting or melting of a double helix) from different organisms would attempt to recombine or hybridize at complementary base positions. The more complementarity there was between hybridized DNA strands, the greater was the intensity of heat needed to break down the bonds holding them together. Consequently, it seemed logical to conclude that the heat (ΔT) it took to disassociate a DNA–DNA hybrid (that is, the more thermally stable the hybrid was), the more similar the annealed strands were in their nucleotide sequences. From this apparent demonstration, one could then invoke the molecular assumption to explain how higher ΔT's, reflecting greater molecular similarity, were also a reflection of closer evolutionary relatedness. As logical, though, as this thought experiment might appear to be, it relies on an assessment of overall similarity, which does not identify the bases or sequence positions that underlie differences in ΔT's. Conversely, similar ΔT's in different taxa might not be the result of the same regions hybridizing.

One of the first applications of the technique of DNA hybridization to evolutionary questions was published in 1968 by R. J. Britten and D. E. Kohne, who were primarily concerned with learning more about the genome. They discovered that the genomes of higher organisms – but not of bacteria or viruses – contained "hundreds of thousands of copies of DNA sequences." However, not only did repeated DNA sequences represent a considerable portion of a genome, Britten and Kohne also found that they were "trivial and permanently inert." Only a small fraction of a genome was composed of unrepeated, unique, single-copy DNA sequences, which apparently constituted the active or functional elements of that genome.

Britten and Kohne also speculated on pathways or mechanisms that might produce genomic change, which they argued must be considered on two different levels: change in nucleotide sequence, and the origin of new families of nucleotide sequences. As Zuckerkandl and Pauling had assumed for differences in hemoglobin, Britten and Kohne proposed that changes in nucleotide sequences – which would be identified as point mutations – occur slowly over time. However, this model would explain only the "divergence of preexisting families" of nucleotide sequences, not the introduction of new families of sequences, as was assumed would eventually happen in the gradual-accumulation-of-change model that dominated molecular systematics. The introduction of new families of sequences, Britten and Kohne alternatively suggested, must "result from relatively sudden events," called *saltatory replications*. Accordingly, "saltatory replications of genes or gene fragments occurring at infrequent intervals during geologic history might have profound and perhaps delayed results on the course of evolution." Although Britten and Kohne's distinctions between types of molecular change were not at the time embraced by molecular systematists, their suggestion of "saltatory replication" would certainly appear to have been borne out with the subsequent identification of vertebrate regulatory genes involved in segmentation – *Hox-a, Hox-b, Hox-c,* and *Hox-d* – as replicates of the orthologous *Antennapedia* gene identified in fruit flies.

One of the early attempts at using DNA hybridization to reconstruct relationships among the primates was published in 1976 by Raoul Benveniste and George Todaro, whose primary focus was actually on the distribution among mammals of the type C viral gene, the presence of which, they

discovered, was particularly characteristic of animals found in Asia, including the orangutan and the gibbon, but which, unexpectedly, was also present in humans. After reviewing these data, Benveniste and Todaro turned to DNA hybridization for sorting out the relationships of various primates. Relying on previous studies, they estimated "the effect of mismatched base-pairs on thermal stability" as being "between 0.7 and 1.7 °C per 1% altered pairs." Since, depending on the animal, nuclear DNA consists of anywhere from 10 to 100 million nucleotide pairs, every 1% of difference in nucleotide sequence between DNA hybrids would result in a drop of about 1.0 °C of the heat necessary to melt the hybrid molecule.

Although Benveniste and Todaro calculated a 1.1% sequence difference between humans and both African apes and a 2.4% difference between humans and the orangutan, their published ranges of ΔT's actually demonstrate overlap between all large-bodied hominoids (although one does not know the identity of nucleotide difference and similarity). Taking into consideration the fact that presumed-to-be-homologous DNA sequences are not the same length (that is, they do not consist of the same number of nucleotides) in all animals, including the large-bodied hominoids (see below for human and orangutan), one also does not know from DNA hybridization experiments the details of how a short sequence from one species aligns with a longer sequence of another species.

Probably because DNA hybridization seemingly provided a way of getting closer to the supposedly most "informative" genetic level, questions about what, exactly, similarity in ΔT's meant were not explored. But other questions were.

In the 1980s, Charles Sibley and Jon Ahlquist reviewed earlier DNA hybridization experiments and concluded that a major problem with them all was that only a limited number of cross hybridizations had been tested. This, Sibley and Ahlquist claimed, was the reason previous studies had such difficulty in refining certain evolutionary relationships, particularly those between humans and the African apes. Although there was not particular morphological support for this theory of relationship, molecular systematists often grouped these three hominoids together. However, the level of resolution in the majority of the molecular work was never fine enough to determine which two of the three hominoids were the most similar (and thus presumably the most closely related).

Sibley and Ahlquist felt another source of error in these earlier studies resulted from using all of an animal's DNA, repeated as well as single-copy, to form hybrids. They argued that it was important to remove all repeated DNA – since it was redundant – and to work only with single-copy DNA.

Before Sibley and Ahlquist decided to tackle the evolutionary relationships of the large-bodied hominoids, they had spent years applying their DNA hybridization technique to the phylogeny of birds, through which they also developed the notion of a uniform average rate of genomic change (UAR). UARs, they believed, characterized the nature of molecular, and thus evolutionary, change in all taxa. The notion of a UAR also seemed to negate the need for debates over constant versus irregularly running molecular clocks, which were directed at individual molecules. As they saw it, the entire genome could change at a uniform average rate, even though the rates

at which individual molecules change may be quite different.

Sibley and Ahlquist argued that the power of DNA hybridization in "discovering" the phylogenetic relationships of organisms came from sampling the entire genome of an animal. They claimed that, since an organism's genome is composed of millions of nucleotides, the "law of large numbers" provides the checks and balances necessary to rule out "false" similarities or parallelisms. Accordingly, since DNA hybrid strands will only link up along those stretches of sequences that are complementary, there should be no question about homology. Conversely, sequences that did not line up were not homologous. At least that was the argument.

Sibley and Ahlquist's melting temperatures for their DNA hybrids produced numbers, which they then converted into phylogenetic distances using a procedure called *average linkage*. "Average linkage" begins "by clustering the closest pair or pairs of taxa," after which "one links the taxa which have the smallest average distance to any existing cluster," and on it goes until "all taxa are linked." The underlying assumption, which permits the linking of the most similar pairs, is that the DNA hybridization technique "measures the net divergence between the homologous nucleotide sequences of the species being compared."

Many aspects of Sibley and Ahlquist's phylogenetic reconstructions of birds were consistent with theories of relatedness derived from study of morphology. However, there were some significant differences, for instance, with regard not only to the broader phylogenetic relationships of, but also to the details of relatedness among, the flycatchers. By the 1980s, however, it was common practice, when molecular and the morphological phylogenies were in discord, to opt for the former. As Caccone and Powel argued in 1989, once the molecular assumption is accepted, overall similarity becomes the yardstick for determining closeness of relatedness. Indeed, whether it is a presumed albumin clock or a UAR, the assumption of ongoing molecular change validates the molecular assumption, which, in turn, demands that a molecular phylogeny is correct in its entirety, even if other sources of information contradict it.

Sibley and Ahlquist's "law of large numbers" and UAR appealed to most molecular systematists. This appeal was probably the primary reason paleontologists finally succumbed to the notion that molecular phylogenies had greater authority in deciphering evolutionary relationships when morphologically based theories were in conflict with them. But not all molecular systematists were convinced. Among them was Alan Templeton, who objected to the use of DNA hybridization because it did not allow one to determine the polarity of the similarity, which is necessary to address the question: "Is similarity due to distant or recent common ancestry?" For, only by studying the actual sequences of nucleotides can one determine the identity and from this attempt to infer the significance of similarity and dissimilarity. Sibley and Ahlquist countered, however, that there was "no reason to expect data derived from base sequences to improve on those from amino acid sequences, which have produced contradictory results." In their support, they also quoted A. E. Friday (who had collaborated with Romero–Herrera on myoglobin sequencing): "Phylogenetic conclusions derived from a study of nucleotide sequences will be subject to the same suspicions as those derived from amino acid sequences."

4
Mitochondrial DNA

As the field of molecular systematics was expanding its sphere of investigation during the 1970s, DNA located outside the nucleus, in a cell's mitochondria, was attracting attention. In a study published in 1979, W. Brown, M. George, and A. Wilson noted that there was more difference between humans and a sampling of Old World monkeys in their mitochondrial (mt) DNA than there was among these same primates in their nuclear DNA. By assuming that the divergence between the human and Old World monkey lineages occurred over 20 million years ago (based on morphological studies of the primate fossil record), Brown et al. concluded that the differences between these humans and Old World monkeys could be explained if nucleotide substitutions occurred at a slower pace –5 to 10 times slower – in nuclear than in mtDNA (in other words, if mtDNA "evolved" 5 to 10 times faster than nuclear DNA). On the basis of this interpretation, Brown et al. calculated that mtDNA data are the most accurate for studying evolutionary events that occurred "within the past 3–10 million years."

In subsequent publications, they proceeded to analyze the mtDNA of the large-bodied hominoids, which, largely because of Sarich and Wilson's molecular clock, were assumed to have diverged within this time period and which, because of this assumption, were thus amenable to such analysis. Although their data showed the fewest differences (interpreted as substitutions) between humans and chimps, Brown et al. concluded that humans were related to an African ape group. Given the molecular assumption, however, the data should have yielded a human–chimpanzee sister group, as Maryellen Ruvolo and a number of collaborators concluded in 1991 from their analysis of mtDNA.

In addition to the fact that mtDNA is single-, not double-stranded, the allure of using mtDNA was and continues to be twofold. One assumption is that, unlike nuclear DNA, mtDNA is supposedly inherited only through the maternal line and thus not subject to the complexities that occur through recombination of maternal and paternal DNA. The second assumption is the existence of a "hotspot" in the D-looped configuration of mtDNA and that this is the primary site of molecular activity. This region is called the *hypervariable zone* and its study has been interpreted as providing evidence of evolutionary change. Although crucial to the use of mtDNA for purposes of phylogenetic reconstruction, these assumptions are probably incorrect.

The introduction of paternal mtDNA into fertilized eggs has been reported in the literature from time to time. For example, in 1992, Allan Wilson and coworkers demonstrated that this happens in mice, as part of the sperm's tail, which does contain mtDNA, penetrates the ovum's membrane. More recently, John Maynard-Smith and coworkers calculated that there had to have been a recombination of maternal and paternal mtDNA during human evolution, and concluded that this possibility should cause systematists to reconsider the seemingly inviolable "fact" that humans and chimpanzees are closely related.

As Erika Hagelberg reviewed at great length, there is increasing indication that mtDNA is not as exempt from paternal inheritance and recombination as was initially believed. With regard to so-called hypervariable sites being regions of preferentially high mutation rates, which therefore lend themselves to phylogenetic analysis,

Hagelberg pointed out that "there is no direct evidence of hypervariability," although "most researchers believe that anomalous patterns of DNA substitution are best explained by mutation." Indeed, she writes, "because the notion of hypervariability fits with the received view of mtDNA clonality [maternal inheritance only], anomalies are seldom questioned." Hagelberg gives an interesting example. Depending on which subject's mtDNA is used, one can reconstruct "our most-recent female common ancestor. . .[as having] lived just 6000 years ago, a date more consistent with Biblical Eve than Mitochondrial Eve." As she cautions: "The picture is far from simple, and it is clear that extreme care must be taken in the interpretation of mtDNA phylogenetic trees in the face of possible recombination. . .[T]here are enough unexplained patterns in mtDNA data to warrant reassessment of the conclusions of many mtDNA studies."

5
DNA Sequences

In spite of Sibley and Ahlquist's and Friday's warnings about nucleotide sequence data not being any more reliable than protein sequence data for reconstructing phylogenetic relationships, the increasing ease with which DNA could be sequenced could not be ignored by molecular systematists. Nuclear as well as mtDNA were sequenced. Regardless of the genetic "level" under scrutiny, the interpretation of similarity or difference in DNA sequences under comparison was still predicated on the molecular assumption. The weight of DNA sequence data as being key to deciphering evolutionary relationships assumed special significance in some areas of molecular systematics because of the supposed information content of nuclear DNA in general. As Elizabeth Bruce and Francisco Ayala wrote as early as 1979 in an article on blood serum proteins: "Information macromolecules – that is, nucleic acids and proteins – document evolutionary history. . .[Thus] degrees of similarity in such macromolecules reflect, on the whole, degrees of phylogenetic propinquity."

Almost coincident with the rise in popularity of comparing DNA sequences for purposes of inferring evolutionary relationships came the cautionary notes. Of particular importance in this regard is the question of alignment, which, in 1991, J. A. Lake was among the first to address. As mentioned above, not all DNA sequences chosen for comparison, if homologous (e.g. representing the same "gene" or segment) are the same length. Therefore, decisions must be made with regard to how to subdivide the shorter sequence in order to align its nucleotides with those of the longer sequence. Typically, the alignment of compared sequences is presented in the literature without justification of the assumptions and decisions that produced the alignment, which, in turn, was then used as the basis for the phylogenetic analysis. But, as Lake warned in the title of his article, "[t]he order of sequence alignment can bias the selection of tree topology." In addition to assumptions that inform the decision to break up a short sequence so that its bases align with those of a longer sequence is the issue of whether, for one or another sequence, bases may have been added or inserted, removed or deleted, or one base substituted for another.

In *what it means to be 98% chimpanzee*, Jonathan Marks provides an example of these problems with DNA sequences from a human and from an orangutan to show three different ways in which

their bases (C,T,G,A) can be aligned and the interpretive consequences. Even beforehand, the assumption must be made that the 40 bases in the human sequence actually have homologous counterparts in the 54 bases in the orangutan sequence.

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HUMAN CCTCCGCCGCGCCG       CTCCGC  GCCGCCGGGCA                 CGGCC               CCGC
ORANG CC                GTCGCCTCCGCCACGCCGCGCCACCGGGCCGGGCCGGCCCGGCCCGCCCCGC

HUMAN          CCTCCGCCGCGCCGCT           CCGCGCCGCCGGGCACGGCCCCGC
ORANG CCGTCGCCTCCGCCACGCCGCGCCACCGGGCCGGGCCGGCCCGGCCCGCCCCGC

HUMAN CCTCCGCCGCGCCG      CT CCGCGCCGCCGGG  CAC   GGCC                          CCGC
ORANG              CCGTCGCCTCCGCCACGCCGCGCCACCGGGCCGGGCCGGCCCGGCCCGCCCCGC
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There may be seven differences or there may be eleven differences, depending on how we decide the bases correspond to each other across the species – and that is, of course, assuming that a one-base gap is also equivalent to a five-base gap and to a base substitution.

As Marks comments:

"Tabulate the differences. The top one invokes five gaps and six base substitutions; the middle has only two gaps but nine base substitutions. And the bottom one has five gaps and only three base substitutions. The three pairs of sequences differ in the assumptions about which base in one species corresponds to which base in the other. While we might, by Occam's Razor, choose the alignment that invokes the fewest inferred hypothetical evolutionary events, we still have to decide whether a gap "equals" a substitution. Does the bottom one win because it has a total of only eight differences? Or might the middle one win because a gap should be considered rare and thereby "worth," say, five base substitutions?

The problem is that we cannot know which is "right," and the one we choose will contain implicit information about what evolutionary events have occurred, which will in turn affect the amount of similarity we tally. How similar is this stretch of DNA between human and orangutan?

In a more general sense, however, the problem of taking *quantitative* estimates of difference between entities that differ in *quality* is prevalent throughout the genetic comparison of human and ape. The comparison of DNA sequences presupposes that there are corresponding, homologous sequences in both species, which of course there must be if such a comparison is actually being undertaken. But other measurements have shown that a chimpanzee cell has 10% more DNA than a human cell. (this doesn't mean anything functionally, since most DNA is functionless.) But how do you work that information into the comparison, or into the 99.44% similarity [between human and chimp]?" [comment added].

These concerns have not, however, been widely appreciated by molecular systematists, especially molecular anthropologists, who not only portray the analysis of DNA sequences as neutral and objective but also use the assumption of relatedness to inform the way in which they analyze

the sequences they have aligned according to certain assumptions. Exemplary in this regard is the multiple DNA sequence analysis Maryellen Ruvolo and collaborators published in 1997, in which they sought to resolve the supposed dilemma of to which African ape humans are more closely related. In order to pursue this question, they assumed first that the orangutan was the sister taxon of a clade or evolutionary group consisting of humans, the chimpanzee, and the gorilla. Consequently, the differences in the orangutan had to be considered primitive relative to any similarities that were delineated between humans and one or the other of the African apes.

With the ever-growing popularity of the parsimony-based phylogenetic computer program PAUP (phylogenetic analysis using parsimony), it is common practice to "root" a phylogenetic analysis in a taxon that is chosen as the primitive outgroup – that is, the taxon that diverged earlier than the others – prior to the analysis taking place. Rooting parsimony or any of the other available clustering analyses (for example, nearest-neighbor joining or maximum likelihood, which are essentially similar to Sibley and Ahlquist's linking technique) in a particular taxon may be necessary for the algorithm to "work." Nevertheless, this procedure artificially determines character polarity since, by definition, the outgroup (the taxon in which the tree is rooted) is defined from the outset as being primitive in its entirety. In turn, the taxa to which the outgroup is the supposed primitive sister taxon are predetermined as being derived in whatever ways they differ from it.

The widespread use of this algorithm-based approach to analyzing nuclear and mtDNA as well as protein sequences presents its own set of problems and assumptions. Consider the molecular assumption: Since molecular change is supposedly continually occurring and being accumulated as a lineage proceeds through time, the degree of molecular similarity reflects the antiquity or recency of lineage divergence. Accordingly, each lineage accumulates it own unique array of molecular changes, which should make a lineage more distinctive (that is, different) the longer it is in existence. Although tautological, this assumption explains why more recently diverged taxa are more similar than more anciently divergent lineages. On the other hand, in order to root an algorithm for purposes of generating presumed phylogenetic relationships, one must assume that the taxon chosen as the earlier-divergent outgroup is totally primitive relative to the taxa to which it is supposed to be the sister taxon. Yet, it is the molecular assumption that validates the use of overall similarity as the key to resolving phylogenetic relationships by contrasting it with the unique differences that earlier-divergent lineages accumulated along their own, unique evolutionary trajectories. Clearly, both assumptions cannot be correct at the same time. Either the earlier divergent-taxa or lineages did not change, but remained primitive (which is the logical extension of identifying a taxon as the outgroup in which to root a computer analysis), or they did change by accumulating their own suites of molecular difference (the basis of the molecular assumption), in which case they are at least in some aspects derived (and uniquely so, for that matter, because of their unique molecular histories) and not primitive relative to the taxa to which they are being compared.

In the realm of morphological systematics, according to Hennigian or cladistic

principles, overall similarity is not *de facto* a clue to evolutionary relatedness. Similarity must be sorted out into features that reflect a hierarchy of inheritance: primitive features from ancient ancestors, and derived features from recent ancestors.

Since the pattern of life is one of a hierarchy of nested sets of smaller and smaller clades (groups of related taxa), that which is considered primitive versus that which is considered derived depends on the level in the hierarchy of nested clades one is investigating. Primitive features – features retained in descendants – do not elucidate the relationships of these taxa. Only derived features can. It is also important conceptually to recognize that a derived feature at one level of the hierarchy is a primitive retention at another. There is no theoretical reason why this approach to systematics cannot be applied to molecular data. The major difficulty is that molecular systematists would have to sample and compare a wide range of taxa. This is the only way in which relative primitiveness and derivedness can be determined. It cannot be justified by *a priori* assumptions of directionality, as underlies the molecular assumption, or by choosing an outgroup on the basis of its presumed evolutionary relationship to other taxa. However, even from the beginning, it is also crucial to realize that shared similarity does not translate directly into a demonstration of relatedness. Taxa may be similar, not because they inherited changes that distinguished a recent common ancestor, but because they share primitive retentions, that is, features that have not changed in a succession of ancestors.

Nevertheless, it is becoming increasingly popular in the literature for molecular studies on the relatedness of taxa to be identified as being "cladistic." One argument is that nucleotide bases – C, G, T, A – represent alternative character states. On one level this may appear logical, but it is actually misleading since none of them represents a character. Phylogenetically relevant alternative molecular character states would be better represented by comparison, for example, of arrangements of "gene" sequences with regard to *cis* and *trans* elements, patterns of introns and exons and of methylation of transposons or other elements, and pathways of molecular communication. Another argument in support of molecular studies being cladistic derives from the claim that molecular similarity is equivalent to synapomorphy; that is, shared similarity represents shared derived character states. This conclusion is, of course, only a restatement of the molecular assumption: the most recently diverged taxa share more recently accumulated (equate with derived) molecular states. Thus, the supposedly shared derived molecular states are delineated *a posteriori*; in other words, after the algorithm of choice has clustered taxa on the basis of their greater or lesser degrees of similarity (depending on the algorithm), typically after rooting the tree in a particular taxon (which, as pointed out above, at once defines it as being primitive and the taxa being compared to it as derived in their shared similarities). This, however, is not how a cladistic analysis proceeds. The endeavor of hypothesizing primitive versus derived character states occurs prior to hypothesizing relationships – which is the only way in which such a methodology can actually be employed.

The assumption of continual molecular change – whether through point mutations affecting nuclear or mtDNA, or altering amino acids in protein sequences – is also of interest. Recall that this idea was initially framed by Zuckerkandl and Pauling

as a way of explaining their data: "overall similarity *must* [emphasis added] be an expression of evolutionary history," with descendants "mutating away" from each other, becoming "gradually more different from each other." It is this assumption that proposes that earlier-divergent taxa accumulate their own molecular differences, while the most recently divergent taxa are similar because of the longer shared history of accumulated molecular changes and shorter time of independent molecular change. The existence of molecular clocks and UAR is predicated on this notion. Nevertheless, it must be recognized that this is an extrapolation – an explanation of how something might come to be. It has not been demonstrated.

The contradiction is that while constant molecular change is predicted through the molecular assumption as occurring during gametogenesis, or in some way as to be passed on to offspring, in molecular biology, it is well known that the only source of constant molecular change is ultraviolet radiation, which produces a mutation rate of 10^{-8}–10^{-9}. But the other element of UV-derived mutation is that it is random, with the potential of affecting either somatic or sex cells and also with regard to the molecule that is affected. Thus, while there might appear to be concordance between the reality of the physical world in which there is a relatively constant UV-provoked mutation and the concept of a constantly "evolving" molecular world, this is an illusion.

The notion of constant and accumulative mutation affecting sex cells is of further interest because it also contradicts the basic tendency of cells to remain in homeostasis. As seen, for example, in the roles of heat shock proteins (HSP) – maintaining cell membrane physical states through lipid transport, eliminating reading errors that occur during transcription or translation, DNA repair, chaperoning other proteins, and ensuring proper folding of proteins as they emerge from the ribosomes – the basic propensity of a cell is to resist change. Intuitively, this should make sense inasmuch as unabated molecular change would undermine the integrity of cell function, as would also be the case with a constant accumulation of point mutations, and more probably lead to the death of organisms than to change.

6
"Evo-devo"

In 1975, Mary-Claire King and Allan Wilson surveyed all available data on blood serum proteins, as well as the results of DNA hybridization, with regard to humans and chimpanzees. Although their publication is cited as having demonstrated the relatedness of these two hominoids, this was not their intention. As they stated: "the only two species which have been compared by all of these methods are chimpanzees...and humans," and thus "a good opportunity is...presented for finding out whether the molecular and organismal estimates of distance agree." The result was that humans and chimpanzees differed in their genetic makeup only by about one percent. King and Wilson concluded that "all the biochemical methods agree in showing that the genetic distance between humans and the chimpanzee is probably too small to account for their substantial organismal differences." In order to explain how humans and chimpanzees could be virtually identical in their genes but markedly different animals, King and Wilson suggested that humans and chimpanzees must be

different in those genes that regulate development.

Since then, studies on the regulation of development have expanded exponentially, not only with regard to distinguishing between regulatory and structural genes, but especially with regard to the array of molecules that induce gene transcription and communicate via signal transduction pathways to produce structure. Interestingly, those animals that have been studied in depth – such as the fruit fly, zebra fish, frog, chick, mouse, human – demonstrate a commonality of "homeotic genes" (which contribute to segmental patterning and segment identify). In turn, through their protein products (transcription factors), homeotic genes control or at least affect gene expression. Time and time again during development, the same proteins (e.g. various growth factors, trans-inducing and bone-modifying proteins) and regulatory genes (and their products) are coopted to produce what in adult organisms are different morphologies.

In 1994, Lewis Wolpert summarized the situation: "During development, differences are generated between cells in the embryo that then lead to spatial organization (pattern formation), changes in form, and the generation of different cell types. Genes control development by controlling cell behavior." But one should not be too gene-centric in envisioning the emergence of form from genes and gene products alone. For, while there might be genetic regulation of some cells' activities, the results (e.g. cellular asymmetry, cell membrane elasticity or rigidity, compressive forces) might produce physical or mechanical responses, which may not themselves be genetically based, but which, nonetheless, greatly affect cell geometry and ultimately organismal form. "Cell behavior," to return to Wolpert, has many different levels of meaning.

In the 1970s, Søren Løvtrup argued that one must recognize the importance of epigenesis in development: especially that changes in properties of the fertilized egg can alter the chronology and spatial organization of patterns of cellular diversification. Since the larger clades of multicellular organisms possess the same kinds of cells, as well as the same chemical substances that form the immediate environment of the cells, variation in the spatial and chronological organization of cellular differentiation must be at least one of the keys to the emergence of evolutionary novelty. For instance, whether a cell divides symmetrically or asymmetrically (which can be affected even by the positions of the chromosomes relative to the center or periphery of the cell) can greatly impact the spatial relationships of cells and, therefore, eventually have an effect on organismal shape. As Pere Alberch has emphasized, the development of organismal form and structure is also a function of the physical and mechanical properties of cells' sizes, shapes, and spatial relationships.

The application to evolutionary questions of discoveries in the regulation of development has given rise to the field of "evo-devo" (evolution and development), in which the interrelationship between the "genetics" and the "epigenetics" of development has become a primary focus. Indeed, Løvtrup's concern with the influence on metazoan development of differences in cellular differentiation during gastrulation appears to be even more germane to an understanding of the emergence of form and its conservation across taxa as well as of the emergence of differences in form, whether their expression constitutes variation (individual differences) or diversity (species differences).

7
Positional Information and Shape

One of the ongoing questions in developmental biology is how cells acquire positional information, not only in terms of entire structures themselves (e.g. where limbs will grow) but also with regard to how cells acquire information to contribute to regional shapes of a structure (e.g. the different segments of a limb).

In invertebrates, wing (e.g. as in a fruit fly) and limb (e.g. as in the brine shrimp-like crustacean, *Artemia*) positioning involves activation of the regulatory genes *nubbin* and *apterous*. In vertebrates, various regulatory genes, especially *Hox* genes, and also *dlx* (distal-less), are known to be involved in segmentation and limb positioning. In fish and tetrapods, the *Hoxd11-13* genes are expressed along the posterior margin of the enlarging limb bud; however, in tetrapods, this homeodomain expands anteriorly across the distal (lower) end of the limb bud. Additionally, in vertebrates, *Hox* genes are not only recruited in the formation of a segmented trunk but also through regional activation, in the segmentation of the hindbrain. Regional activation of *sonic hedgehog*, however, in part, underlies forebrain segmentation.

Eye development is also of interest in this regard. For although what used to be thought of as "master-control genes," such as *Pax-6*, were found to participate in signal transduction pathways leading to eye formation in invertebrates (typically multilensed, rigid) as well as in vertebrates (single lensed, deformable), there is at least one element that vertebrates have in common: Even though there are differences among vertebrates so far studied with regard to when in ontogeny and how often and in how many different regions the *Rx* gene (which is also recruited in fruit-fly eye development) is activated, it is always expressed in the vertebrate forebrain. Thus, at one level, one can hypothesize that the last common ancestor of vertebrates was characterized by early activation of the *Rx* gene in the presumptive forebrain and that differences between taxa are the result of differences in other aspects of *Rx* gene expression: for example, the different proteins in the lenses of amphibians and mammals may be due in part to down- or upstream effects of the *Rx* gene being expressed later on in development in the amphibian (frogs) retina, whereas in mammals (mice), the *Rx* gene is expressed early on in the presumptive eye itself.

In addition to considering morphological differences in light of differences in regional (as well as in overlapping) domains of regulatory gene expression, it is becoming increasingly clear that differences in fields of molecular gradients (morphogenetic fields) also play a role. As C. Owen Lovejoy, M. J. Cohn, and T. D. White hypothesized in 1999 in their discussion of the evolution of human pelvic form, "if a particular PI [positional information] gradient were to span *n* cell diameters, and those cells defined the ultimate anteroposterior dimension of the presumptive ilium (superoinferior in the adult human), then a slight increase in the steepness of its slope would cause that signal to span fewer cells, 'distorting' the presumptive anlagen and substantially altering downstream adult morphology." In other words, although it would seem to be a process involving myriad steps, "the transformation of the common ancestral pelvis [in its entirety] into that of early hominids may have been as 'simple' as a slight modification of a gradient" [comment added]. Thus, in addition to differences in gene expression and pathways of molecular communication,

as well as to the physical and mechanical consequences of cellular organization, morphological novelty in metazoans (and presumably plants as well) may also be affected by altering the domains of morphogenetic fields.

But what is the source of differences in gene or molecular gradient expression? Lovejoy et al. suggest that one need not seek the answer in mutation, which is a position that Sean Carroll has recently also strongly argued.

8
"Mutation"

The concept of "mutation" is about as slippery as that of a "gene." It means different things to different researchers, and, interestingly, the differing concepts seem to "work" in their disparate intellectual contexts. With regard to mutation, the "textbook" notions of preceding decades included point mutation, gene duplication, and chromosomal rearrangement. The latter was basic to the earlier experimental studies and theoretical considerations of the fruit-fly geneticist, Theodosius Dobzhansky. Dobzhansky's emphasis on chromosomal rearrangement as a potential source of evolutionary novelty was subsequently adopted by the developmental biologist, Richard Goldschmidt, in his theory of systemic mutation, which he argued would lead to the abrupt appearance of novel form. Unfortunately, Goldschmidt is best remembered, and consequently criticized, for suggesting that such novelties would emerge in individuals he identified as "hopeful monsters."

One of Goldschmidt's major theoretical thrusts, however, was distinguishing between what he identified as micromutation (leading to variation and microevolution) and macromutation (leading to the origin of species or evolution). The small mutations that fruit-fly population geneticists, such as Thomas Hunt Morgan, inferred lay behind small phenotypic changes, Goldschmidt identified as micromutations, which, he argued, led only to the survival of species, not to their origin. The latter required a larger source of genetic disturbance, and for that he turned to chromosomal rearrangement. The logic is understandable: If the chromosome theory was correct (that, indeed, units of heredity or genes were contained on chromosomes – as Morgan presented it, like beads in a necklace), then manipulating them on a grand scale (producing a systemic mutation) might yield evolutionarily significant novelty, that is, new species. A major problem with Goldschmidt's theory, however, was that he did not provide a mechanism by which more than one individual would be the bearer of the novelty and, thus, of the systemic mutation underlying it.

Point mutations, commonly the result of UV radiation, are random with regard to affecting somatic or sex cells. In addition, if they do not interfere with cell function, such point mutations are not a reliable source of potential genetic and subsequently morphological novelty. Indeed, it appears that point mutations do not often cause any noticeable effect, either genetically or phenotypically. Gene duplication – as seen, for instance, in the emergence of *Hoxa-d* – does sometimes occur, but knockout experiments have demonstrated that duplication typically reflects redundancy, not a source of phenotypic novelty.

It may be true that manipulation of levels of thyroxin or retinoic acid during ontogeny can affect the size of an organism, or the shapes of some of its features, just

as a mother's diet can affect methylation in its fetus or fetuses, and thus aspects of her progeny's postnatal growth. However, these disturbances only affect an individual during its lifetime, and should not be expected to be repeated across generations and under the influence of fluctuating environmental stimuli (e.g. diet, temperature, amount of daylight). The problems, then, that still must be addressed are as follows: How does a genetic or cellular change remain "fixed" or constant, and how do many individuals come to bear it?

It is important to realize that there is a difference between change at the genetic level and what is perceived as phenotypic change. Common in the literature on the genetics of evolution is the mistake of conflating the two as constituting macromutation – a misconception that derives from the confusion Dobzhansky introduced with regard to the terms micromutation and macromutation when, in 1941, he sought to discredit Goldschmidt. Nevertheless, especially with the increasing awareness from molecular biology that there are not "genes for" features, we must be vigilant in making a distinction between what appears morphologically to have been the result of a macromutation (e.g. developing feathers instead of scales) and the underlying genetic–epigenetic interactive pathway.

9 Toward a Theory of Evolutionary Change

The question at hand, then, is the articulation of a mechanism that can first provide the potential for genetic novelty. Building on my original theory for the sudden appearance of morphological novelty (through the silent spread of recessive "mutations"), the molecular biologist, Bruno Maresca, and I have proposed that the opportunity for genetic novelty may lie in overstressing cells to the extent that their HSPs can no longer maintain genetic homeostasis; that is, they cannot fulfill their roles as chaperones, respond to the needs of the cell membrane, and, perhaps most importantly, for this discussion, properly fold other proteins and assist in DNA repair. Although first identified in heat shock experiments, HSPs can be affected by a variety of stresses, including diet (saturated vs unsaturated fatty acids), wind, aridity, and cold. Since most multicellular plants and animals possess HSPs, the theory is more widely applicable than metazoan-centric Darwinian and neo-Darwinians models of evolutionary change, the latter of which relies on unwarranted extrapolations from fruit-fly population genetics.

Since most multicellular organisms have a window of heat shock response, they can "adapt" to normal fluctuations in their environmental circumstances. If environmental change exceeds this window (as when seasons change), most organisms can "reset" it, often in less than two months. Until this window is reset, the stress induces an increase in HSP production. If, however, there is a spike in environmental stress that exceeds an organism's ability to reset its HSP response, HSP function will fail, and opportunities for introducing genetic novelty will emerge – especially as a result of improper protein folding and inefficient DNA repair. In the former situation, improperly folded proteins may, for example, no longer recognize (or be recognized by) promoter or enhancer regions to which they would normally bind, but they may now be capable of binding to different sites. An obvious result could be the activation or deactivation of a

"gene" or "genes," and, thus, the creation of one or more new developmentally significant signal transduction pathways. With regard to inefficient DNA repair, genetic novelty of a different sort can be introduced, with obvious potential consequences. In both cases, however, the fact that the environmental stress will be at least regional (if not global), the circumstances exist for more, perhaps many more, than one individual of a species to be affected (not, however, necessarily in the same way).

However, while it might be tempting to extrapolate immediately from these possible sources of genetic novelty to the emergence of evolutionarily relevant morphological novelty, one must be cautious. First, the effects of extreme environmental spikes on HSPs must be actualized during gametogenesis. If they are not, offspring will not be affected. Second, if the effects do not kill the individuals that inherit any of these genetically based novelties, they will probably not be expressed; that is, these genetic novelties will be in the "recessive" state. Consequently, there will not be an immediate phenotypic reflection of these genetic changes. In the recessive state, however, they can spread "silently" throughout the population, until it becomes sufficiently saturated with heterozygotes that homozygotes for the genetic novelty will be produced. If the resultant phenotypic expression – cellular or greater – does not kill its bearers, they may continue to reproduce themselves, as heterozygotes will also contribute to the numbers of individuals bearing the phenotypic novelty. Thus, the spread of a genetic basis for potential phenotypic novelty may take numerous generations before there is any statistical possibility of the phenotype being expressed. In other words, there will be a temporal disjunction between the disruption of cellular and genetic homeostasis, and what will be seen as the abrupt or sudden appearance of phenotypic novelty, and in some number of individuals. In addition, one must bear in mind that, during periods of "silently spreading" genetic novelty, there could be other environmental spikes that would contribute to the pool of potential for genetic novelty and also then phenotypic novelty (however defined). Superficially, this process – or at least the sudden appearance of phenotypic novelty – may seem macromutational, but, clearly, it is not, at least in the original sense of Goldschmidt or even that of Dobzhansky. Indeed, something as simple as slight changes in protein folding could have major cascading morphological effects.

10
Molecules and Systematics: Looking Toward the Future

It may be widely believed, and even true at some level, that, as Sean Carroll has recently reiterated, "genomes diverge as a function of time." However, the observation that genomes may be different (in whatever ways difference, and similarity, may be identified and defined) does not in and of itself provide clues to how this difference was achieved. No doubt, some difference is due to the rare and random effects of UV radiation. In addition, genomic difference may be due to failures in DNA repair. There may be something intuitively appealing about Sibley and Ahlquist's the "law of large numbers" – the idea that organisms are closely related because they share "lots" of their genome. However, as Jonathan Marks points out, humans share

about 25% of their genome with bananas. Essentially, there is nothing in an observation of genomic difference or similarity that directly translates into the "molecular assumption" and, consequently, a theory of evolutionary relatedness.

Can, then, molecular information be useful in systematics and phylogenetic reconstruction?

The answer is yes, but it will have to be at the level of cell biology and pathways of molecular communication. As King and Wilson came close to predicting many decades ago, it is not through the study of molecular or genomic similarity of organisms that we will come to understand their biology, but through the investigation of those elements that underlie the development of their biology. This makes sense. For, if something as simple as the inactivation or deletion of a transcriptional enhancer can result in a more caudal repositioning of the sacrum, or if the expansion of a morphogenetic gradient can transform in its entirety a narrow pelvic girdle with tall, thin ilial blades into a broad, deep, and squat structure, then it is by seeking to identify the similarity or difference in these molecular events that we may more profitably explore the molecular basis of morphology and, consequently, the evolutionary relationships of complex organisms.

This is, perhaps, a timely occasion to both question and expand our perceptions of what is or will be evolutionarily revealing at the molecular level. There has been a steady increase in the number of studies that demonstrate virtual molecular identity between taxa that are morphologically very different and then express amazement at this apparent contradiction. As Sean Carroll pointed out with regard to the importance placed on the human and chimpanzee-genome projects – especially since so much money has been poured into them in the hope that forthcoming comparisons will instantaneously provide answers to any questions – demonstrating molecular similarity does not translate into deciphering the pathways that make these organisms so different in hard- and soft-tissue anatomy, physiology, reproductive biology, cognitive abilities, and behavior. Here, the "law of large numbers" fails to be enlightening. For, in contrast to the bacterial world, in the metazoan world, a one-to-one correspondence between a "gene" (a sequence of nucleotides bound by start and stop codons) and a "gene product" (a protein or amino acid sequence) is not there. In multicellular animals, RNA essentially directs the "show," for example, in reading select bases and splicing specific introns, as it composes different proteins from the same stretches of DNA. The surprise "The International Chimpanzee Chromosome 22 Consortium" had at finding upon comparing human chromosome 21 with its apparent orthologue in the chimpanzee, chromosome 22 – not only that these hominoids differ by 83% in their amino acid sequences but also that this large difference is produced from very similar DNA sequences – should serve as a lesson: While there may be appeal to the "law of large numbers" that comparison of chromosomes and especially of entire genomes purportedly represents, in the end, this molecular level may not be the evolutionarily informative hotspot everyone has been seeking.

See also Gene Mapping and Chromosome Evolution by Fluorescence–Activated Chromosome Sorting; Genetic Intelligence, Evolution of; Genetic Variation and Molecular Evolution; Immunoassays.

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