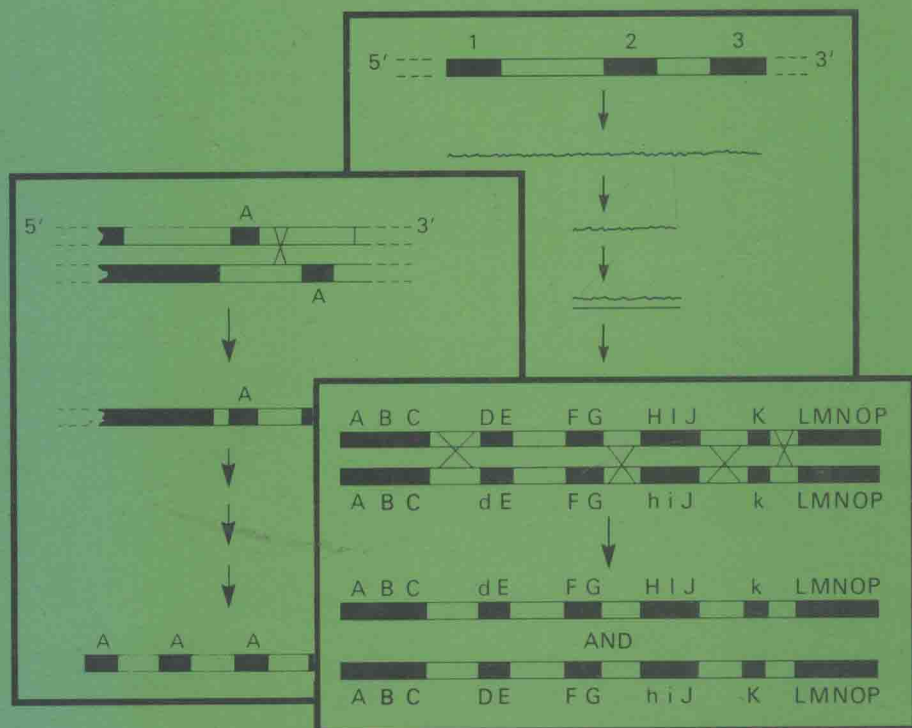


**MOLECULAR GENETICS
AND
COMPARATIVE EVOLUTION**

J. LANGRIDGE



Molecular Genetics and Comparative Evolution

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PREFACE

An understanding of evolution, by which is meant the gradual development of complex organisms from their primeval beginnings, has been gradually advancing only from the early nineteenth century. Before that time, the doctrine of special creation, based on the first two chapters of the book of Genesis, held that the world and all the organisms in it were created by God in 4305 BC. Not until 1801 when Lamarck proposed an inheritance of acquired characters was 'descent with modification' commonly accepted. But the full mechanism remained unknown even though Darwin and Wallace provided evidence in 1858 that evolution occurs by the natural selection of inherited variations. In the modern theory of neo-Darwinism, natural selection is combined with Mendelian genetics with emphasis on the random nature of mutation and the importance of isolating mechanisms. However, the theory is still incomplete in that it does not account for the frequently episodic nature of phylogenetic change and the prolonged evolutionary stasis of many groups, and in that it is unable to predict future evolutionary trends.

In this book we will attempt to contribute to a more complete theory by combining molecular, genetical and evolutionary data and reconsidering the evolutionary significance of genetic variation. Accordingly, this book stresses, not unity of process, but evolutionary differences as expressed in the various living phyla and classes. If we examine the variety of mechanisms generating change in organisms, their distinctive levels of complexity and integration, the adaptive nature of their existences and other matters related to the ease and direction of evolution, certain deviations from accepted evolutionary principles become apparent. They seem to show that, depending on the group under examination, evolution may involve different mechanisms of genetic change, take a variety of courses and reach quite diverse end-points.

These conclusions also take into account molecular data on the origins and relationships of genes. However, the molecular information that bears on the mechanisms of evolutionary change is still small in amount and tentative in significance. I hope that the book will be capable of withstanding the expansion or modification, and even the removal of pieces of evidence shown to be false, that future research will undoubtedly necessitate. So, in the writing, I have tried to keep in mind the caveat of the protozoologist Minchin (1912): 'The views put forward by any man but represent the visions conjured up by his imagination, more or less limited, or intuition, more or less fallacious, of an infinite world of natural phenomena'.

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INTRODUCTION

Strictly speaking, the secure data identifying the fact and course of evolution come only from fossil remains of organisms from the remote past. In this sense, its data base is like that of stratigraphical geology, although not as complete. And all that the fossil record tells us is what has happened; it says nothing about how it happened. So the reason for evolutionary change must be derived from other data which are quite independent of its physical evidence. These data are also, of necessity, observational, for the extreme slowness of evolution means that it cannot be experimentally produced. Thus it has been observed that there is a continual generation of genetic change by processes assumed to be much the same in all organisms. A second observation is the decisive role of natural selection, which is assumed to be all-pervasive. The dynamic component of modern evolutionary theory is then provided by the principles of population genetics as applied to these processes of mutation and selection.

From the above premises, a coherent theory of the evolutionary process is expressed as a set of governing principles. These are: that spontaneous gene changes (mutations) provide genetic variations in gene expression (phenotype) which are then subjected to positive or negative selection by factors of the environment - genetic changes incorporated in the population are thus usually adaptive ones that enhance the survival or reproduction of organisms; further, that evolutionary change is most often by the gradual accumulation of successive point mutations, due to one or a few alterations in the nucleotides of the genetic material, that result in the appearance of a new or modified structure or function. With a gradualistic course of evolution thus envisaged, the transformation from one adaptive category, usually a population of interbreeding organisms, to another represented by a species, is an unbroken process with further changes of precisely the same kind leading in turn to higher taxonomic units.

The species is taken to be the basic evolutionary unit and evolutionary change from one taxonomic category or taxon to another progresses in the direction: species, genus, family and order.

These principles have been deduced, for the most part, from the study of evolutionary phenomena in the animal kingdom, which has been justified on the grounds that only the vertebrates and the shelled invertebrates leave reliable fossil evidence of the course and direction of evolutionary change. The other four kingdoms of organisms, the prokaryotes, protista, fungi and plants, have been little considered except where their evolution fits the animal pattern. The few treatises on bacterial or plant evolution owe much of their interpretation to the extrapolation of principles derived from the animals.

We may assume that all forms of life started from a single origin about 4000 million years ago and thereafter they became diversified by their differential acquisition of novel forms of organisation. These new organisational features include organelles, multicellularity, cellular differentiation, morphogenesis and nervous systems. Only the more advanced animals have acquired all these new features, which make the nature and extent of their evolution different from that in other organisms. Evolution in animals is mostly based on changes in developmental processes which are much simpler in fungi and plants and practically absent in prokaryotes. These simpler organisms lack the constraints to changes in gene expression that are imposed by the sequential and interactive nature of the development of animal embryos. A further difference is that some adaptations in higher vertebrates may not have genetic causes but be reflections of learning activities which, in turn, are affected by experience. Indeed, it is possible that, in many higher animals, genetic change gives only a rough fit to the environment, with close fitting being provided by behavioural modification.

A genetic advance that has affected the neo-Darwinian view is the use of molecular data to provide a new means of establishing evolutionary mechanisms and relationships independent of fossil evidence. The mechanisms responsible for genetic diversity are various and probably differently distributed in diverse groups. For example, it now appears, at least in some groups, that evolutionary novelty is generated by different types of recombinational variation and not by point mutation.

The above considerations, among others, suggest that it may be informative to take a comparative approach by subdividing evolution according to whether it is rapid, slow or static in a particular group, what the focus of change may be and what mechanisms are involved. Little that is useful can be added to other current evolutionary controversies about continuity or periodicity, adaptation or drift and the role of sexuality, so they will not be topics of this book.

In the first part of the book we consider the various phases through which evolution has gone. Most organisms comprise a number of distinct features such as metabolism, subcellular structure, genome organisation, development and nervous system, so evolution may be subdivided into types depending on which feature undergoes adaptive changes. All features, of

course, will be subject to mutational alteration, but changes in some of them have little phenotypic expression, in others they will be mostly deleterious, and only in some will viable changes be selected in the appropriate environment.

Certain of these features, like metabolic pathways and intracellular structure, now undergo few changes that are likely to be adaptive. Their early evolution has provided a satisfactory basis for all forms of life but they are so basic to the organism that they must now remain static or fixed within narrow tolerances. Others, however, like development and, in animals, behaviour, may still vary in significant ways, especially through changes in hormonal activity in development, and learning processes in behaviour. Whereas the basic frameworks of these features may alter very infrequently, modifications of their expression are possible by genetic alterations in regulation.

A survey of types of evolution indicates that, in all but the prokaryotes and the simplest eukaryotes, the main means of evolving since the Proterozoic eon is by developmental change. Behavioural change may also have a role in the evolution of higher vertebrates, but there is little evidence for it as yet.

The second part of the book examines genetic changes with time: over a few tens of years (toxin resistance), several thousand years (selective breeding), several million years (differentiation of lower taxa) and many million years (formation of orders and classes).

Genetic resistance to toxins is usually a matter of metabolic adjustment, involving a limited number of minor changes because major changes are lethal. Nevertheless, many of the adjustments are large enough to be deleterious in the absence of the toxin, and represent compromises between death from toxicity and debilitation from the alteration of essential metabolism. Some groups like bacteria and insects readily become resistant to toxins because they can acquire (bacteria) or have partly evolved (insects) genetic systems for detoxification.

Evidence suggests that the practice of artificial breeding for economic purposes results in developmental changes mostly brought about by the selection of alterations in the hormonal control systems of animals and possibly of plants. It may be that the formation of species and genera also occurs in part by hormonal changes, but their origin has not been examined from that point of view.

The genetic processes concerned with the intermediate-term evolution of species and genera have been shown repeatedly to involve changes in gene regulation. The altered trans-acting factors implicated in plant and animal breeding are overshadowed in frequency and effect by the cis-located alterations of species differentiation (the terms 'cis' and 'trans' are explained in Section 2.2.8). Some cause morphological changes by altering the regulation of genes responsible for development, while others affect the developmental regulation of genes unconcerned with development itself. Apparently, alterations are usually in short flanking sequences of a gene which are sites for the binding of trans-acting factors in gene regulation. As a consequence, gene expression may be shifted with respect to

developmental stage, tissue or organ, or to environmental signals.

Marked differences exist in the evolution of prokaryotes, plants and animals at higher taxonomic levels. Since the prokaryotes have so little morphological development, they only serve to emphasise the paucity of evolution in its absence. The few aspects of spore development that some possess occur by a modification of the mechanism for metabolic adjustment to adverse environments; namely, an alteration in the transcriptional specificity of RNA polymerase. The plants have a simple form of development linked to environmental signals. They are characterised by a facility for morphological change that results in numerous species and genera, a low occurrence of pleiotropy and a high incidence of parallelism and convergence. There is no evidence that they have mechanisms for the evolution of orders and classes that are additional to those involved in plant breeding and in the formation of subordinate taxa. In the animals, in contrast, marked evolutionary changes appear frequently to be due to alterations in the morphogenic movement of cells, in cell adhesiveness and in induction-reaction processes during ontogeny.

The different means of generating genetic change are discussed in Part 3. There appear to be three broad classes: point mutations of one or a few nucleotides; alterations in the structure or number of chromosomes; and reshufflings of gene parts. The last class, which we can call loosely 'recombinational', occurs by processes of segment transfer, gene conversion, reverse transcription and transposition. As far as we can see at present, these three sorts of genetic variation may have had different roles in evolution: chromosomal changes seem to be of negligible importance because they rarely have advantageous phenotypic effects; point mutations alter genes slightly to make minor adaptive adjustments; and the formation of new or much altered genes probably depends on recombinational processes.

Part 4 of the book considers the evidence showing that the evolution of a group does not usually continue indefinitely. Each major group of organisms underwent an evolutionary radiation of novel characters sometime in the past, but many remained relatively static thereafter. The fossil record shows that stasis in innovative evolution usually means the termination of evolution in that group. Some static groups like the amphibians are declining, although environmental opportunities for novel evolution appear to continue. Others like the insects are very successful because they are in equilibrium with their environments and small ecotypic adjustments are sufficient to keep them there. However, nearly all groups are capable of producing species and genera by random events, which may be an inevitable consequence of a large population size.

The differences in evolutionary attainment in the various groups seems to be caused in part by a particular group adopting one adaptive strategy rather than another. When the potentialities of the chosen strategy have been exhausted, the group may have gone too far in one direction to shift to the strategy previously passed by. So the bacteria, by not acquiring or evolving mitochondria for efficient energetic metabolism, remain unicellular and microscopic; the fungi, by relying on absorption rather

than photosynthesis, can exist only as parasites or saprophytes; and the plants, by retaining their cellulose walls, have shut themselves off from higher developmental and nervous systems.

The differences between groups in evolutionary potential may also be due in part to their having somewhat different mechanisms for the generation of genetic change. Major phenotypic characters, in particular, probably require the formation of one or more new genes by the assembly of parts of previously evolved genes. This is a process that seems to depend on the presence in the genes of nonprotein coding regions, or intervening sequences, of appropriate number, size and translational phase (see Section 4.5.6.5). Such intervening sequences are, however, absent in prokaryotes and most unicellular eukaryotes, they are much reduced in size in invertebrates and fungi, and in irregular translational phases in many of the genes unique to flowering plants. The unique genes of mammals, on the other hand, typically have more and larger intervening sequences of fairly uniform phase than do the genes common to vertebrates and other organisms.

The comparative evolutionary approach thus suggests that the focus of evolution has changed broadly from metabolism to development to nervous system. As the type of evolution has altered, so also has the mechanism, probably from mutational to recombinational. The development of a particular structure or function frequently blocks future innovative evolution, causing a decline in the evolution of many groups since the Cambrian.

PART 1

Succeeding Phases of Evolution

For nearly four thousand million years, evolution has been at work fashioning every aspect of an organism from its chemical composition to its nervous basis of behaviour. As we might expect, there have been successive phases during the history of life where evolution has concentrated on one aspect more than another. In part this has been due to the necessity of developing such attributes of life as metabolism as rapidly as possible in early stages, and in part it is because one feature of an organism must be evolved first to provide the basis for the production of another feature such as development. Consequently, the overall course of evolution may be divided into a number of different types or stages according to the property of the organisms acted upon, each with its individual mechanism and significance. Thus evolution may be biochemical (metabolic), macromolecular, subcellular, chromosomal, genomic, developmental or behavioural, some of which may be more important in certain episodes of evolution and in some groups of organisms than in others. Moreover, many of these evolutionary activities were essentially completed in the Proterozoic, allowing the nature and rate of the evolutionary process to alter markedly in the succeeding Phanerozoic eon (see Appendix 2 for the geological time scale). The major types of evolution are considered in the following pages, with the conclusion that the evolutionary focus since well before the Proterozoic eon ended has been mainly on matters of development, and perhaps latterly, in animals, of behaviour.

1.1 *Metabolic Evolution*

Although very important in the early establishment of life, metabolic evolution now appears to be of much less consequence. The basic genetic and enzymatic framework of energetic, synthetic and degradative reactions must have been established very early and has persisted with only minor change since its origin.

Much of this early evolution must have concerned the basic problem of

energy supply. The evolution of an energy-generating system is essentially the development of a means of synthesising adenosine triphosphate or perhaps similar phosphorus compounds, so that synthetic and other activities requiring free energy can occur.

In their evolution during the Proterozoic, the prokaryotes developed all the main types of energy-yielding metabolism: fermentation, where organic compounds are both the donors and acceptors of electrons; anaerobic respiration, where the ultimate electron acceptor is an oxidised inorganic compound; aerobic respiration, where the ultimate electron acceptor is molecular oxygen; and photosynthesis, where light is the source of energy (Figure 1).

The initial course of biological evolution is thought to have been largely determined by metabolic inventions especially as regards the availability of oxygen. The first organisms to produce oxygen in their metabolism were the cyanobacteria which succeeded various forms of anaerobic heterotrophs, photoautotrophs and perhaps methanogens. This oxygen may first have been absorbed in the oxidation of inorganic compounds, but later it accumulated in the atmosphere. At an oxygen concentration near 1% of the present level, which may have been reached 1300 million years ago (Cloud, 1978), the evolution of efficient respiration became possible. The oxygen of respiration has probably been responsible for the extinction of many anaerobic organisms, for the dominance and variety of aerobic bacteria and eventually for the evolution of the eukaryotic type of cell. However, according to Morowitz (1971), the energy levels per unit mass of living material, whether bacterial cells or mammalian tissue cells, are constant at about five kilocalories per mole higher than the same material in the nonliving state. This implies that an increase in energetic complexity has not accompanied evolutionary advance.

The first known metazoa are found in the Ediacarian sediments with an age of nearly 700 million years. The thin flattened shapes of the Ediacarian worms are believed to have been necessitated by the short diffusion paths possible with low oxygen tensions (Raff and Raff, 1970). The lack of hard parts (shells, cuticles or carapaces) in these early faunas was perhaps because the formation of these structures requires collagen, and collagen synthesis needs molecular oxygen (Towe, 1970).

The other main aspect of metabolism is the synthesis of compounds necessary for the living cell. Although biosynthetic pathways are practically identical in all organisms in which they occur, they appear to have had several evolutionary origins. The little evidence available suggests that some of the more ancient syntheses have followed prebiological pathways by evolving the intermediates and the genes and enzymes responsible, backward from the original metabolite (e.g. the genes for aspartate and homoserine kinases in threonine biosynthesis; Truffa-Bachi et al., 1975). Some catabolic reactions which yield energy from intermediate stages may have evolved stepwise from the initial substrate to the final product (e.g. carbohydrate breakdown by fermentation; Degani

<u>Energy Sequence</u>		<u>Electron Donors</u>	<u>Electron Acceptors</u>
abiological energy sources	spontaneously formed organic phosphates ↓	-	-
anaerobic respiration	substrate level phosphorylation ↓	free hydrogen or carbohydrates	oxidised elements or inorganic compounds later replaced by pyridines and still later by pyridine nucleotide (NAD)
origin of electron transport	'oxidative' phosphorylation ↓	reduced organic and inorganic compounds	electron transfer from NAD to flavoprotein and then on to porphyrins and eventually to oxidised inorganic compounds
evolution of chlorophyll molecules from porphyrins of anaerobic respiration	photoassimilation ↓	partly reduced inorganic compounds	quinone and cytochrome
production of oxygen	photosynthesis ↓	hydroxyl ions from the photolysis of water	additional electron carrier ferredoxin evolved to receive electrons from irradiated chlorophyll
complete oxidation of organic substances to CO ₂	aerobic respiration	organic and reduced compounds	electrons now transferable to oxygen

Figure 1 A possible sequence for the evolution of energy metabolism.

and Halmann, 1967). A third form of evolution, for pathways where the intermediate steps are without selective value, may have involved the duplication of genes with other functions, and their mutational alteration, to give enzymes of altered specificity catalysing a new metabolic sequence (e.g. the catabolism of certain aromatic compounds by oxidases which do not produce ATP for energy (Ornston and Stanier, 1966; Figure 2).

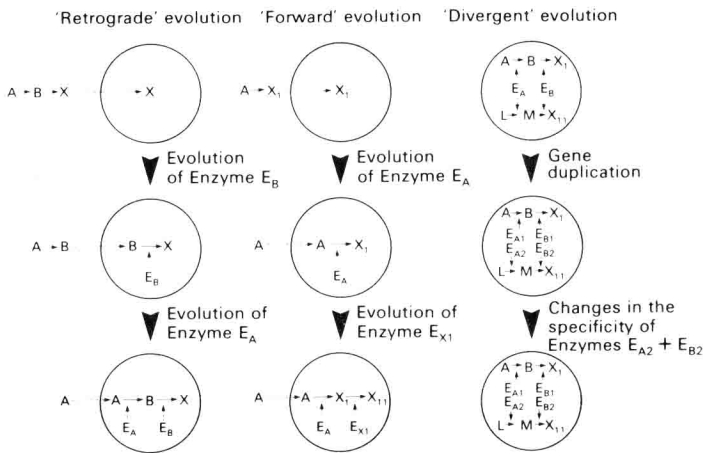


Figure 2 Patterns for the evolution of metabolic pathways. The circles represent protocells. Spontaneous reactions occur outside the protocells and dashed lines show the diffusion of extracellular substances into the protocells. X , X_1 , X_{11} are metabolites required by the protocell; E_A , E_B etc. are enzymes evolved by the protocells.

Baldwin (1949) examined animal metabolism in relation to environment and concluded that 'there exists a common fundamental chemical ground-plan of composition and metabolism to which all animals, and very probably other living organisms also, conform'. In this connection, Britten and Davidson (1971) have pointed out that lower and higher organisms appear to have perhaps 90% of their enzymes in common. Also Medawar (1967) has remarked that he knows of 'no new kind of chemical compound that has come into being over a period of evolution that began long before animals became differentiated from plants. Nor has there been any increase in chemical complexity; no chemically definable substance in any higher organism, for example, is more complex than a bacterial endotoxin'. Apparently a common

biochemical heritage was established very early in the evolution of life. Since then, further biochemical evolution has mainly been concerned with variations in anabolism, as in the distinctive metabolic processes of blood flagellates, and in catabolism such as adaptive changes in the breakdown of nitrogenous waste compounds. Plants are exceptional in this respect for they synthesise a variety of secondary organic compounds usually from sugars, organic acids and amino acids; over 80% of all known organic compounds of natural origin have been isolated from plants (Swain, 1974). There is one aspect of biochemical evolution that may still be occurring frequently: modification of enzyme control systems, as shown by the great variety of regulatory controls in organisms that have an otherwise stereotyped metabolism. This is further discussed below (Section 2.3.1).

1.2 *Macromolecular Evolution*

Although evolution by the mutation and selection of nucleic acids and proteins for functional efficiency is very ancient, macromolecular sequences are still highly variable. Ayala (1984) has calculated, taking into account data from sequential electrophoresis, heat denaturation and peptide mapping, that the protein-coding genes of flowering plants are on average 27% heterozygous, those of invertebrates 22%, and those of vertebrates 28%; because of codon redundancy (see Section 3.1.2), the nucleotide variation will be even greater. There appears, however, to be no correlation between either reproductive isolation or rates of morphological evolution and degree or rate of change in protein sequence (Langridge, 1987). This conclusion accords with the proposition that the degree of amino acid difference is mainly a function of the time that the organisms containing the coding genes have been separated.

The proposition that amino acid substitutions can occur in a metronomic or regular manner has been called the 'molecular clock' hypothesis. It implies that a fraction of DNA nucleotide substitutions, including most third-base replacements in coding triplets and some of those altering protein sequence, is neutral or nearly so with respect to natural selection. The most direct test of this proposal would be to show that some at least of the alleles producing protein variants in a population are indifferent to selection, but for technical reasons this has proved difficult. A consequence of the proposition is that, following the origin of one group from another, their common genes and proteins should become progressively more different as time elapses. This prediction is supported by the finding that the degree of change in amino acid sequence in homologous proteins is approximately proportional to the duration, based on fossil evidence, that various mammalian species have been separated (Fitch and Langley, 1976; Figure 3). The existence of a molecular clock in the genome that acts to produce time-dependent divergence in protein molecules is reasonably well established. However, it is a clock that runs at different speeds for different proteins, and it can fluctuate in rate over long evolutionary periods.