Evolving Insights into the Laws of Nature for Biological Evolution

Werner Arber

It was shortly after the middle of the 19th century that scientific investigations in the disciplines of evolutionary biology, of genetics and of nucleic acids biochemistry took their start, largely based on the observation of phenotypic traits of plants and animals. But for a long time it remained unknown how such traits could be determined and transmitted from generation to generation.

Microbial genetics starting around 1940 were then able to reveal that phenotypic traits of single-cellular bacteria are determined by deoxyribonucleic acid (DNA), but not by other kinds of biological macromolecules (1). Shortly thereafter the double-helical structure of DNA filaments became known (2) and it was suggested that genetic information is stored in the specific linear sequences of nucleotides of the DNA (3). It was also proposed that phenotypic variants might depend on occasionally occurring alterations in the nucleotide sequences of the chromosomes composing the genome.

It was already at the 1953 Cold Spring Harbor Symposium on Quantitative Biology that Watson and Crick pointed out that short-living isomeric forms of nucleotides can give rise upon DNA replication to an alternative base pairing and thus to a nucleotide substitution in the genetic message (4). This should not be interpreted as an error. From today's point of view, we rather conclude that Nature uses the phenomenon of isomeric conformational shifts to occasionally give rise to one specific kind of genetic variants. In the meantime, it has become known that Nature uses a remarkable number of different molecular mechanisms to produce alterations in the genomic nucleotide sequences at quite low rates (5).

Experimental results show that, by far, not all spontaneously occurring DNA sequence alterations are favorable and provide to the organisms a selective advantage. More often nucleotide sequence changes are unfavorable and provide a selective disadvantage, whereas many other nucleotide changes are without immediate influence on life processes and remain silent/neutral. In the light of this situation we cannot see any evidence that new spontaneously occurring mutations would be precisely directed in response to an identified need.

Let us now direct our attention to Figure 1, which outlines in the upper part the effects of the three pillars of biological evolution and in the lower left part the specific molecular mechanisms known to contribute to genetic variation (5). Without occasional genetic variation, populations of organisms of a given species would not undergo biological evolution at their population level. We can conclude that genetic variation is the driving force of biological evolution. Natural selection depends on the physico-chemical composition of a given habitat and on the presence of different kinds of organisms in a particular ecosystem. It is natural selec-

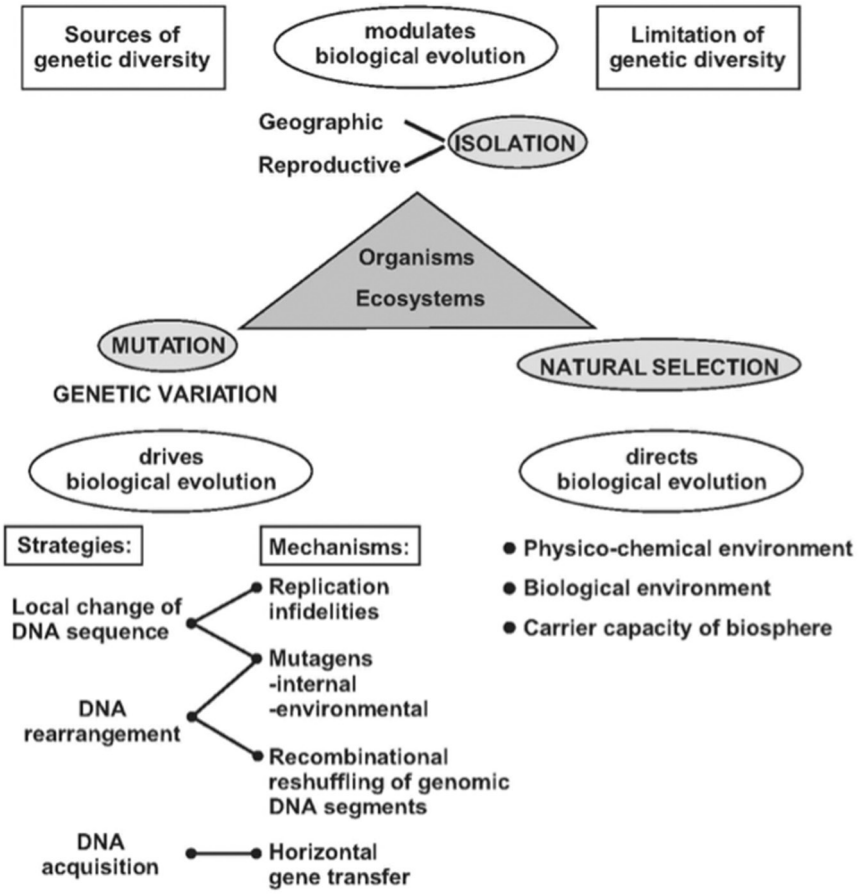

Figure 1. Genetic variation and molecular evolution (from Ref. 5).

tion, together with available genetic variants, that determines the directions taken by biological evolution. Finally, both geographic and reproductive isolations modulate biological evolution. Natural reality renders the pattern of biological evolution somewhat more complex, since a number of different kinds of organisms normally inhabit any given ecosystem and can influence each other. Striking examples are the microbiomes, microbial populations routinely found on and in plants and animals (6).

In its lower left part, Fig. 1 lists groups of known specific mechanisms of genetic variation as replication infidelities, as caused by chemical and physical mutagens, as intragenomic reshuffling of DNA segments and finally, as horizontal transfer of genetic information from one kind of living organism to another (7). Up to now, some of these mechanisms have already been well characterized, in particular for a few microorganisms.

One of the conclusions to be drawn from the insights obtained into spontaneous genetic variation is that Mother Nature follows three different natural strategies to produce genetic variants:

(a) To produce a local change in the parental nucleotide sequence. This can be a nucleotide substitution, as already discussed above, or the deletion of one or a few adjacent nucleotides, the additional insertion of one or a few adjacent nucleotides or the mingling of a few nucleotides.

(b) The second natural strategy of spontaneous genetic variation brings about an intragenomic rearrangement involving a segment of DNA. This can be an amplification of genetic information, an inversion of a DNA segment, a segment-wise deletion or the translocation of a DNA segment known as mobile genetic element.

(c) Mobile genetic elements, as well as other factors, are also known to occasionally cause the horizontal transfer of a segment of genetic information from one kind of organism to another one. This latter natural strategy of genetic variation is here called DNA acquisition.

Each of the three natural strategies of genetic variation contributes with a different quality to the progress of biological evolution. (a) The local DNA sequence change can occasionally bring about a step-wise improvement of an available biological function. This natural strategy is the basis for the "molecular clock", an indication of the evolutionary distance between different kinds of organisms. (b) Intragenomic DNA rearrangements generally depend on specific kinds of enzymes, which can bring about novel combinations of available genetic capacities, such as the occa-

sional fusion between two different functional domains or the provision of an alternative expression control element to an open reading frame of a functional gene. (c) The DNA acquisition strategy is a sharing in successful developments made by others. This is a very efficient strategy and it can, by chance, provide a particular capacity to the receiving organism in just one step of evolutionary progress. Both DNA acquisition and intragenomic DNA rearrangements can explain a sudden emergence of a novel property of an organism.

In an overall view we can conclude that natural reality actively takes care of biological evolution by self-organization. In this long-term process, gene products acting as variation generators and/or as modulators of the rate of genetic variation are actively involved. We call the underlying genetic determinants *evolution genes* (7). Their products act in close cooperation with a number of non-genetic elements, such as structural, conformational flexibility and limited stability of biologically active molecules, environmental mutagens or random encounter (e.g. of a transducing bacteriophage with an alternative host bacterium). Great care is thereby generally taken by Mother Nature to highly respect the genetic stability of most individuals in a population, and this goal can be reached by a number of mechanistically different approaches.

In this context, it may be helpful to remember that biological evolution progresses extremely slowly, and so does cosmic evolution, including the evolution of habitats on our planet Earth. This contrasts with the remarkable speed and high efficiency of specific biological interactions, as it is seen, for example, in the interaction of a purified enzyme with its specific substrate serving to manufacture a specific reaction product. It is for this principal timing difference that we, human beings, can only with difficulty become aware of evolution and its steady but very slow progress.

As far as we know, evolution genes seem to be present in all kinds of living beings, microorganisms, plants, animals and humans. They have resided in genomes possibly since the early times of biological evolution, as have housekeeping genes. We come therefore to the novel insight of a principal duality of the genome (8). It is well known that many genes in the genome work for the immediate benefit of the individual during its lifespan; they work for the fulfillment of the life of each individual. In contrast, the products of evolution genes serve for the benefit of the evolutionary development of populations and therefore for the expansion of life and biodiversity. In other words, we can conclude that Creation is, although quite slow, a permanent process.

As we have already discussed, a number of different specific molecular mechanisms have become known to contribute to each of the three natural strategies of genetic variation. So far, we still only have fairly poor insights into the relative rates of contributions to overall mutagenesis by each available specific variation mechanism.

Microbial genetics offers an interesting opportunity to investigate the sources of the prevailing mechanisms of spontaneous genetic variation causing lethal mutagenesis. Lysogenic bacteria maintain and propagate the genome of bacteriophages. In some lysogens the phage genome is integrated into the bacterial genome. In other lysogens the phage genome is maintained as an autonomously replicating plasmid. This is the case for *E. coli* bacteria lysogenic for bacteriophage P1. In an experimental study P1-lysogens were propagated in the laboratory for three months by considerable dilution every morning, followed by exponential growth during the day, until saturation. With the culture obtained after three months of serial growth, individual bacterial colonies were obtained after spreading appropriate dilutions on Petri-plates. By UV irradiation phage production was then induced and revealed by using appropriate replica plating methodology capable of rendering the presence of infective P1 phage particles visible. In this experiment only relatively few of the colonies tested did not reveal the presence of infective phage. But in all colonies studied the presence of the P1 plasmid in the non-induced bacteria was verified. By DNA extraction and appropriate hybridization methods it was then shown that in 95% of the analyzed independent lethal phage mutants a transposable insertion sequence (IS) element from the host genome had become translocated into the P1 plasmid genome. Only 5% of the lethal P1 mutants studied were identified as local sequence alterations. We can conclude that intracellular IS transposition is a major source of lethal phage mutagenesis in this laboratory experiment growing the P1-lysogenic culture for many generations. Whereas the phage P1 genome is quite densely packed with essential genes for the production of infective P1 particles, the sites of insertion of individual bacterial IS elements showed a drastically non-random distribution. The most often encountered IS2 element obviously preferred to insert into a 1,500 bp long sequence of the 90,000 bp long P1 genome (9). But within this region insertion was largely random with regard to both the actual site of insertion and its narrow nucleotide sequence (10). In contrast, IS30 was found only three times and in each case its insertion had occurred precisely into the same nucleotide sequence located also in the 1,500 bp stretch of the P1 genome, twice in one direction and once

in the opposite direction. These results are consistent with previous reports on insertion selection criteria for different bacterial IS elements.

In contrast to the laboratory experience reported here, microbial life in natural ecosystems occurs mostly in mixed populations containing many different kinds of living organisms. Under these latter living conditions, horizontal gene transfer by DNA transformation, by conjugation and by bacteriophage mediated transduction can also occur in addition to intragenomic genetic variation. Thereby, various different factors and criteria seriously limit the chance of success of acquisition of foreign genetic information. In brief, limiting factors are cellular surface compatibilities, restriction-modification systems enabling recipient cells to distinguish invasive foreign DNA from the cell's own DNA and, last but not least, functional compatibilities for propagation of horizontally transferred stretches of DNA and functional harmony upon the expression of acquired genetic information. In view of all these limitations to horizontal gene transfer we can conclude that the natural strategy of genetic variation by DNA acquisition is normally most successful if it occurs in small steps. But the common language of different kinds of living organisms, i.e. the universality of the genetic code, highly favors the potential use of any genetic information acquired by horizontal gene transfer (11).

Whereas horizontal gene transfer has so far mostly been studied with microorganisms, in particular with bacteria and their bacteriophages, this phenomenon had been ignored for a long time in regard to eukaryotic genetics. But novel research methodologies, such as nucleotide sequence comparisons between different kinds of organisms, have recently provided good evidence that occasional horizontal gene transfer may play a general role in the evolutionary progress of all kinds of living organisms.

Already Charles Darwin had drawn a tree of evolution symbolically showing that today's living organisms have a common origin. Neither Darwin nor today's scientists are able to know whether life on our planet Earth has only one or many independent origins. In view of the DNA acquisition strategy of genetic variation we can now draw randomly placed horizontal connectors (12) between branches of the tree of evolution (Fig. 2). Each of these connectors serves once for the horizontal transfer of a small genomic segment from a donor organism to a recipient one, whereas entire genomes undergo vertical flux from generation to generation. A lesson to be learned from these insights is that today's living organisms not only have a common origin but also a common future. This insight into one of the fundamental laws of nature must serve us, human beings, to se-

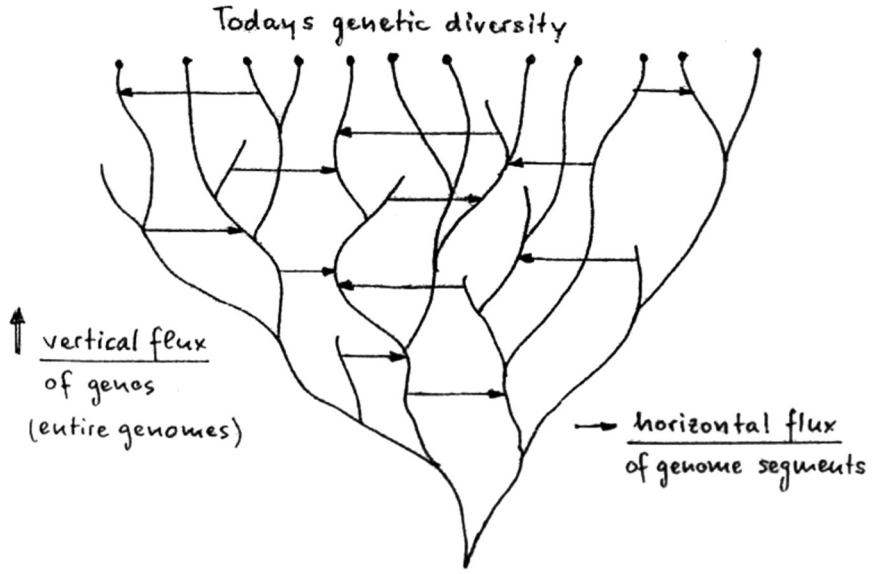

Figure 2. Updated tree of evolution with branches for vertical flux of genomes and connectors between branches symbolizing occasional horizontal flux of a small segment of genetic information by horizontal gene transfer (from Ref. 11).

riously take care not to destroy the still remarkably rich biodiversity with its great number of different functional genes and natural habitats. We are on the way to better understanding the systemic aspects of the evolving living world in its slowly evolving habitats (13). It is due to our increasing scientific knowledge on life and its specific functions that we reach good insights into the basic laws of Nature for life processes, including evolutionary progress. A general conclusion from the accumulated knowledge presented here is that Mother Nature is remarkably inventive and succeeds to reach particular purposes often by a number of different specific approaches. We realize that evolution of forms of life and of their habitats is an ongoing process by self-organization that we can call *permanent creation*.

References

1. Avery, O.T., Mac Leod, C.M. and Mc-Carty, M. (1944). Studies on the chemical nature of the substance inducing transformation of Pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from Pneumococcus type III. *J. Exp. Med.*, 79, 137-158.
2. Watson, J.D. and Crick, F.H.C. (1953). Molecular structure of nucleic acids. A

structure for deoxyribose nucleic acid. *Nature*, 171, 737-738.
3. Watson, J.D. and Crick, F.H.C (1953). Genetical implications of the structure of deoxyribonucleic acid. *Nature*, 171, 964-967.
4. Watson, J.D. and Crick, F.H.C. (1953). The structure of DNA. *Cold Spring Harbor Symp. Quant. Biol.*, XVIII, 123-131.
5. Arber, W. (2007) Genetic variation and molecular evolution. In: Meyers, R.A. (ed.). *Genomics and Genetics*, Wiley-VCH, Weinheim, Vol. 1, 385-406.
6. Blaser, M., Bork, P., Fraser, C., Knight, R. and Wang, J. (2013). The microbiome explored: recent insights and future challenges. *Nature Rev. Microbiol.*, 11, 213-217.
7. Arber, W. (2000). Genetic variation: molecular mechanisms and impact on microbial evolution. *FEMS Microbiol. Rev.*, 24, 1-7.
8. Arber, W. (2005). Dual nature of the genome: Genes for the individual life and genes for the evolutionary progress of the population. *IUBMB Life*, 57, 263-266.
9. Sengstag, C. and Arber, W. (1983). IS2 insertion is a major cause of spontaneous mutagenesis of the bacteriophage P1: non-random distribution of target sites. *EMBO J.*, 2, 67-71.
10. Sengstag, C., Shepherd, J.C.W. and Arber, W. (1983). The sequence of the bacteriophage P1 genome region serving as hot target for IS2 insertion. *EMBO J.*, 2, 1777-1781.
11. Arber, W. (2006). The evolutionary strategy of DNA acquisition as a possible reason for a universal genetic code. *Hist. Phil. Life Sci.*, 28, 525-532.
12. Arber, W. (1991). Elements in microbial evolution. *J. Mol. Evol.*, 33, 4-12.
13. Arber, W. (2009). Systemic aspects of biological evolution. *J. Biotech.*, 144, 242-244.