Evolution of prokaryotic genomes*

(Spontaneous mutagenesis; replication infidelity; transposons; IS; DNA rearrangements; gene acquisition; selection; molecular evolution; variation generator; biodiversity)

Werner Arber

Department of Microbiology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Received by G. Bernardi: 7 June 1993; Accepted: 11 June 1993; Received at publishers: 2 August 1993

SUMMARY

Molecular genetics, which has its roots mainly in the development of microbial genetics in the middle of this century, not only greatly facilitates investigations of essential cellular functions, but also offers a means to better understand evolutionary progress. Spontaneous mutagenesis, the driving force of biological evolution, depends on a multitude of mechanistically distinct processes, many of which are already quite well understood. Often, enzymes act as variation generators, and natural gene vectors help to spread functional domains, entire genes and groups of genes across natural isolation barriers. In this overview, particular attention is given to comparing three selected natural strategies for the generation of genetic diversity: nucleotide substitution, DNA rearrangements, and gene acquisition. All of these mechanisms, as well as many others, appear to fulfill their specific roles in microbial evolution. Rather than being the result of an accumulation of errors, biological evolution may depend on a multitude of specific biological functions, as well as on a certain degree of intrinsic structural flexibility of biological molecules.

INTRODUCTION

It was in the year of publication of the DNA double helix model by Watson and Crick (1953a,b) that I entered research in microbial genetics as a postgraduate student at the University of Geneva. As one of my first tasks I was assigned to prepare a journal club seminar on the Nature papers on the double helix model. Together with the 40th anniversary of these publications I can thus also celebrate my personal 40th jubilee of research in molecular genetics. I am therefore particularly honored to present a contribution to this symposium.

Correspondence to: Dr. W. Arber, Dept. of Microbiology, Biozentrum, University of Basel, Klingelbergstr. 70, CH-4056 Basel, Switzerland. Tel. (41-61) 2672130; Fax (41-61) 2672118.


Abbreviations: bp, base pair(s); IS, insertion sequence; RFLP, restriction fragment length polymorphism.

(a) Molecular genetics has its roots in the middle of our century

If we identify the birth of molecular genetics with the publication of the double helix model for DNA 40 years ago, we may wonder how long the time of pregnancy preceding that birth had lasted. In my view, this roughly covered the decade spanning from 1943 to 1953. In these ten years, a number of natural concepts relevant for the development of molecular genetics were discovered and at least partially unraveled. Without any claim for completeness I would like to briefly evoke some of these important issues.

(1) In the 1940s, it became obvious that bacteria and bacteriophages have genes and that these can undergo mutation. Important contributions to this insight were made by Delbrück, Luria, Tatum, Hershey and others. In an original approach called fluctuation test, Luria and Delbrück (1943) showed that spontaneous mutations of
bacteria occur independently of the agent used for selection of such mutants. Making use of phenotypic differences between strains of Pneumococci, Avery et al. (1944) presented solid evidence that genetic information is carried on DNA molecules. A few years later, this concept found an independent support for genetic information of bacteriophage (Hershey and Chase, 1952).

(2) Within this same decade, three different mechanisms of horizontal transfer of genetic information between bacterial strains were described. The first of these mechanisms is the uptake of free DNA by bacterial strains. This phenomenon is called transformation (Avery et al., 1944). Using different mutant strains of *Escherichia coli*, Lederberg and Tatum (1946; Lederberg, 1947) discovered bacterial conjugation in which genetic information is transferred from a donor to a recipient cell upon close contact between the participating cells. Upon investigation of the potential for conjugation of *Salmonella typhimurium* strains, Zinder and Lederberg (1952) discovered transduction in which horizontal transfer of host genes occurs with bacteriophage as a natural gene vector.

It soon became obvious that natural gene vectors are also involved in conjugation. These belong to the small autonomously replicating DNA molecules called plasmids. It is interesting to note that these three basic processes of horizontal gene transfer - transformation, conjugation and transduction - were discovered within a few years and that, with the only exception of the possibility of cell fusion which has later been shown to occur under particular conditions with some bacterial strains (Fodor and Alfeldi, 1976; Schaeffer et al., 1976), no other process of horizontal gene transfer has been discovered within the last 40 years.

(3) In the year 1952 several independent reports were published on a phenomenon then called host-controlled modification of bacterial viruses. These studies had been carried out with *Escherichia coli* (Luria and Human, 1952, Bertani and Weigle, 1953), *Salmonella typhimurium* (Anderson and Felix, 1952) and *Staphylococcus aureus* (Ralston and Kruger, 1952). Later investigations showed that the restriction-modification systems responsible for this phenomenon set natural limits to horizontal gene transfer (Arber, 1965). In recent years, the use of restriction enzymes greatly facilitated the application of molecular genetic techniques to segments of chromosomal DNA of any organism.

(4) The description of mobile genetic elements has its roots both in plant genetics and in microbial genetics. The pioneering work of McClintock (1950) established in a classical genetic approach that some chromosomally located genetic information can be a source of genetic instability by acting as mediator of genetic rearrangements. Within the same decade, the unraveling of the phenomenon of phage lysogeny by Lwoff and Gutmann (1950) introduced the concept of prophage as an inheritable element of lysogenic bacteria and its underlying potential for a temporary but firm association between a viral genome and its host chromosome.

(5) The concept of filamentous double-stranded DNA molecules of high molecular weight with a potential for semi-conservative replication (Watson and Crick, 1953a,b) represented a very important basis for the further investigation and deeper understanding of the phenomena listed above. With this realm of new knowledge molecular genetics had excellent prospects for its development.

(b) Spontaneous mutations

We find it appropriate to define as spontaneous mutation any alteration in the nucleotide sequence of a genome occurring independently of the reflected intervention of an investigator. This somewhat unconventional definition includes all changes in the inherited linear sequences of nucleotides of a genome, independent of the molecular reactions mediating the change and independent of whether such change results in an alteration of phenotypic traits or not.

It is a long experience of geneticists that mutations may often be lethal so that the mutant cannot undergo further reproduction. In most cases this inhibits a profound experimental study. Many other mutations may be without immediate influence on the life processes and thus remain neutral or silent. Of easier access to genetic studies are mutations which inhibit life processes in one way or other and therefore give selective disadvantage to the mutant as compared to its parent. More rarely, a mutation may result in a selective advantage. Because of the effect of selection the latter 'useful' mutations are of primary evolutionary relevance.

Since lethal mutations represent an important fraction of all spontaneous mutation events one can generally postulate that for any species a tolerable mutation frequency should be lower than one mutation per genome and generation. It is appropriate to note here, however, that this definition lacks precision, since the generation time is difficult to define unambiguously for all possible physiological and environmental conditions. Indeed, while this definition is easy to apply to exponentially growing organisms, it becomes problematic in the stationary phase in which growth is at most residual.

For the workhorse of molecular genetics, the bacterium *Escherichia coli* K-12, the rate of discernible spontaneous mutations is in the order of $10^{-6}$ per base pair and generation (Drake, 1991). This corresponds to between 1‰ and 1% of new mutant cells in each generation. This mutation rate appears on the one hand to be low enough
to ensure a certain genetic stability needed for any species of living organisms to be maintained in the biosphere, and on the other hand it offers enough genetic variation to provide for a potential of genetic adaptation to new environmental conditions.

Because of their haploidy, bacteria and their viruses and plasmids are most appropriate organisms for the study of spontaneous mutation. On its genome of about 4.7 \times 10^6 bp, *Escherichia coli* may accommodate a few thousand genes of which roughly 1500 have already been identified and characterized to a considerable extent (Bachmann, 1990). This gives an excellent basis for further investigations. In addition, these microorganisms have the advantage of short generation times, which under appropriate growth conditions can easily reach as little as 20–30 min. Therefore, under conditions of exponential growth, large populations can arise in small volumes in short periods of time, which opens doors to population genetic studies. For all of these reasons, molecular processes are readily amenable to experimental studies on the generation of genetic varieties, i.e., on the basis of genetic diversity and biological evolution.

Within the last 50 years, work particularly with microorganisms led us to a rich stock of knowledge on mechanisms relevant for spontaneous mutation. Very importantly, spontaneous mutation is not due to a single mechanism but it has its source in a wide diversity of mechanistically very different processes, each of which may contribute in its characteristic way and with its characteristic rate, which can also depend on the environmental conditions such as the ambient temperature, to the overall production of spontaneous mutants. We find it convenient to group these mechanisms into four distinct categories, namely (1) replication infidelities, (2) effects of external and internal environmental mutagens, (3) DNA rearrangements and (4) acquisition of genetic information. Each of these categories again includes a variety of specific mechanisms. In addition, some of the observed processes may overlap with more than one of the listed categories. For example, intrinsic limits of structural stability of nucleotides can be seen as an internal environmental mutagen but it also has its relevance for replication infidelity. Another example is the possibility to reincorporate into the genome a segment of genetic information which had been replicated at the RNA level with an increased chance of mutation before its retrotranscription into DNA.

It is not possible to comment in this review on all possible mechanisms of spontaneous mutagenesis and we will limit ourselves to a few selected areas, namely nucleotide substitution, site-specific DNA inversion, transposition and gene acquisition.

(c) Do replication infidelities constitute errors?

In growing bacterial cells, the replication fork migrates along the DNA molecule with a speed corresponding to about 1000 bp per second. Complementary base pairing between the parental and the newly synthesized DNA strands depends on the atomic structure of the nucleotides and their electron distribution. It is well known that nucleotides besides their standard form can take unusual short-living tautomeric forms. Under these conditions, adenine in tautomeric form was proposed to undergo base pairing with cytosine and thymine in its tautomeric form pairing with guanine (Watson and Crick, 1953b). In this light, mispairings resulting from DNA replication can be attributed to the presence of short-living tautomeric forms, although experimental evidence for this explanation is still difficult to obtain (Goodman et al., 1993). While many mispairings resulting from DNA replication can be corrected rapidly by enzymatically guided repair processes, a few sequence alterations may remain unrepaired and give rise to substitution mutations. In view of this explanation, it appears inadequate to call such mutations 'errors' as if at the time of the incorporation, appropriate base pairing would not have been reached. Under this optics and in view of the structural flexibility of bioorganic molecules, we prefer to refer to this source of mutation upon DNA replication as replication infidelity or reproductive infidelity.

(d) Site-specific recombination can generate genetic variation upon use of secondary crossing-over sites

Site-specific DNA inversion systems have been thoroughly studied both in vivo and in vitro with bacteria, bacteriophages and plasmids (Glasgow et al., 1989). An example is the periodic inversion of a segment of the bacteriophage P1 genome. This segment contains two alternative C-terminal parts of a gene for phage tail fibers. A gene for site-specific recombinase is carried on the P1 genome adjacent to the invertible segment and it is constitutively expressed. The resulting Cin recombinase acts at two 26-bp long crossing-over sites which are carried in inverted orientation and it thereby inverts the DNA segment located between these sites. In the case of the P1 tail fiber gene, the alternative fusion between an N-terminal constant part and one of two possible C-terminal variable parts by repeated DNA inversion gives rise to two different tail fiber proteins which determine different host ranges of the phage (Iida, 1984). Therefore and because DNA inversion occurs every few generations, phage P1 populations are usually mixtures between particles of two different host ranges.

For the purpose of studies on the role of the two central bp in the crossing-over sites, Shigeru Iida, at the time of his stay in our laboratory, had constructed plas-
mids carrying besides the gene for site-specific DNA inversion only one site for efficient crossing-over rather than the usual two (Iida et al., 1984; Iida and Hiestand-Nauer, 1986; 1987). Under these conditions DNA inversion is rare but it can also occur. This was seen because these plasmids also carried a reading frame, the expression of which gave rise to a selectable phenotype such as kanamycin resistance. While in the original form of this plasmid none of the promoters present on the plasmid initiated expression of kanamycin resistance, after DNA rearrangement such expression could arise from one of the promoters. Upon molecular investigation many of the selected mutants showed to be due to DNA inversion between the single consensus crossing-over site and any one of a number of different secondary crossing-over sites (Iida and Hiestand-Nauer, 1987; see also Arber, 1990; 1991). Some of these were shown to be used repetitively but with much lower frequency than is seen for inversion of DNA segments carried between two consensus crossing-over sites. Interestingly, there is no specific pattern of consensus nucleotide preservation to be detected if a number of different crossing-over sites are compared. However, the observation that several of the studied secondary crossing-over sites served more than once independently for DNA inversion indicates a non-randomness of selection for secondary crossing-over sites. Since wide deviations from the consensus are tolerable, a large number of secondary crossing-over sites can be used each with its characteristic low frequency. We attribute DNA inversion at secondary crossing-over sites to the intrinsic structural flexibility both of the involved enzyme and its double-stranded DNA substrate rather than to errors of the enzyme mediating the recombination.

In the literature, site-specific DNA inversion is often considered as a control element for gene expression. However, DNA inversion seems to be largely an aleatoric process rather than to respond to specific environmental conditions. Its effects are relevant for populations rather than for individual organisms. A flip-flop system at the DNA level can be of a certain advantage to a species since it can provide to a population some flexibility in the adaptation to periodic fluctuations of the living conditions. One may wonder, however, whether that kind of advantage represents indeed the selective force which had been exerted in the past to ensure the maintenance of DNA inversion systems as part of microbial genomes. Alternatively, selection might have been exerted by the potential of DNA inversion systems to act as variation generators by their relatively rare action on secondary crossing-over sites. This can give rise to novel nucleotide sequences. Of particular interest are gene fusions resulting in mosaics composed of different functional domains or other kinds of sequence motives. There is no reason to believe that such gene fusions would occur in a strictly directed way. Rather their origin is partially aleatoric and would depend on the fortuitous presence of sequences which can serve as secondary crossover sites. This should leave enough freedom to have tried out a wide variety of possible fusion products in bacterial populations in the course of time. Rarely produced successful combinations are expected to be favored by selection on the basis of improved fitness under particular ecological conditions. Similarly, novel operon fusions resulting from DNA inversion may sometimes present a selective advantage and thus be maintained and enriched in populations. The evolutionary relevance of such new recombinants is obvious. Site-specific recombination systems do not allow recombination at secondary crossover sites to occur frequently enough to endanger the genetic stability of the concerned strains. Therefore we believe that the potential for the generation of variation at the population level might represent a selective force strong enough to explain the presence of DNA inversion systems in the genomes of bacteria and of their viruses and plasmids.

(e) Mobile genetic elements contribute essentially to the generation of genetic variation

Insertion sequence (IS) elements, transposons and at least some viral viruses have been defined as mobile genetic elements (Campbell et al., 1979). Such elements can form part of microbial genomes. They may change location, a process which is called simple transposition. Although the transposition activities can depend on host factors, each particular element has its proper specificities with regard to transposition mechanisms and the selection of target sites. Many mobile genetic elements such as IS elements resident in the E. coli genome show transposition rates in the order of about 10^{-6} per element and cell generation. In the E. coli chromosome there are about ten different kinds of mobile elements, each in a small number of copies so that a total of 1–2% of the bacterial chromosome represent mobile genetic elements (Birkenbihl and Vielmetter, 1989). Besides simple transposition IS elements can give rise to more complex DNA rearrangements including DNA inversion, deletion, the fusion or cointegration of two DNA molecules and gene amplification. Part of these DNA rearrangements are mediated by enzymes, called transposases, expressed by the mobile elements themselves. Thereby target selection is usually neither fully random nor fully site-specific. While some IS elements show a pronounced degree of site-specificity in the selection of their transposition target, but can occasionally also use secondary target sites, others preferentially select their transposition targets in specific DNA regions but use a large number of possible
integration sites within the preferred region of insertion (Sengstag and Arber, 1983; 1987). In contrast to these IS-promoted transpositional DNA rearrangements different copies of a given IS element carried in a genome can also serve for general recombination in which the element passively offers its sequences as an extended homology.

Transposition rates can depend on physiological and environmental conditions. It is of interest to note that transposition is not limited to the phase of exponential growth. Rather it can also occur in the stationary phase. Therefore transposition together with other mechanisms of spontaneous mutagenesis can contribute to the generation of genetic variation in resting bacteria. We have explored this potential with the P1 prophage (Arber et al., 1979) and more recently with the E. coli genome. A study of restriction fragment length polymorphism (RFLP) made with the standard strain W3110 of Escherichia coli K-12 revealed a wide variety of different genome structures among subclones isolated from an old laboratory culture which had resided for the last 30 years in a stab collection. Stabs are small glass vials with solid growth medium into which bacteria are inoculated. After one day of growth these vials are closed with paraffined corks so that no evaporation is possible. Surprisingly, bacteria keep their viability for long periods of storage of stabs at room temperature. Our studies revealed that polymorphism increases upon storage. This becomes obvious if DNA from subclones is extracted, cleaved with a restriction enzyme, the fragments separated by gel electrophoresis and then hybridized with DNA probes taken from mobile genetic elements. Under the particular conditions studied in our laboratory, a hybridization probe prepared with an internal sequence of IS5 revealed a particularly strong polymorphism. While the polymorphism seen with probes from several other IS elements was less pronounced but still considerable as compared to the quite high genetic stability revealed using DNA probes from single chromosomal genes. From the study of more than 100 subclones of the old culture of strain W3110 and by application of a computer program taking into account the relative number of structural differences seen with the RFLP analysis, a parsimonious pedigree could be derived which clearly illustrates an amazing degree of genetic plasticity of the chromosome of this strain (T. Naas, M. Blot, W. Fitch and W.A., to be published). As a matter of fact, the unrooted pedigree of this old E. coli culture could not reveal any longer the genetic origin of this strain. These studies indicate that genetic rearrangements can also occur in resting bacteria in confirmation of an old analogous conclusion made for spontaneous mutagenesis per se (Ryan, 1955). This finding has its particular evolutionary and ecological significance, since it is well known that bacteria spend only a limited fraction of their time under conditions of exponential growth.

Several bacterial IS elements have been shown to give rise to composite transposons. These are mobile genetic elements flanked by two identical IS copies and carrying in between these copies one or several genes unrelated to transposition. Such composite transposons can originate at any region in the bacterial chromosome when it had successively been the target for simple transposition of a specific IS element to both sides of the concerned bacterial genes (Iida et al., 1981).

(f) Gene acquisition

Transposition of an IS element as well as of a composite transposon can occur to natural gene vectors, such as transferable plasmids or viral genomes present in a cell. This can give rise to horizontal transfer of the mobilized genes to other bacterial strains in conjugation or in phage mediated transduction, respectively. After uptake by a recipient cell, genes carried on a transposon can become incorporated into the recipient genome by transposition without a requirement of sequence homology. Transposable elements offer therefore appropriate means both to charge natural gene vectors with genetic traits of the donor genome and to mediate a stable association of the transferred genes with the recipient genome. These processes have played an important role in recent times in the dispersion of drug resistance characters to wide varieties of enterobacterial strains. As a matter of fact, horizontal transfer of such genes is likely to have always occurred in the past, but it is only recently, upon a wide use of antibiotics in human and veterinary medicine, that strong selection was exerted on rare enterobacterial cells which had spontaneously acquired resistance-conferring genes.

Gene acquisition can also occur independently of transposable elements. Uptake of DNA molecules in transformation as well as phage-mediated general transduction of chromosomal DNA segments are well known examples.

A number of factors impose in part severe limitations to gene acquisition. These factors include the following barriers. First of all, the surface of the recipient cell must be compatible with the infection process and a successful uptake of horizontally transferred DNA. Second, penetrating DNA is often screened by restriction-modification systems able to recognize if this DNA is foreign, in which case it is cut into fragments. Interestingly, such fragments may be particularly recombinogenic in the short time before they become exonucleolytically degraded. In this view, restriction-modification systems on the one hand limit the frequency of gene acquisition and on the other hand help acquisition to occur in small steps. Third, acquired DNA is only useful for a recipient cell line if it becomes inherited into the progeny. This requires integration into the genome or maintenance on an autono-
mous replicon. Finally, if functions of the acquired genes are expressed, they must be compatible with the functional harmony of the recipient cell. We see here an additional factor in favor of the acquisition in small steps. Indeed, it is likely that simultaneous acquisition of many functions has a higher probability to result in functional incompatibility than the acquisition of a single gene or a single functional domain.

(g) The tree of microbial evolution represents a network

In the chapter on spontaneous mutagenesis, we grouped the different mechanisms leading to spontaneous mutation into four categories. The first three of these act at given rates on the entire genome of cells as members of an evolving population. In addition, at any moment a cell may also undergo acquisition of one or a few gene functions by horizontal transfer. Therefore, the classically drawn evolutionary tree of microorganisms should be equipped with horizontal shunts through which once in a while genetic information developed in one branch of the tree can be made available to a member of another branch (Arber, 1991). In this view, future biological evolution of any branch of the tree may not only depend on further variation occurring to the existing genome, it may also benefit from the acquisition of a novel function which had been developed in another branch of the tree.

(h) Comparison of efficiencies of some of the evolutionarily relevant developments

Let us compare the relative efficiency per event for three of the key processes discussed in this paper: acquisition, development by DNA rearrangement and development by nucleotide substitution. Between these three distinct processes, we postulate a considerable gradient of decreasing efficiency per single event. Gene acquisition may represent the most successful and a single step of nucleotide substitution the least efficient of the three processes considered, while DNA rearrangement may have an intermediate rate of success. This statement is based on the following considerations.

In the acquisition in small steps, in which a single domain, a single gene or a few genes often representing an operon are added to the genetic potential of the recipient or sometimes substitute homologous functions of the recipient, the success rate of acquisition may be quite high, particularly if accessory genes are involved. This is well seen in the success of the horizontal spreading of genes encoding antibiotic resistance. Thanks to the universality of the genetic code a biological function which had been developed in one branch of the evolutionary tree may have a good chance for success to also serve in another branch. In this view, acquisition can be considered as a strategy of sharing in the success of others.

Evolutionary development by DNA rearrangement can bring the improvement of available capacities, being it by operon fusion, by gene fusion or also by a position effect depending on the location of particular genes on a genome. Many of such often aleatoric rearrangements may be lethal or in another way detrimental. Thus the efficiency per rearrangement event with regard to evolutionary success may be smaller than in gene acquisition.

Probably the least efficient process, always considered per event, is nucleotide substitution. Indeed, it takes a large number of substitutions to develop a new biological function, and even the chance to considerably improve an existing function in a single random step of substitution may be relatively low. Although substitution may be less often lethal than DNA rearrangement, it may take many more substitution steps to achieve evolutionary progress than by DNA rearrangement.

Needless to state that any of these processes as well as any other process of mutagenesis may each have its strategic importance in its proper way and be necessary and relevant for harmonious microbial evolution.

(i) Uniqueness of nucleotide sequences

A simple numerical consideration may help to understand that biological evolution would hardly be successful if it had to start from zero in each branch of the evolutionary tree for each novel biological function to be developed. Although we are far from understanding and from having general rules to explain the evolutionary passage from one function to another, we are ready to assume that a biological function once lost from the biosphere cannot be expected to reappear rapidly as a result of a new development.

The following theoretical considerations can give insight into the uniqueness of nucleotide sequences. Let us look at a nucleotide sequence of 1000-bp length composed of the four different nucleotides. For such a sequence, the number of possible unique sequences is $4^{1000}$ which equals $10^{602}$. A rough estimate of the number of living cells in the biosphere indicates the steady existence of about $10^{30}$ living cells on our planet. This is based on an estimation of the total volume of living matter and the average volume of single living cells. If we assume that life on our planet existed since about $3 \times 10^9$ years which corresponds to $10^{15}$ min and if we further assume that $10^{30}$ cells were present since these early times, that each of the cells carried in its genome $10^5$ segments of 1000 bp length and finally that in each segment one new mutation was tried out per minute, we come to a number of sequences so far tested of $10^{50}$, i.e., $10^{30}$ cells multiplied by $10^5$ genome segments multiplied by $10^{15}$ min. Amazingly, this quite high number of $10^{50}$ sequences is far from the even much higher number of $10^{600}$ possible
nucleotide sequences per segment. Thus, about $10^{120}$ possible sequences could not yet have been tested up to now. These considerations are limited by the fact that we do not know how many different sequences give rise to one and the same biological function. However, one may doubt very much in view of the tremendous potential for different sequences and for their combinations that all possible forms of life have already shown up on our planet. It is thus a reasonable assumption that the acquisition strategy with a sharing in the success of others represented indeed an important component of the successful evolution of forms of life on our planet in the past. If so, it is obvious that the same role has to be attributed to acquisition in future biological evolution. This argumentation should be taken into account in any debate on the importance of biodiversity.

(j) **Biodiversity reflects the present state of biological evolution**

In Fig. 1 we present a summary of the factors relevant for biological evolution. On the left are listed the four categories of mechanisms of spontaneous mutagenesis already discussed. All of these mechanisms contribute to the steady generation of genetic diversity. However, total diversity is severely limited by sampling dictated by the size of the biosphere. This together with natural selection by living conditions steadily reduces the diversity while the latter is replenished by the genetic instability. Additional reduction in diversity is brought about by a number of enzymatically guided repair processes which mainly limit the mutagenesis rates of infidelity in reproduction and of at least some environmental mutagens. In Fig. 1 we have taken into account that a relatively high degree of diversity can be maintained on the planet due to cloistering effects by reproductive and geographic isolations.

At any time, the observed biodiversity reflects the present state of biological evolution. The possibilities for future biological evolution largely depend on the kind and on the diversity of genetic information presently available.

We are aware that microgenetic diversity within preexisting genes may be regenerated relatively rapidly in large populations of a single species. In contrast, the generation of different species and diversified forms of life in the course of evolution may take much longer times. For both of these developmental processes, a deeper understanding of the multitude of molecular mechanisms contributing to genetic variation has become possible through the development of molecular genetics, and it is to be expected that additional investigations in these lines may bring about important contributions to pending ecological questions.

(k) **Some biological functions fulfill needs for the development of populations rather than needs of individual lives**

In this presentation we took the attitude not to consider the biological evolution to be the result of accumulated errors. Rather we believe that a number of activities of specific biological functions have their principle role either as generators of sequence varieties or as promoting agents of lateral gene transfer. Such functions may be single enzymes or enzyme systems or they may be more complex entities such as organelles. Interestingly, these kinds of biological functions are encoded for on the genome of each individual of a population. Therefore, on the same genome we expect besides many genes required to meet the needs of the individual lives, e.g., the housekeeping genes, also other genes which in contrast are not required for the individual life that spans in bacteria from one cell division to the next. Genes of this latter category exert their functions at the population level. Individual cells hit by such a gene function will more often suffer than benefit from the gene activity. This is due to the fact that evolutionary developments do not seem to be directed to a particular goal. Rather variety generation has a partly aleatoric nature. It should be noted in this context that whenever a gene product becomes available in a cell, it may often be used for more than one purpose, provided that its functions serve to these purposes. Therefore, some gene products may serve for the needs of individual lives as well as for population dynamics and evolutionary developments. But many functions clearly associated with the evolutionary development have, as
far as we can see, no obvious relevance for essential functions of individual cells.

An interesting distinction between the two broad categories of biological functions is brought about by striking differences in codon usage (Médigue et al., 1991). This may indicate that variation generating genes may themselves be more successful in horizontal spreading than genes stringently required for each life span.

The conclusions drawn in this contribution are based on investigations with prokaryotic microorganisms. Possibly some of them are also relevant for eukaryotic organisms being it for their longterm evolution or for the somatic developmental diversification. In this view, scientists exploring functions assumed to be expressed on the basis of available nucleotide sequences might be well advised to specifically look also at potential functions exerting their activities at population levels and mainly serving evolutionary and developmental purposes.

REFERENCES


