# SCOPE 44 - Introduction of Genetically Modified Organisms into the Environment

# 3 Impact of Human Civilization on Biological Evolution

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Essential strategies of genetic engineering are of direct evolutionary relevance. These include the transfer of genetic information between different kinds of organisms, site-directed mutagenesis, and the deliberate release of genetically altered organisms into the environment. The evaluation

of risks inherent to these strategies should thus be based on an understanding of mechanisms acting in natural biological evolution, in particular with regard to spontaneous mutagenesis, selection processes, and isolation principles. These considerations can also reveal other impacts that the cultural evolution of mankind has on biological evolution. An appreciation of the relative importance of any single human intervention with ecological conditions in the biosphere can help us to put technological applications into a broad perspective with regard to their potential risks and to propose appropriate measures to prevent undesirable interference with the natural course of evolution.

## 3.1 A MULTITUDE OF DIFFERENT MECHANISMS CONTRIBUTES TO SPONTANEOUS MUTAGENESIS

For the purpose of our considerations we define a mutation as an alteration in the nucleotide sequence of a given genome. Such an alteration can be lethal and thus difficult to investigate experimentally, or it can alter a phenotypic trait and thus represent a mutation as defined in genetic experimentation, or else it can be silent. The latter type of genetic alteration can become functionally relevant at some later time, in conjunction with subsequent alterations of the genetic message. It is generally accepted that any living organism can tolerate only mutation rates affecting biological functions considerably less often than once per generation.

One source of mutation is the *limited fidelity of DNA replication.* These so-called replication errors group nucleotide substitutions, small deletions, small insertions, and other types of replication slippage products. Spontaneous mutation may also result from the intervention of *environmental mutagens* such as chemicals or radiation. Often these alter the chemical structure of one or a few nucleotides, or they can provoke breaks in the DNA chains.

Many living organisms possess elaborate *repair systems* which provide means to restore a majority of DNA sequence alterations resulting from incorrect replication and from the action of environmental mutagens.

Other important sources of spontaneous mutations are various, mostly *enzyme-mediated recombination processes.* In these reactions, existing nucleotide sequences are reassembled in a new order. Often these are internal rearrangements of DNA already forming part of the cellular genome, but sometimes invading external DNA is involved in recombinational mutation processes, e.g. upon integration of a viral genome into chromosomal DNA. Some of the recombinational mutations cause the duplication of certain DNA segments. On the other hand, these processes can also lead to deletion formation. Generally speaking, some recombination processes are conservative with regard to the number of nucleotides of the participating genomes, others not.

Systems of *general genetic recombination* are known to exist in many organisms. Their strategy is based on the recognition of extended nucleotide sequence homologies, which then serve for reciprocal recombination. These enzymes are known to function in some repair processes, e.g. where recombination between two damaged sister DNA molecules can serve for the restoration of an intact genome. General recombination helps to provide a wide genetic diversity upon sexual reproduction. It also directs recombination at homologous segments, e.g. transposable

genetic elements, located at different sites of DNA molecules. These reactions of unequal crossing-over can give rise to DNA duplication, DNA inversion, and deletion formation. These processes are of immediate evolutionary relevance.

*Transposition* of mobile genetic elements is a recombination process, the biological significance of which seems largely to be evolutionary. In this process, DNA segments of defined length are translocated to other sites within the same or another DNA molecule. Depending on the transposable element involved, the criteria of target selection vary. Some transposable elements strictly select their transposition target on the basis of its nucleotide sequence. Others transpose more randomly into various sites. However, this does not necessarily mean that no selection is applied at all. A good example is IS2. This transposable element prefers to transpose into particular DNA segments, but within these segments, IS2 may insert into many different sites, which show no sequence homology among each other (Sengstag and Arber, 1987).

Well-known examples of *site-specific recombination* are the integration of bacteriophage genomes into the bacterial chromosome (Weisberg and Landy, 1983) and microbial DNA inversion systems (Plasterk and Van de Putte, 1984; Iida and Hiestand-Nauer, 1986, 1987). An essential part of the sites of recombination in such systems is determined by a consensus sequence. However, at decreasing frequencies, nucleotide sequences, which increasingly differ from the consensus, may still serve for recombination. Such recombination at secondary crossing-over sites open many possibilities to procure new DNA arrangements in microbial populations at low rates. Some o these rearrangements may represent new gene structures, which could be of utility in changing environmental conditions.

Still other, rare, recombination processes may be due to the action of enzymes, the principal role of which is to cut and to religate DNA strands, such as topoisomerases (e.g. DNA gyrase) and restriction endonucleases. How many of the so-called *illegitimate recombinants* are to be attributed to these processes or also to site-specific recombinations exerted on rarely used secondary sites remains to be seen.

In conclusion, a multitude of different mechanisms contributes to the formation of spontaneous mutations. Only some of these mechanisms can be considered to act fully at random in space and in time (Arber, 1984). Others might become activated under particular conditions, they might act specifically at particular locations on DNA molecules, or they might make use of randomness to a limited extent as part of a strategy serving to increase the chance of producing unique new DNA arrangements in order to provide a very wide genetic variability within populations of organisms.

## 3.2 LEAKINESS IN GENETIC ISOLATION PROVIDES MEANS FOR HORIZONTAL EVOLUTION

Efficient means of *reproductive isolation* inhibit the exchange of genomes between organisms that are not closely related. This principle is the basis of speciation and has certainly contributed to the genetic diversification.

Isolation barriers also limit the acceptance of small parts of a foreign genome, which might be spread around by natural gene vectors such as viruses or which might simply penetrate as free DNA molecules into a cell. Although invading DNA has to overcome a number of different barriers, such as the cell wall, restriction systems, lacking means to become established and to undergo propagation, the spontaneous acquisition of foreign genes has often been reported. It is particularly well documented with microorganisms.

In microbial systems, the following processes of horizontal gene flux are well known. In *transformation,* free DNA penetrates directly into a recipient cell. In *transduction,* genes from a donor cell become packaged into a viral particle, which then infects a recipient cell. In *conjugation,* a conjugative plasmid procures the efficient contact between two partner cells and it mediates the transfer of genes from the donor cell to the recipient cell.

In some of these processes *vector DNA molecules* form an essential part of the transfer system. This is the case in specialized transduction, where a viral genome serves as vector, and in conjugation, where a conjugative plasmid serves as vector. These vectors can become charged with small segments of the genome of the donor strain by some of the recombination processes already described above. These same processes can also help to stably establish a transferred gene segment in the infected recipient strain.

Because of isolation barriers, the successful transfer of genes between two different bacterial strains is relatively rare. The observed leakiness in the isolation appears to allow for a more efficient acceptance of small segments of foreign DNA than of larger DNA segments. The addition of single genes to a genome may also conceptually appear to be a better evolutionary strategy than an exchange of entire chromosomes. Virus-mediated horizontal transfer of one or a few genes has also been observed between several higher organisms. Hence we see in the horizontal, lateral gene transfer a perhaps general strategy of biological evolution.

While in *vertical evolution* entire genomes gradually undergo sequence alterations by various processes of spontaneous mutagenesis, in *horizontal evolution* the uptake of a small DNA segment, one or a few genes at once or even parts of a functional gene, can represent to the recipient cell an important acquisition, which might help it to cope with a new environmental constraint. In this view, the tree of biological evolution should be represented as a network, as represented in Figure 3.1.

## 3.3 SELECTION PLAYS AN UNCONTESTED ROLE IN BIOLOGICAL EVOLUTION

The world-wide spreading of drug resistance to enteric bacteria in the last few decades represents a convincing testimony for horizontal gene flux, and it also illustrates the potency of efficient selection. Molecular genetic analysis of antibiotic resistance traits, which had recently been acquired by enteric bacteria, has shown that these characters are often determined by genes carried on natural gene vectors and that many of them are also contained in transposable elements. There is no indication that either horizontal gene flux or transposition would be inducible by the presence of antibiotics. Therefore the wide use of antibiotics in human and in veterinary medicine in the last few decades must have influenced the widespread establishment of resistance genes by selection. Many of these genes were obviously acquired by horizontal

transfer from other organisms. This is a rare event, but in the presence of antibiotics the very rare resistance recipient cells were heavily favoured and must have rapidly overgrown the non-resistant members of the microbial populations. Within 10 to 20 years of intensive use of antibiotics a high proportion of enteric bacteria have world-wide become resistant to one or often several antibiotics. This is a good measure for how fast potent selection can become evolutionary effective.

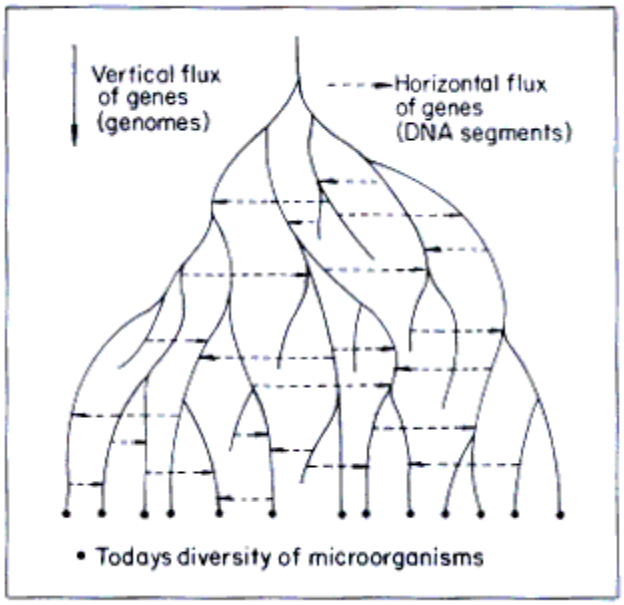


**Figure 3.1** Microbial evolution represented as a network. As a function of time, genomes evolve by spontaneous mutagenesis as they are vertically transmitted from generation to generation. Relatively small segments of genomes are horizontally transmitted between different microbial species by any of a number of different mechanisms of interspecific gene transfer. This evolutionary tree with horizontal shunts between different branches should be regarded as multi-dimensional rather than two dimensional as drawn in this presentation.

Evolutionary selection does not always act as immediately as in the application of antibiotics. Selective effects are normally based on more of less favourable living conditions. Under given conditions, some members of a mixed population of living organisms may propagate more readily than others, so that in the course of several generations the composition of the population may gradually change in favour of those organisms that find the living conditions most appropriate. The effect of selection becomes strongly manifest only in the course of time.

## 3.4 CULTURAL EVOLUTION MORE AND MORE INFLUENCES SELECTION AND THUS BIOLOGICAL EVOLUTION

Besides the described effect of the use of antibiotics many other human activities, as a matter of fact a great number of technological applications made with the motivation to serve for the benefit of mankind, may in the long term considerably change selection criteria in the biosphere

and thus exert a direct influence on biological evolution. These effects of our civilization can be grouped into two categories.

The first category concerns the *management of land* for agriculture and human living. In these cultivated areas the original living conditions are drastically altered. Natural ecological equilibria are destroyed and often replaced by ecologically unstable plantations, frequently as monocultures `protected' by appropriate interventions from competing weeds and from animals interfering with the growth of the crop. Under these artificially maintained conditions, natural biological evolution cannot function properly, so that these vast areas cannot any longer serve as breeding grounds for evolutionarily relevant genetic variants. As a result of this anthropogenic selection, many original inhabitants of these areas die out. The situation becomes particularly grave upon the transformation of forests into intensively used agricultural land.

The second category of influences of the civilization on the course of biological evolution acts through the increasing *pollution of soil, air, and water.* Pollution is also spread through the air and through the water to those areas of the globe where human activities have not yet exerted a direct influence on the living conditions. These conditions suffer from deterioration under the increasing concentrations of pollutants. Therefore evolutionary relevant selection also undergoes changes in the so-called wilderness, which includes virgin forests, high mountain regions, deserts, and also the oceans. Practically no region of the planet is protected from such effects. Again one must expect a drastic reduction in biological diversity as a result of these interferences at the level of evolutionary selection.

Both from the massive transformation of land for direct human use as well as from the influence of increasing concentrations of pollutants including carbon dioxide one expects considerable world-wide climatic changes. This can again affect efficiently the selection processes acting in biological evolution.

### 3.5 MAINTENANCE OF A RICH BIOLOGICAL DIVERSITY IS ESSENTIAL BOTH FOR FUTURE BIOLOGICAL EVOLUTION AND FOR FUTURE CULTURAL EVOLUTION

Mainly for the reasons discussed above, mankind is in the process of involuntarily reducing genetic diversity. Not only are many living species dying out but others, particularly those of agricultural importance, steadily lose their diversity of genetic variations as a result of present-day agricultural practices. In the context of the issues discussed here, two strong reasons should motivate us to call for urgent measures to preserve the rich genetic diversity:

1. As we have outlined above, genetic rearrangements and in particular horizontal gene flux are important sources for spontaneous mutations, a basic condition for living organisms to cope with changing environmental conditions. Any reduction in genetic diversity reduces the evolutionary possibilities.
2. Biotechnological applications are likely to widely influence the life of human societies of the future. Many gene products may serve in the future as biologically active products in medicine and in many other fields of human activities. It is impossible to predict today which these applications will be the most beneficial. Nor do we know in which living

organisms potentially useful genes are. With every species that dies out genes for interesting products could become host for ever. Genes should be considered as unique resources for the human civilization of the future. A rich genetic diversity should also be maintained for these reasons.

In summary, it is our responsibility to safeguard the genetic richness as a basis for normal biological evolution and for the further progress of our civilization.

## 3.6 THE SPONTANEOUS APPEARANCE OF A HIGHLY SPECIFIC, NEW NUCLEOTIDE SEQUENCE IS STATISTICALLY EXTREMELY RARE

The nucleotide sequence of a gene of average size is about 1000 bp. This sequence is built by a specific linear arrangement of 4 different nucleotide. Therefore, one expects $4^{1000}$, that is $10^{602}$, different specific 1000-bp sequences to be possible.

Based on an estimation of the volume of the biosphere of our planet and on our knowledge of the volumes of living cells, one can estimate the biosphere to contain roughly $10^{30}$ living cells. Life exists on earth for $3 \times 10^9$ years, that is $10^{17}$ seconds. Assuming that each living cell would try out one new nucleotide arrangement every second, which would correspond to an excessively high mutation rate, only about $10^{47}$ of the $10^{602}$ possibilities of specific gene structures could have been tested by today.

Although it is clear that many slightly different nucleotide sequences can code for the same biological function, this simple calculation does suggest that the spontaneous generation of a particular biological function might be a rare event. For this reason, the preservation of existing genes and thus of a high genetic diversity can prevent us from the permanent loss of existing biological functions that could become of increasing importance at some future time. It is unlikely that the gene for a particular biological function can be generated *de novo* any time that there is a need for it.

## 3.7 CONCLUSIONS WITH RESPECT TO THE RELEASE OF GENETICALLY DESIGNED ORGANISMS IN THE ENVIRONMENT

### 3.7.1 *IN VITRO* RECOMBINATION BYPASSES ISOLATION BARRIERS

One of the strategies of gene technology implies the horizontal transfer of a gene into a cell of another species of organisms, e.g. a mouse gene into a bacterial cell. As we have discussed, horizontal gene transfer is an important principle acting in natural biological evolution. However, relatively little is yet known about these phenomena, particularly on the range of organisms into which genes usually become transferred horizontally. It is clear, however, that in *in vitro* recombination, existing isolation barriers are often bypassed. In other words, the natural leakiness of isolation barriers becomes increased in the *in vitro* gene transfer. Biological evolution is deliberately guided into a chosen direction. Although biological evolution could go into the same direction also by natural means, the experimental approach brings an increase in the rate of such occurrences. The evolutionary appearance of a new gene combination is not any longer a matter of chance; it is the reflected goal of the project.

### 3.7.2 SITE-DIRECTED MUTAGENESIS ALSO INFLUENCES EVOLUTION DELIBERATELY

Site-directed mutagenesis serves on the one hand to explore gene functions systematically and on the other hand to specifically alter, e.g. improve, gene functions, once their mechanisms have been clarified. Again, any such genetic alteration could also occur spontaneously, but the probability of most specific genetic alterations is low. Hence genetic experimentation also influences evolution at the level of mutation, and this again into a designed direction. Alteration of the genetic message can either alter the specificity of the gene product or the efficiency of gene expression. Both of these influences can represent evolutionary steps.

### 3.7.3 GENETIC EXPERIMENTATION CAN BYPASS PRIMARY SELECTION

In genetic experimentation, molecular as well as classical, large clones of new genetic combinations can be produced in the absence of naturally acting selection principles. This can bypass the need to establish founder colonies, an often stringent condition which stands in the way to the success of a new trait in ecological populations.

### 3.7.4 *IN VITRO* RECOMBINED GENES CAN LATER BECOME TRANSFERRED HORIZONTALLY UNDER NATURAL CONDITIONS

There is no reason not to assume that cloned genes as well as any other gene could, at one time or another, become transferred horizontally to other organisms forming part of the ecological population in which the host of the cloned gene might live after its release into the environment. At this level, natural isolation barriers are expected to work properly and display their normal leakiness.

### 3.7.5 FURTHER POINTS TO BE CONSIDERED FOR A WISE, RESPONSIBLE APPLICATION OF RECOMBINANT DNA STRATEGIES

With respect to biological evolution, recombinant DNA techniques hardly represent entirely new aspects. Leakiness of genetic isolation, spontaneous mutagenesis, and mechanisms to bypass selective forces are known to exist under natural conditions. However, genetic experimentation, whether molecular or classical, can interfere with natural evolution (1) by the design of the experiment which can push evolution into a chosen direction and (2) by the possibility of introducing altered organisms at high concentrations into the environment. Particular attention should be given to these aspects. In view of the discussed importance of maintaining a high genetic diversity in our biosphere, any experimental design should avoid causing directly or indirectly a reduction in the genetic richness. For example, if a new trait or a new property is to be introduced into a crop of agricultural importance, care should be taken not to use a single clone. Rather, the gene for the particular trait should be introduced into a large number of different natural varieties of the crop, a measure which could help to preserve the richness of the natural gene pool of the concerned species. This kind of strategy may not be in the immediate interest of those propagating commercial applications. However, a wide scientific consent of their general importance could help such measures to be followed.

For a better evaluation of the long-term effects that the introduction of genetically designed organisms into the environment can have, a better understanding of biological evolution is an urgent need. Research in these fields deserves particular attention. Its results can also help us in the appreciation of any of the many different other influences of human civilization on biological evolution, such as those outlined above. Release of genetically modified organisms, if it respects a high genetic diversity, is likely to interfere with natural biological evolution to a much lesser extent than many other human activities, particularly if these alter the living conditions and thus evolutionary active selection.

The principle that any functional gene represents a unique entity of imminent importance for future biological as well as cultural evolution deserves a wide recognition by the scientific community. This could also help its acceptance by the general public. This is a prerequisite for politically effective measures to be taken in view of diverting the imminent threat that genetic diversity becomes drastically reduced within the next few human generations. Recombinant DNA techniques are not the main cause of this danger; rather, if applied with responsibility and wisdom, it is likely to serve mankind to remedy the present ecological abuses and thus to preserve a high genetic diversity.

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