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Overview

Vertical and horizontal gene transfer in lichens

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Abstract

Lichens are symbiotic organisms, which consist of a mycobiont (fungus) and a photobiont (green alga or cyanobacteria). They inhabit a wide range of ecological niches. Genetic variations among different lichen populations reflect both vertical and horizontal gene transfer. Vertical gene transfer refers to aseuxal and sexual propagation of lichens. Horizontal gene transfer occurs in each symbiont separately, between that symbiont and the organism of some other species. Horizontal gene transfer enables adaptation through long time periods, while vertical gene transfer enables adaptation to sudden environmental changes. Genetic structures of lichen populations, together with vertical and horizontal gene transfer patterns, provide insight in the past evolutionary events and present changes that lichens are going through. Lichens have many different roles in the environment, bringing benefits to other organisms. Studying genetic variation and gene transfer patterns offers improvement and development of conservational strategies for lichen protection and survival.

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SYSTEMATICS, ECOLOGY AND DISTRIBUTION OF LICHENS

Lichens are symbiotic organisms with dual nature. They are composed of at least two organisms: a fungus and an alga or cyanobacteria, or, in rare cases, of organisms from all three kingdoms. Lichens are classified as fungi, since fungi are their constant component *(1)*. Simon Schwendener recognized them as single organisms in 1867. Investigating thallus morphology and lichen anatomy, he concluded that gonidia and fibers, two main morphological parts of lichen, are genetically different. Gonidia are more algae-like and the fibers, therefore, must be fungal hyphae growing on algal cells. Schwendener also proposed that successful lichen resynthesis in a laboratory could be done much easier if fungal ascospores were cultivated on algal colonies. His thesis was later confirmed by Rees, Stahl and Bonnier who resynthesized and cultivated lichens in the laboratory *(2)*.

Lichens are a polyphyletic group of organisms – they have multiple origins and do not share common ancestors; they have no common characteristics except the ones that allow them to hold together symbionts they are formed from. The total number of lichen species is estimated to be around 18,000. Mostly they belong to the fungi division Ascomycota. Only the small number that usually inhabits tropical habitats belongs to the division of Basidiomycota. It is believed that the lichen forming process appeared several times during the evolution of a few fungi groups. Presently living lichens are the result of such a punctual evolution *(1)*.

The annual lichen growth is around 0, 5 mm but the total thallus size can vary between few millimeters and 2 meters. Their visual appearance varies greatly. Beside the color variety of their thalli, they differ in the thalli shape. Pigments, secondary metabolites and the type of surface they grow on define the color of the lichen thalli. As pioneer species they can grow in areas where no other species grew before them. They grow on wood, soil, mosses, rocks, even, as epiphylle, on leaves of some tropical plants. They usually grow as terrestrial autotrophs but they can be found in the stream waters (e.g. *Peltigera hydrothyria*) and in the zones of flux and reflux on rocky shores (e.g. *Lichina* spp.). They dominate in the vegetation of polar and subpolar habitats and represent a great part of the vegetation of mountain and rainforest ecosystems (i.e. rainforests of the southern hemisphere and taiga of the northern hemisphere). As for all poikylohydric organisms, their distribution and habitats as well as the genetic structure of populations are influenced by the water status of the environment *(1)*.

The two main components of lichens, a fungus and an alga or cyanobacteria, are called mycobionts and photobionts, respectively.

Mycobionts

Although fungi by themselves can act as a saprobic or parasitic organisms, when lichenized, they obtain carbohydrates and nitrogen from their photobiont (i.e. green alga or cyanobacteria). Fungi that can lichenize belong to the division Ascomycota (cca 13,000 species) and the division Basidiomycota (cca 400 species) *(1)*.

Lichenized fungi can be grown in aposymbiotic state, but in nature, they can only be found in symbiosis with a photobiont. These fungi are therefore ecologically obligate but physiologically facultative biotrophs *(1)*.

There are certain adaptations fungi had to develop in order to be capable of lichenization. Those include changing the type of nutrition and adaptation to cohabitation with photobiont cells. The most visible adaptation is at the level of thallus morphology when fungus is in symbiotic state. Symbiotic thallus of most fruticose and foliose lichens has internal aerial hyphae and peripheral conglutinate, tissue-like zones. In aposymbiotic state aerial hyphae are at the periphery of tissue-like zones. Aerial hyphal cell walls become more hydrophobic in lichen. Hydrophobicity of cell walls is achieved through the synthesis of hydrophobins, small fungal proteins that form a hydrophobic film in the thallus layer containing photobiont cells. Hydrophobicity ensures the aerated environment for efficient gas exchange *(1)*.

Photobionts

The green algae genera *Trebouxia* and *Trentepohlia* and cyanobacteria (phycobionts) genera *Nostoc* and less often *Gleocapsa* and *Scytonema* are the most common photobionts in lichens. The majority of algae are from class of green algae, which share many characteristics and pigmentation with land plants (e.g. chlorophyll a and b) *(1)*.

The type of carbohydrate metabolites and how they are transported from autotrophic photobiont to heterotrophic mycobiont depends on the photobiont. In case of green algae, carbohydrate metabolites are sugar alcohols (e.g. manitol, arabitol). If photobiont is cyanobacteria then the carbohydrate metabolite is glucose *(1)*.

Only when grown in laboratory, cyanobacteria in lichen can be determined precisely. In general, determination of cyanobacterial species is based on characteristic developmental stages when they are in aposymbiotic state. When a cyanobacterium is the photobiont, developmental stages are not continuous. The morphology of the cyanobacterial cells is then changed and there are more heterocysts (i.e. specialized cells for atmospheric nitrogen fixation, containing nitrogenase). For this reason PCR method can be used for determination of cyanobacteria directly from thallus DNA sequences characteristic for 16S rRNA *(1)*.

Coccoid, sarcinoid and filamentous green algae can form lichens. Filamentous green algae are usually reduced to short fragments or single cells. This makes the production of vegetative spores easier. Their determination can be done morphologically, using standard preparation methods (e.g. squashing), but for more precise determination (i.e. exact species), laboratory cultivation is necessary *(1)*.

When there is a symbiosis of all three kingdoms (Monera, Plantae and Fungi), cephalodia are present – small, round, inner or outer thallus structures that contain cyanobacteria. Formation of thick cortical layer around cephalodia provides the optimal microaerobic conditions for maximal nitrogen fixation. In such lichen species green alga is a primary photobiont and cyanobacterium is a secondary photobiont *(1)*.

Many morphological, anatomical, ecological and genetic variants of lichens result from both vertical and horizontal gene transfer in fungi, algae and cyanobacteria.

VERTICAL GENE TRANSFER

Vertical gene transfer represents gene flow from parents to offspring; it is a gene transfer through successive lineages of a species. Vertical transfer mainly follows Mendel's laws of inheritance and it happens only between organisms of the same species. It can result only in modifying already existing phenotypes in a certain species and not in the production of completely new ones. The changes are introduced through the point mutations, gene rearrangements and gene duplications. Vertical gene transfer in lichens is therefore explained in terms of asexual and sexual reproduction. Asexual reproduction represents an effective survival strategy for populations in competitive, but at the same time ecologically stable, habitats. Sexual reproduction ensures a great

number of different genotypes in a certain population and it strengthens the chance of survival in a competitive environment *(3)*.

Many symbiotic associations were hypothesized to result from parallel cladogenesis and coevolution (i.e. the genetic change of an organism as a response to the genetic change of a related organism). Such assumption in lichen symbioses could be accepted only in cases where vertical transmission of photobiont occurs, i.e. in lichens that reproduce asexually. Successive vertical photobiont transmission through fungal lineages would require coevolution of algal or cyanobacterial genotypes with fungal genotype. In this case a certain need for transmission of a photobiont would exist, due to a requirement for simultaneous evolution of two symbiont genotypes that would supplement each other *(4)*. However, morphological studies of lichen thallus have shown that there was no coevolution between lichen symbionts. If parallel evolution of symbionts happens, it would result in equal numbers of symbiont species. Because fungal genotypes are much more diverse than the algal, we know that two symbionts undergo separate evolutions. It appears that mycobionts select algal genotypes, choosing the most compatible one at the particular moment of their evolution *(4)*.

Horizontal photobiont transmission occurs during both asexual and sexual lichen reproduction. Mycobionts in asexually reproducing lichens can switch the already existing photobiont with the new compatible photobiont available from the environment. Such photobiont switching (horizontal transmission) occurs in the early stages of thalli development and is reported in several parasitic lichen species *(1, 4)*. During sexual reproduction, dispersed fungal ascospores need to find new compatible photobiont. It results with a new combination of algal and fungal genotypes *(4)*.

Despite the denied coevolution hypothesis, preferential algal genotypes do exist. Although fungal genotypes are very diverse, very few different algal genotypes can be compatible for a new symbiosis formation. This phenomenon might be explained by long periods of fungal genotype divergence, higher mutation rate in fungi than in algal genomes, strong fungal selection of algal species or population processes (i.e. fertility, mortality and migration) leading to the algal genotype fixation *(4)*. Genotype distribution of a lichen population is conditioned not only by the preferential algal genotype (some fungi species are more specific in photobiont choice, some are less specific) but also by altitude, air pollution rate, plant population of a certain habitat and the preferential reproduction mode *(5)*.

Interestingly, in very few lichen species, same organism can reproduce both asexually and sexually.

Asexual reproduction

Thallus fragmentation is the simplest way of lichen reproduction. Fragments are made of fungal and algal or cyanobacterial cells. Thallus fragments are capable of regenerating the whole lichen individual genetically identical to parental lichen. Special fruiting bodies on lichen thalli contain diaspores consisting of one algal or cyanobacterial cell circled with fungal hyphae. Isidia and soralia are the most frequent structures that enable the simplest symbiont dispersal. Isidia are abundant in *Peltigera* and *Parmotrema* species. They can be cup-shaped or in the coral shape, branched or non-branched. Isidia are the cortical layer extensions. They facilitate the gas exchange between thallus and atmosphere and enlarge photosynthetically active thallus surface. They can be easily removed from thallus enabling symbiont dispersal. Soredia are powdery propagules consisting of hyphae wrapped around algal or cyanobacterial cell. Soredia are diffusely situated throughout the thalli surface or placed in soralia (i.e. special structures containing large amount of soredia). Proliferating medullary and algal layer form soralia. Raindrops disperse soredia. Soredia are characteristic for *Lobaria* and *Melanelia* species *(1)*.

In some cases, asexual reproduction appears to be more successful than sexual reproduction, due to the abundancy of both mycobiont and photobiont partners in the same vegetative spore *(1)*. It increases the chance of efficient lichen growth. However, vegetative spores are heavier than sexual spores so dispersal is restricted to short distances *(5)*. Consequently, genetic structure of a population inhabiting certain habitat becomes uniform.

Asexual reproduction represents an efficient way of reproducing, enabling long-term and successful dispersal of diaspores if the environment remains unchanged. However, long-distance clonal propagation could be possible, too. Herbivorous invertebrates, strong winds and rain can disperse diaspores over long distances *(6)*. It is believed that asexual reproduction is an evolutionary strategy that enables the individual to increase its abundance in a population of a certain habitat. After a while, both asexual and sexual spores are formed *(6)*.

Lobaria pulmonaria is a model lichen amongst those that reproduce asexually because of the exclusiveness of a photobiont choice (Figure 1A). Except green alga *Dictyochloropsis reticulata, L. pulmonaria* can have a cyanobacterium as the second photobiont. If the dominant reproductive mode was sexual, it would be much more complicated to establish new symbiotic organism on a new habitat. Mycobiont would have to connect not only with compatible algal but also with compatible cyanobacterial cells. It is, therefore, more complicated than the asexual propagation. For this reason, *L. pulmonaria* prefers asexual reproduction, even though it can acquire both reproduction strategies *(3)*.

Asexual reproduction provides stable genetic structures in populations of *L. pulmonaria*. Stable genetic structures ensure the transmission of locally well-adapted spores and genes they carry. Adaptational changes happen through recombination and mutations. Mycobionts adapt via recombination and mutations while photobionts adapt almost exclusively via mutations *(7)*. Better

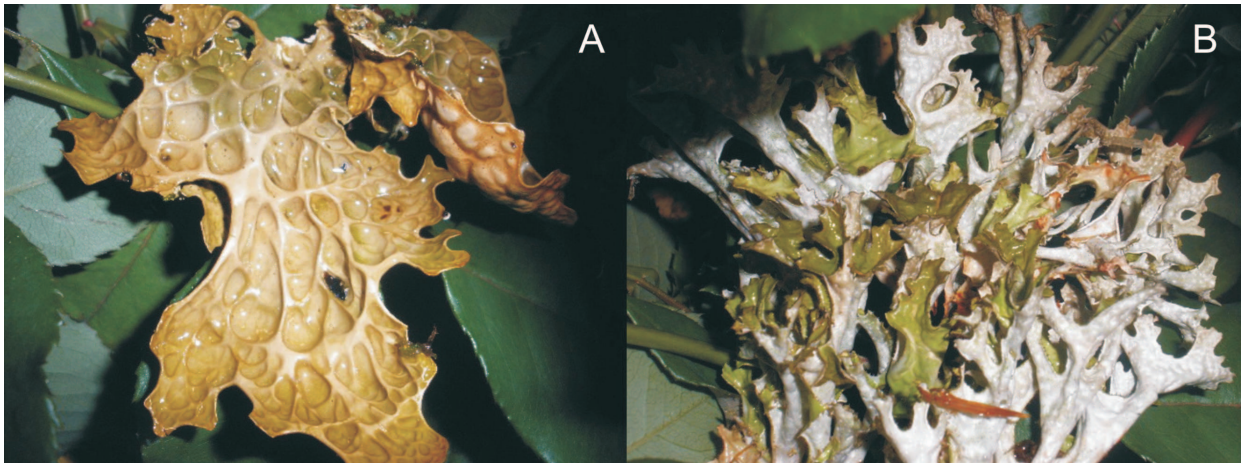

Figure 1. *A)* Lobaria pulmonaria *(L.) Hoffm., B)* Cetraria islandica *(L.) Ach.*

adaption to a certain environment can happen via sexual reproduction. This is, however, a very rare event limited to extreme habitats *(8)*.

Sexual reproduction

Ascomycota fungi cannot be separated to female and male individuals. Every single haploid mycellium can produce both female and male gametes (i.e. microconidia and macroconidia-sexually reproductive spores). They can also form gametangia (i.e. ascogonia-cells for asexual reproduction). The mycobiont spores are situated inside asci that are placed in hymenium. Hymenium is a tissue layer in hymenophore of a fruiting body (ascocarp or basidiocarp). The most frequent fruiting bodies on lichen thalli are apothecia and perithecia in lichens with Ascomycota mycobiont. Basidiocarp is the fruiting body on thalli of lichen with Basidiomycota mycobiont *(1)*. Every postmeiotic ascus contains four haploid nuclei that mostly divide once more. In the end, every ascus contains 8 nuclei. Paraphyses are long, sterile filamentous hyphal end cells that encircle every ascus. They have a double role. They secrete mucose and synthetize pigments in peak cells. As ascospores mature, the water pressure rises because of the secreted mucose, and asci are pressed. When asci mature enough and the environmental conditions are adequate, asci can rupture if the water pressure is high enough, freeing mature ascospores. Ascospore diploidization in lichen can happen by means of self-fertilization and non-self-fertilization.

Sexual reproduction of Ascomycota fungi is under the control of mating type (MAT) genes. Heterothallic species (not capable of self-fertilization) have two alleles of those genes: *MAT 1-1* and *MAT 1-2*. Since there is a big difference between them amongst organisms of the same species, the term idiomorph is used in such cases *(1)*. Heterothallic species can mate only with individuals carrying complementary idiomorph. Homothallic species (capable of self-fertillization) have only one idiomorph, which can be either *MAT 1-1* or *MAT 1-2*. Rarely, elements of both idiomorphs (i.e. the result of unequal recombination) can be present in haploid mycelium forming one idiomorph *(9)*. Sexual reproduction of homothallic species can occur with any idiomorph in other mating individuals, not necessarily complementary ones *(1)*.

MAT genes code for transcription factors that are of crucial importance in regulation of early and late stages of sexual cycle *(10)*. Additionally, they can be involved in vegetative reproduction where they contribute to the development of morphological reproductive structures on lichen thalli *(10)*. They have a role in a process of selecting the compatible reproduction partner. Mating type region of these genes is organized as heterochromatin and special histone methylation patterns dictate what kind of reproduction will take place in a current life stage (sexual/asexual) *(11)*.

The evolution of *MAT* genes is very fast, even though in certain species they need to remain conserved *(1)*. *MAT 1-1* is coding for a protein containing a conserved alpha-box motif. *MAT 1-2* is coding for a protein containing conserved DNA-binding domain called *HMG box*. It was shown that those two domains are phylogenetically closely related despite the low similarity in their gene sequences *(12)*.

Diploid ascospores must find a compatible photobiont before new lichen can develop although prethallus state can be formed even with the incompatible one *(1)*.

As mentioned before, recombination and mutation are the main factors shaping the genetic structure of mycobionts while in photobionts mutations shape genetic structure *(7)*. Sexual reproduction enables emergence of additional genetic variations allowing best adaptations to the environmental conditions. The genome discrepancies are important because they ensure fast adaptation of an organism to the sudden environmental changes and provide higher survival rate. Less genome discrepancy in population leads to lower survival rate and slower evolution processes *(13)*. Sexually reproducible species usually inhabit open, agricultural lands with frequent ecological changes *(12)*.

HORIZONTAL GENE TRANSFER

In the 1960s Lynn Margulis explained the process of primary endosymbiosis and introduced the phenomenon today known as the horizontal gene transfer. She proposed that chloroplasts and mitochondria have prokaryotic origin. Her hypothesis was based on the observation that each contains their own genome, which replicates independently of the nuclear genome. Some of the nuclear genes code for mitochondrial and chloroplast proteins. As proposed by endosymbiotic theory, those genes were horizontally transferred to the nucleus from mitochondria and chloroplasts *(14)*.

Endosymbiotic theory states that chloroplasts of photosynthetic eukaryotes have evolved through the process of primary endosymbiosis between heterotrophic eukaryote and photosynthetic cyanobacteria. The other important concept is that green and red algal plastids were horizontally transferred to the phylogenetically unrelated organisms by means of secondary endosymbiosis. Heterotrophic eukaryote invaginated photoautotrophic eukaryote and gained its photosynthetic apparatus. Plastids of green and red algae are the remains of photoautotrophic eukaryote *(14)*.

Genome sequencing techniques provided a tool for comparing individual organisms of the same or different species making it possible to track changes and their origins. Horizontal gene transfer was confirmed and it became evident that mutations, gene rearrangements, gene duplications and homologous recombination are not the only sources of genome changes.

Horizontal gene transfer (HGT) is a transfer of genes between the organisms of different species during one lifetime. It can occur between organisms of different life domains but is much more often between phylogeneticalally closely related organisms (i.e. between organisms which are closest to their most recent common ancestor) *(15)*.

Since it is considered as an important way of gaining evolutionary novelties, HGT brings into question neo-Darwinian concept of gradually gaining new traits and functions *(15)*. Horizontal gene transfer is a very strong force in the evolution of Archaea and Bacteria as well as of single-celled and multicellular eukaryotic organisms *(16)*. It was discovered by analyzing multidrug resistance patterns among the world bacterial populations and led toward the formation of the complexity hypothesis *(17, 18)*.

The complexity hypothesis includes two main concepts important for HGT: functional group and gene complexity.

Genes can be divided into two functional groups based on the frequency of their horizontal transfer. First group includes genes that are not subjected to the frequent horizontal transfer. Those genes are called informational genes: genes active in DNA replication, transcription and translation as well as genes that code for protein products involved in multiple interactions with other proteins and molecules. Second group includes operational genes. These are housekeeping genes and genes whose products do not have multiple roles in complex metabolic pathways. They are frequently involved in the process of horizontal gene transfer *(15)*.

Gene complexity, defined as a total number of molecular interactions of a gene product, is a far more important factor for integrating and maintaining a horizontally transferred gene than the functional group that gene belongs to. Genes that code for multi-interacting and multifunctional proteins are expected to be removed from the host organism. Genes that code for a new membrane cell receptor will be kept and incorporated in the network of regulatory interactions very slowly, and usually at the periphery of those networks *(15)*.

Whether the transferred gene will be incorporated and kept in the genome depends upon natural selection. If it brings selective advantage to the fitness of a specific individual it will probably be transmitted to future generations and kept in the population. If an organism has no benefit, the gene will not be transmitted to the offspring through the vertical transfer leading to its elimination from the population *(15)*.

Successful horizontal gene transfer includes two steps: successful transfer of the genetic material and survival of transferred genes through generations. Gaining of new traits via horizontal gene transfer demands the realization of these three conditions:

1) there must be a way to transfer the donor DNA into a recipient;
2) transferred sequences should be incorporated into the host genome (or connect with autonomously replicative element or survive as episome);
3) incorporated genes must be expressed and provide some benefits for the host organism *(15)*.

The key evidence for concluding whether the new sequence is horizontally transferred or not, is the genetic analysis of the transferred sequence itself. It is necessary to find whether the transferred sequence is different from the rest of the genome and in which way it distinguishes from vertically transferred genes. Such analyses are based on the fact that horizontally transferred sequences can be found among closely related strains and species and they can be detected using specific markers *(19)*.

Some of the properties and functions that can be gained through horizontal gene transfer are: antibiotic resistance, virulence (e.g. plasmids or pathogenicity islands) and expansion of metabolic pathways (e.g. synthesis of new primary and secondary metabolites, which enable development in new ecological niches). Gaining new properties might result in adaptation in new ecological niches and potentially in speciation *(20)*.

One of the most important differences between horizontal and vertical gene transfer is in how long it takes for a gene transfer event to induce changes in the genome. It takes much longer for horizontal gene transfer to create

new phenotypes as well as changes in the genomes and phylogenetic relations. Vertical gene transfer (includes mutations, gene rearrangements and other intragenic changes in DNA sequence) can result in forming new variations in existing phenotypes much quicker *(15)*.

Horizontal gene transfer in cyanobacteria

Cyanophages – viruses that infect cyanobacteria, are one of the most important ways of horizontal gene transfer among cyanobacteria. They are capable of rapid sequence transfer between two cyanobacterial organisms and also between cyanobacteria and purple bacteria (Proteobacteria), green sulfur bacteria (Chlorobi), green filamentous bacteria (Chloroflexi) and Gram-positive heliobacteria (Firmicutes) *(21)*.

Cyanobacterial genes can be divided into two groups due to the frequency of their horizontal transfer: core genes and shell genes. Genes coding for complex systems with many macromolecular interactions are less prone to horizontal gene transfer than the genes coding for small complexes of several gene products. Thus, photosynthetic and ribosomal genes, which share similar evolutionary histories, belong to the cyanobacterial genome core. They are resistant to the horizontal gene transfer and tend to cluster into putative operons, containing two to four genes that are highly conserved among all cyanobacteria and plastids of higher plants. Genes coding for proteins of the periphery of the photosynthetic apparatus (i.e. functional "add-ons") and for proteins whose function is still not well known, are prone to horizontal gene transfer and belong to the cyanobacterial genome shell *(21)*.

HGT probably played a crucial role in the recent modern cyanobacteria evolution. Their ability to uptake atmospheric nitrogen is a novelty when compared to their most recent common ancestor. In the late Archean to early Proterozoic eons they obtained ability to fix nitrogen due to *nif* operon that was possibly horizontally transferred from heterotrophic prokaryote to their genome *(21)*.

Horizontal gene transfer in algae

Seven different groups of algae (Haptophyta, Heterokontophyta, Cryptomonada, Dinoflagellata, Apicomplexa, Euglenophyta and Chlorarachniophytes) evolved due to the secondary endosymbiosis. The evidence for such evolution can be found in plastids of two groups of marine flagellate algae: Chloroarachniophytes and Cryptomonada. Secondary endosymbiosis is a two-step process. In the first step, a cyanobacteria is engulfed by heterotrophic eukaryote and eventually becomes a plastid. Part of cyanobacterial genes are horizontally transferred to the primary host nucleus. In the second step, eukaryote containing primary endosymbiont is engulfed by a second eukaryote and it becomes a secondary endosymbiont – the new plastid. Along with the horizontal transfer of secondary endosymbiont genes, the cyanobacterial genes of a primary endosymbiont are transferred to the new host nucleus. The nucleus of the primary host reduces and becomes a nucleomorph set between inner and outer membrane pair of a new plastid *(22)*.

As a consequence, nuclear algal genome codes for the majority of plastid proteins. These proteins are subsequently targeted to the primary and secondary plastids by N-terminal targeting peptides. Transit peptide mediates the transfer of proteins to the primary plastid. The protein transfer to the secondary plastid is mediated by a signal peptide and a transit peptide. Signal peptide targets proteins to the endomembrane system and transit peptide targets proteins to the secondary plastid *(22)*.

The model chlorarachniophyte *Bigelowiella natans* was used for analysis of horizontal gene transfer in algae. Although the majority of plastid-targeted genes were transferred from the symbiont to the host nucleus, a significant number of plastid-targeted genes were horizontally transferred from other sources. Some of those sources are: streptophite algae, red algae and red algal endosymbiotic plastids. The type of nutrition in algae has an important role for the extent of horizontal gene transfer. *B.natans* is a mixotroph, and such organisms are considered to be prone to receiving horizontally transferred genes *(22)*.

Horizontal gene transfer in fungi

It is generally accepted that horizontal gene transfer affects the prokaryotes more than the eukaryotes. Nevertheless, fungi have developed several mechanisms that make horizontal gene transfer easy. How they function is still poorly understood. Bacterial conjugational plasmids are possibly implicated in horizontal gene transfer from bacteria to fungi *(23)*. Some fungi (e.g. *Saccharomyces cerevisiae*) can be transformed with *Agrobacterium tumefaciens (24)*.

One of the important processes in fungi is anastomosis – fusing of the same or different hyphae. If the origin of fusion comes from several different spores, conidial anastomosis tubes arise, providing gas exchange, nutrients delivery and common developmental regulation. Additionally, they provide a possibility to exchange genetic material between young gametophytes, which enables them to act as universal coordinated individual. Fungi have developed mechanisms to recognize foreign DNA; however, there is evidence for anastomosis tubes formation between different species of pathogenic fungi *(25)*.

Despite the mechanisms that make HGT in fungi easier than in other eukaryotes, there are still certain barriers. Some of them are: nucleus is attached to the membrane making it difficult for the new sequence to enter the genome, in different species different intron processing is needed, gene promoters are often non-compatible and gene code is variable *(24)*.

Despite those obstacles, fungi have obtained many useful properties and functions via HGT. Genes trans-

ferred from prokaryotes provided fungi with a capacity to synthesize biotin and produce sulphates from several different organic sources. It also enabled growth in anaerobic conditions, wine fermentation, different toxins production and pathogenicity *(24)*.

Moreover, HGT in fungi has been reported in several directions. It is most easily detected from the prokaryote to fungus. The problem of alternative intron processing which exists in eukaryotes does not exist in prokaryotes; the number and variety of bacterial populations is greater than the number of eukaryotic populations; bacterial genes of some metabolic pathways can be clustered in operon which means that the transfer of a relatively small DNA fragment can transfer the whole metabolic pathway. Examples for transfer from prokaryote to fungus are genes *BIO3* and *BIO4* of biotin metabolic pathway in *S.cerevisiae (24)*. Those genes have different donors although they code for proteins implicated in two subsequent steps of the same biotin synthesis pathway. The donor for *BIO3* gene is gamma – proteo bacterium and for *BIO4* gene is alpha – proteo bacterium *(26)*.

The transfer from one to the other fungus has been reported from *Aspergillus nidulans* to *Podospora anserina (27)*. In that transfer, 23 genes of sterigmatocystin (toxic secondary metabolite) metabolic pathway are transferred allowing better adaptation to cellular oxidative stress, survival of limiting nutrient occurence and development of cellular defense mechanism *(28)*. This transfer favors the hypothesis of great contribution of horizontal gene transfer to metabolic advancements in fungi.

Despite the rarity of horizontal transfers between plants and fungi, the transfer of four plant genes has been reported in phylogenetic analyses of plant and fungi genomes. Fungi that horizontally received genes are: *Botrytis cinerea, Batrachochytrium dendrobatidis* and *Laccaria bicolor*. Transfers to *B. dendrobatidis* and *L. bicolor* involve a potential prokaryote intermediate. Genes transferred from lower plants (e.g. bryophyte *Physcomitrella patens*) to fungi mostly code for sugar transporters and transporters of small solutes found in soil or siderophore carriers. These horizontal transfers enabled exploatation of soil environments *(29)*.

Although complex, and at the same time easier than in other eukaryotes, horizontal gene transfer in fungi is important for their evolution in terms of choosing the optimal ecological niches, pathogenicity and changes in metabolic properties that allow them to inhabit new environments, synthetize new metabolites and absorb different sugars and other metabolic compounds.

Horizontal gene transfer in lichens

The molecular biology of lichens is still insufficiently explained. The basic question of all lichen molecular research is the way in which the symbiosis between fungus and alga is established and which processes it includes. The key fungi genes in the process of symbiosis are: genes coding for proteins involved in self and non-self-recognition, lipid metabolism genes, genes coding for glucose repressible proteins, an oxidoreductase, a dioxigenase, a conserved hypothetical protein and probably the most important – hydrophobins. Hydrophobins are proteins in the cell wall of fungal cells that are crucial in the symbiosis establishment; they are the ones holding mycobiont and photobiont together. Algal genes important in the process of symbiosis establishment are: gene coding for hitinase like protein, gene involved in aminoacid metabolism, gene coding for dynein related protein, gene coding for arginine methyltransferase. Through the process of symbiosis establishment, the regulation of the expression of both mycobiont and photobiont genes is modified in order to allow better adaptation to newly arisen symbiosis *(30)*.

Horizontal gene transfer between mycobiont and photobiont is still unrecorded, but it was shown that mycobiont and photobiont can be recipients of closely related gene groups such as ammonium transporters (AMT) and methylammonium permeases (MEP) *(31)*.

The question that arises is whether the horizontal gene transfer between photobiont and mycobiont really is necessary. It is likely that symbionts, through the process of symbiosis itself, use whatever they need from all three different kingdoms. It is, therefore, possible that mycobiont and photobionts adaptations are necessary for connecting three separate evolutionary pathways. Lichen symbionts might be taking advantage of separate cyanobacterial, green algal and fungal adaptations to the environment and to each other. In that case, there is no need for gene transfer between those three groups of organisms when they form single symbiotic organism.

On the other hand, lichens are hard to cultivate in laboratory conditions and most of mycobionts do not exist as free-living organisms. For this reason there might be no records of horizontal gene transfer between a mycobiont and a photobiont. Without a reference genome available for investigation it cannot be concluded what the causes for different changes/adaptation of genomes are.

Whether, and in what proportion, the survival success of lichens as symbiotic organisms and their evolution rate depends on horizontal gene transfer between mycobiont and photobiont thus remains to be investigated.

DISCUSSION

There are several different processes in genome evolution. Symbiotic organisms like lichens, go through all of them. Three kingdoms (Monera, Plantae and Fungi) include potential mycobiont and photobiont partners. Each kingdom, besides the horizontally interlinked evolution with other kingdoms, evolves separately. Lichens use the most favorable combination from the evolution of all three kingdoms.

Vertical gene transfer in all lichen components ensures the best possible adaptation to sudden environmental changes. Asexual reproduction dominates in continuous habitats and sexual reproduction dominates

in fast changing habitats. The prevalence of certain reproduction mode depends on lichen species and habitat. Moreover, in sexually reproducible populations, small spore size ensures long distance dispersal and colonization of new habitats. Long distance colonization includes volcanic islands, polluted urban habitats that had not been colonized for a long time, destroyed woodlands and areas that have been covered with ice for a long time *(5,8)*. On the other hand, horizontally transferred genes in cyanobacteria, algae and fungi enable lichens to adapt over the long period and to make the best possible symbiotic combination of mycobiont and photobiont available in the specific environment.

Lichens can serve as a shelter for different organisms enabling their survival and can act as pioneers of vegetation in areas were other species cannot survive.

Because of their secondary metabolites and enzymes, lichens can be used as pharmaceuticals. Polysaccharides of the species *Umbilicaria esculenta* are shown to be useful in HIV treatment *(32)*. They have inhibitory effects on HIV replication under the laboratory conditions. The most well-known commercially used species is *Cetraria islandica* (Figure 1B). Its secondary metabolite is used in the production of the cough syrup. The *Lobaria pulmonaria* extract is quite effective in prion degradation. It contains a serine protease that degrades a misfolded prion protein, the causative agent of spongiform encephalopaties *(34)*. The secondary metabolites can also be used for perfume production. Some lichen species are edible and some are used for fabric colouring. Unfortunately, extensive exploitation has led to a reduced size of some lichen populations *(1)*.

Bearing in mind the importance of lichens as the founder organisms of newly forming vegetation and their potential use as therapeutics, it is important to develop conservation strategies that will counteract their current exploitation. Additionally, lichens can serve as a model for detection of environmental changes. Horizontal and vertical gene transfer can therefore provide insight into genetic fluctuations that occur as an answer to the current ecological conditions in the environment. Intensive research of gene transfer can detect certain patterns of gene changes in time and modes of adaptation resulting from gene transfer. Present state of lichen populations reflects what environmental changes lichens are currently adapting to as well as what should be changed or preserved in order to maintain the diversity of different species and genotypes among symbionts.

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