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Recycling of pathogenic microbes through survival in ice

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Summary Viable microorganisms (e.g. fungi, bacteria, Archaea and viruses) are distributed by wind over great distances, including globally. Microbes may settle out of the atmosphere or may be incorporated into fog, rain, sleet, hail, or snow. These organisms fall into lakes, streams, oceans, or onto the land or glaciers. When they become incorporated into environmental ice (e.g. glaciers, ice sheets, and snow), those that survive freezing and thawing may persist for years, centuries, millennia, or longer. Once they melt from the ice, they may enter contemporary populations. This mixing of ancient and modern genotypes (i.e. temporal gene flow, or what we term "genome recycling") may lead to a change of allele proportions in the population, which may have effects on mutation rates, fitness, survival, pathogenicity and other characteristics of the organisms. Pathogenic microbes that survive freezing and thawing (e.g. influenza viruses, polioviruses, caliciviruses and tobamoviruses) can remain in these icy reservoirs long enough to avoid resistance mechanisms of the hosts, thereby conveying a selective advantage to these pathogens over those that cannot survive in ice. Ice is an abiotic reservoir of microbes that has been ignored in surveillance activities for human diseases.

Introduction

Our view of habitats suitable for microorganisms has changed over the past several decades. Although the biosphere has four major reservoirs for microorganisms; soil, air, water and ice; the adaptability of microbes to the extremes of these substrates is amazing. Soil contains a vast diversity of microbial forms that play important functional and ecological roles in ecosystems. Metabolically active microbes have been found kilometers underground, kilometers into the atmosphere, and almost everywhere between these two extremes [2–4,10,12,17,19]. Water, like soil and air, is more than a reservoir because it serves multiple purposes, including providing suitable habitats for microbes, moderating temperatures, mediating chemical reactions, and transporting food, as well as the microbes themselves. Ice is a unique repository because it concentrates time and acts as

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an historical trap for organisms from different time periods. It provides insights into past communities as well as an indication of the influx of ancient microorganisms into contemporary communities. More importantly, it may trap pathogenic microbes and later release them into a population of hosts that lack resistance to the pathogens.

Migration, mixing and gene flow

Environmental ice (e.g. glaciers, ice fields, snow and permafrosts) provides a mechanism for temporal gene flow, which we have termed "genome recycling" (Fig. 1). It is dependent on the revival and establishment of organisms once they emerge from the ice, migration to a suitable location, and gene flow into the extant populations. This can only occur if the organisms are in sufficient numbers and the genes survive and propagate within extant populations [20]. This is dependent on the presence of environmental conditions, transport mechanisms, population sizes and the fitness of alleles. Depending on local and global weather patterns, as well as substrates for specific organisms, the probability of growth, migration, deposition, release and gene flow will necessarily vary from one year to the next. Portions of the pathogen population can complete the cycle by becoming frozen into another ice matrix.

Glacial cycles

Environmental ice carries a diverse mixture of organisms. Glaciers and ice fields entrap and release huge numbers of microbes, sometimes for more than 500,000 years. Since the last glacial maximum from 18,000 to 25,000 years ago, glaciers have been releasing enormous numbers of ancient microorganisms as they recede. Over the past 1000 years, the rate of glacier retreat appears to have increased. More recent accelerations in glacial recession have been recorded, a major one during the 19th century, and a further acceleration during the 20th century. For example, approximately 95% of the ice on Mt. Kilamanjaro has melted during the past 100 years. Hubbard Glacier in Alaska has receded over 50 km during the past 1000 years, with a substantial portion of the loss occurring during the past century. The Rhone Glacier in central Switzerland has receded 3 km in the past 400 years, representing a loss of over 10^8 m^3 of ice [14]. Similarly, the Aletsch Glacier in Switzerland has receded over 2.5 km during the past 100 years, representing a loss of over 10^{10} m^3 of ice. Ice ages tend to decrease the release and mixing rates, while periods of global warming, such as the current one, increase the rates.

Based on results from several studies [1,7—9, 13,21,23], approximately 10^3—10^7 viable microbes are present in each liter of environmental ice meltwater. This translates into an annual release

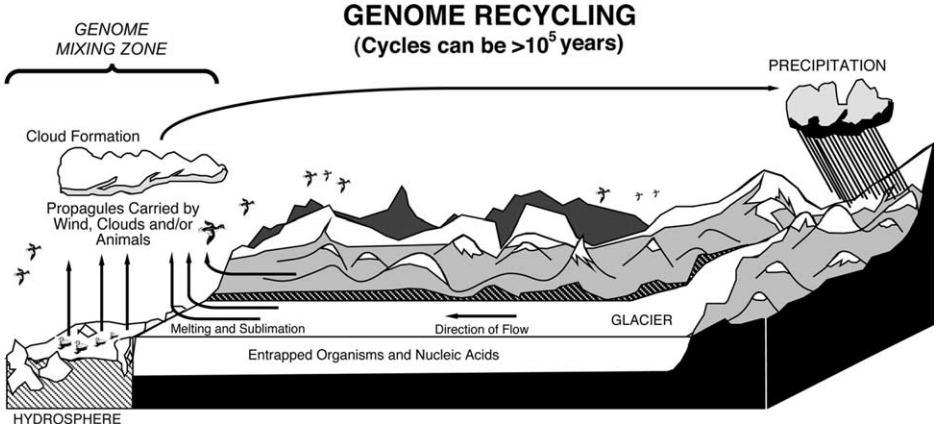

Figure 1 General scheme of Genome Recycling through glacial ice. Organisms comprising a subset of a population travel into the atmosphere by wind, vulcanism, bolide impacts, animals, etc. They are then deposited onto the glacier by precipitation (which deposits not only the organisms from the clouds, but also forces microbes to the ground from the atmosphere through which the precipitation falls) or simply by falling onto the glacial surface. The organisms are then entrapped in the glacier for days, years, centuries, millennia, or longer (depending on the length and speed of the glacier, as well as long term trends in temperature). The organisms then melt from the glacier and flow into the hydrosphere (streams, rivers, lakes, seas and oceans) or are picked up by wind or animals and transported to other locales. In this mixing zone (essentially the contemporary environment), given the proper substrate, they may grow and reproduce, eventually interacting with other extant populations of the same species and/or with susceptible hosts.

of at least 10^{17}–10^{21} viable microbes (including fungi, bacteria and viruses) from environmental ice, which is equivalent to 10^{3}–10^{7} metric tons of microbial biomass. If spread evenly over the surface of the Earth, this would be approximately 10^{2}–10^{6} propagules per species per square meter. Tens of thousands of species may be represented. Microbes are found in clouds, fog, precipitation and elsewhere in the atmosphere [5] indicating that transportation mechanisms are readily available. Animals, oceans, streams, and other forms of transportation also are common. Depending on wind currents, season and precipitation, propagules may be distributed worldwide, thus increasing the chances of landing on a suitable substrate. This represents a migrating population large enough to become established and to interact genetically with contemporary populations virtually anywhere on Earth.

Many of the organisms entrapped in glaciers are pathogens of various organisms, including humans. Strains of pathogenic organisms (e.g. influenzaviruses, caliciviruses, polioviruses and tobamoviruses) may disappear for decades, centuries, or longer, and then reappear to infect virgin host populations [6,22]. Thus, when they are released, they may be able to easily infect a population of hosts that lacks immunity or defenses against these particular strains of microbes. However, previous studies and health surveillance efforts have ignored these reservoirs of potentially harmful microbes. Understanding the process of release of organisms from melting glaciers is important to our understanding of epidemics or pandemics that may be caused by these pathogens. The appearance of identical disease strains separated by decades and longer has been previously reported. For example, the appearance of genetically identical influenza A (an RNA virus) strains separated by decades of absence, has been reported [22]. The sudden appearances, disappearances, and reappearances of many marine caliciviruses could be satisfactorily explained by a polar ice entrapment and release mechanism. One example is the appearance on just one occasion of vesicular exanthema of swine virus types J and K in New Jersey, their disappearance and then sudden reappearance 24 years later in bowhead whales in Barrow, Alaska, and in California sea lions [24]. Entrapment in, and later release from, environmental ice, could explain these mysterious recurrences.

Genome recycling predicts that at each point on the glacier there will exist a mixture of the genomes, implying that there is no clear separation between ancient and modern genomes, and that there is constant mixing. From our comparisons of ice-entrapped versus contemporary microbes, we conclude that the present environment consists of a mixture of genomes that have been entrapped in glaciers at vastly different past times [6,7,15,16,29]. Ancient organisms melt from the ice and enter the contemporary population of conspecific organisms. They can then have direct effects on the population through gene flow. This can affect the allele frequencies, but also effectively lengthens the life cycle of the organism. In fact, some of these microbes may have some of the longest life cycles of all organisms, if entrapment in glaciers is considered. Genome recycling may be yet another mechanism whereby microbes are able to survive detrimental events on Earth, including bolide impacts, epidemics, drought, host resistance, volcanism and ice ages. It also provides a means to store alleles on a long-term basis. These alleles may be beneficial once the organisms re-emerge from the glacier and enter a changed environment.

Effects on evolutionary processes

One of the predictions of genome recycling is that measured rates of nucleotide change should be lower than the basal rates of mutation within isolated populations. Because of the cessation or extreme retardation of growth and metabolic processes, microbes entrapped in ice are expected to exhibit nucleotide change rates close to zero, while an actively replicating population would exhibit higher rates of change, due to errors incurred during genome replication (which is expected to be highest for RNA viruses). When the populations mix, the resulting rate would be between these extremes (see [20] for discussion of allele mixing and gene flow). Previously, it has been noted that the mutation rates of microbes are lower than the predicted rates based on genome size, genome complexity, generation time, and experimental mutation rates [11,25]. We calculated rates of nucleotide change for *Cladosporium cladosporioides* (data from [15,16]), *Bacillus subtilis* (data from [29]), and the RNA virus tobacco mosaic tobamovirus (data from [6]) by comparing sequences (nuclear ribosomal DNA internal transcribed spacers, prokaryotic ribosomal DNA internal transcribed spacer, and coat protein gene, respectively) from ice samples that were up to 140,000 years old. The rates ranged from 4×10^{-5} to 8×10^{-5} per site per year. While this degree of similarity might be expected for DNA-based genomes, rates of change for RNA viruses were

expected to be higher. This apparent damping of mutation rates might be caused by the effects of genome recycling.

Conclusions

Although genome recycling has yet to be unequivocally demonstrated, the evidence collected so far is compelling. The results from many studies are consistent with the predictions of genome recycling. The major prediction is that there exists a homogenization of genomes due to gene flow between ancient and modern conspecific organisms or via introgression among heterologous organisms. The evidence for long term survival in ice is backed by a broad range of studies from workers throughout the world, thus increasing the likelihood that the process of genome recycling is possible. The problem now is to address a strategy for rigorously testing for further evidence of genome recycling. If small populations are represented in the ice, then it will be difficult to measure. The small populations would not allow accurate measurements of the variability of each population or an estimate of the total degree of variability for the species. However, if global mixing is in operation and large populations are assayed from the ice, then the measurements can be interpreted, since large numbers of organisms could be assayed in order to accurately assess genetic diversity within each ice core sample. Although this is challenging, testing for genome recycling in glaciers is possible. At the very least, surveillance should be initiated to determine the quantities of pathogens that are contained within environmental ice (e.g. glaciers, snow and lake ice), as well as the quantities released on a yearly basis. Influenza A is a likely first choice, since it is a significant human pathogen [26—28], it survives freezing [18], and is known to be present in subarctic lakes frequented by migratory waterfowl (Rogers, Zhang, Shoham and Gilichinsky, unpublished results). Another virus that survives freezing [18] is poliovirus. Since attempts are underway to eradicate this virus worldwide, it is important to determine whether any reservoirs of this virus exist in environmental ice.

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