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Abiotic Gene Transfer: Rare or Rampant?

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Abstract Phylogenetic studies reveal that horizontal gene transfer (HGT) plays a prominent role in evolution and genetic variability of life. Five biotic mechanisms of HGT among prokaryotic organisms have been extensively characterized: conjugation, competence, transduction, gene transfer agent particles, and transitory fusion with recombination, but it is not known whether they can account for all natural HGT. It is even less clear how HGT could have occurred before any of these mechanisms had developed. Here, we consider contemporary conditions and experiments on microorganisms to estimate possible roles of abiotic HGT—currently and throughout evolution. Candidate mechanisms include freeze-and-thaw, microbeads-agitation, and electroporation-based transformation, and we posit that these laboratory techniques have analogues in nature acting as mechanisms of abiotic HGT: freeze-and-thaw cycles in polar waters, agitation by sand at foreshores and riverbeds, and lightning-triggered electroporation in near-surface aqueous habitats. We derive conservative order-of-magnitude estimates for rates of microorganisms subjected to freeze-and-thaw cycles, sand agitation, and lightning-triggered electroporation, at 10^{24}, 10^{19}, and 10^{17} per year, respectively. Considering the yield of viable transformants, which is by far the highest in electroporation, we argue this may still favor lightning-triggered transformation over the other two mechanisms. Electroporation-based gene transfer also appears to be the most general of these abiotic candidates, and perhaps even of all known HGT mechanisms. Future studies should provide improved estimates of gene transfer rates and cell viability, currently and in the past, but to assess the importance of abiotic HGT in nature will likely require substantial progress—also in knowledge of biotic HGT.

Keywords Horizontal gene transfer · Evolution · Freeze-and-thaw transformation · Sand-agitation transformation · Lightning-triggered transformation · Electrotransformation

Introduction

Over the last quarter-century, progress in DNA sequencing has revolutionized our understanding of evolutionary relationships between organisms, but it also revealed that evolution does not proceed solely by gradual divergence of species driven by inherited random mutations and their natural selection but also by heritable interchange of genetic material among species (horizontal gene transfer—HGT). This started to emerge in the early 1990s from comparing genomes, which revealed a number of bacterial genes present in eukaryotic species yet absent from any archaeal species, though eukarya are phylogenetically closer to archaea than to bacteria (Doolittle et al. 1990; Smith et al. 1992; Hilario and Gogarten 1993; Lawrence and Ochman 1998), and it was further corroborated by comparing nucleotide sequences in individual highly-conserved genes, from which it emerged that phylogenetic trees inferred from different such genes can differ considerably (Doolittle 1999; Zhaxybayeva and Gogarten 2004).

HGT is by now widely recognized as a major contributor to genetic variability of prokaryotes (Bapteste et al.

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2009), and five natural mechanisms of such transfer have so far been extensively documented:

- *competence* uptake by an organism of DNA from its surroundings (the term *transformation* is also often used for this mechanism, but this is ambiguous, as the same term is also applied more generally to any heritable incorporation of exogenous genetic material into the organism);
- *conjugation* transfer of DNA between two organisms in direct contact;
- *transduction* transfer of DNA from one organism into another via a viral infection;
- *GTA-mediated transfer* transfer of DNA by *gene transfer agents* (*GTAs*), virus-like particles that are synthesized by certain bacteria, embed fragments of their DNA, and upon release transfer these fragments into other bacteria in a transduction-like manner;
- *transitory fusion with recombination* pairwise fusion of certain archaea into a hybrid, followed by homologous recombination of their DNA, and the hybrid's fission back into two separate archaea.

This listing order reflects the chronology of discovery: competence was first documented in 1928 in *Streptococcus pneumoniae* (Griffith 1928), conjugation in 1946 in *Escherichia coli* (Lederberg and Tatum 1946), transduction in 1952 in *Salmonella enterica* (Zinder and Lederberg 1952), GTA-mediated transfer in 1974 in *Rhodobacter capsulatus* (Marrs 1974), and transitory fusion with recombination in 1989 in *Haloferax volcanii* (Rosenshine et al. 1989).

Thus, by the time the importance of HGT for prokaryotic evolution began to be appreciated, these five mechanisms had already been discovered, albeit perhaps only the first three were universally recognized and widely investigated. It was also rather clear by then that these mechanisms are all biotic, i.e., actively performed by the organisms involved in HGT, and based on proteins these organisms synthesize, which implied that each of these evolution-accelerating mechanisms is itself a product of evolution. Therefore, while a consensus was gradually forming that HGT has been ongoing since the earliest stages of evolution (see e.g., Fig. 3 in Doolittle 1999; Fig. 1 in Smets and Barkay 2005; Fig. 1 in Koonin 2009), an outstanding question persisted and still persists: how could HGT have proceeded before any of its biotic mechanisms had developed?

It also seems questionable whether each occurrence of HGT identified to date can be explained by the five biotic mechanisms outlined above; although competence, conjugation, and transduction are found in both prokaryotic kingdoms, i.e., both in archaea (Luo and Wasserfallen 2001; Johnsborg et al. 2007) and in bacteria (Chen et al. 2005; Johnsborg et al. 2007; Cann 2015), it is far from clear whether in every archaeal and bacterial species at least one of them can occur. Namely, it is estimated that only about 1 % of all bacterial species are naturally competent (Brochier-Armanet and Moreira 2015), and likely an even lower fraction of archaeal ones (Johnsborg et al. 2007; Lipscomb et al. 2011); a further hindrance to competence is rapid degradation of free DNA in natural habitats (Lorenz and Wackernagel 1994). For conjugation, though one recent study estimates it to be the most important of the HGT mechanisms (Halary et al. 2010), its efficiency quickly decreases with increasing genetic distance, as the protein machinery utilized is highly adjusted to a particular organism's envelope (Guglielmini et al. 2013). Regarding transduction, most viruses infect selectively, only transferring genes among genetically very close organisms (bacterial phages often even only bind to a single strain of a single bacterial species). And finally, there is increasing evidence of HGT in unicellular eukaryotic microorganisms, in particular microalgae and yeasts (Keeling and Palmer 2008), although neither conjugation nor competence exist in eukaryotes, nor are they in general infectable by bacteriophages or archaeal phages.

As so often in biology, there are exceptions to the general limitations. Among competent prokaryotes, it was shown that the bacterium *Acinetobacter baylyi* can take up and express even highly fragmented and degraded DNA (Overballe-Petersen et al. 2013), and this ability may be present in many more naturally competent bacteria. It is also known that mechanisms similar to conjugation can lead to gene transfer from various bacteria into certain eukaryotes: from *Escherichia coli* into the yeast *Saccharomyces cerevisiae* (Heinemann and Sprague 1989), from *Agrobacterium tumefaciens* into cells of some flowering plants where they induce tumorigenesis (Zupan et al. 2000), and from *E. coli* into Chinese hamster ovary cells (Waters 2001); still, except for *A. tumefaciens*-induced tumorigenesis in plants that occurs amply in nature, the other instances of transfer were only attained in laboratories with plasmid DNA pre-engineered for stability and transferability. Regarding transduction, several viruses with a broad host range and/or adaptive host specificity are known to exist both in bacteria (Koskella and Meaden 2013) and eukaryotes (Bandin and Dopazo 2011), and bacteriophages can be modified—at least artificially—for gene delivery into eukaryotic cells (Poul and Marks 1999).

A lesson on the (in)completeness of our knowledge and understanding in this field can also be learned from the tardiness with which GTA-mediated transfer in bacteria and recombinant transitory fusion in archaea were acknowledged as legitimate mechanisms of HGT. While—as cited above—their discoveries date to 1974 and 1989, respectively, many prominent reviews of natural HGT mechanisms left them unmentioned even over the last decade (Chen et al. 2005; Thomas and Nielsen 2005; Boto

2010; Syvanen 2012). Yet, other research suggests that this view may be subjective: some recent studies estimated that GTAs are a major mechanism of HGT (Kristensen et al. 2010; Lang et al. 2012) and perhaps even the prevalent one in the oceans (McDaniel et al. 2010), and others assessed that recombinant hybrids contribute importantly to HGT in certain archaea (Naor et al. 2012; Papke et al. 2015). And finally, while these findings are gradually recognized as a basis for extending the list of HGT mechanisms from three to five, other recent studies suggest that the extension may not stop there, as there may well be other biotic HGT mechanisms, such as DNA-packing vesicles formed by the budding of the outer membrane in Gram-negative bacteria (Kolling and Matthews 1999; Yaron et al. 2000; Renelli et al. 2004; Mashburn-Warren and Whiteley 2006), and transfer of DNA through intercellular nanotube bridges forming among some bacteria when they are sufficiently close to each other (Dubey and Ben-Yehuda 2011).

With so many exceptions, and exceptions to these exceptions, it is likely impossible to pinpoint a case of HGT for which it could be inferred, let alone rigorously proved, that none of the known biotic HGT mechanisms, or yet unknown natural adaptations thereof, can explain its occurrence. Thus, while our knowledge and understanding of HGT is rapidly increasing, we are far from a reliable assessment of the relative importance of each of the known HGT mechanisms, and farther still from a steadfast conclusion that the known HGT mechanisms are the only such mechanisms.

We have recently suggested that lightning-triggered electroporation of organisms' envelopes acts as a natural abiotic mechanism of HGT, causing both DNA release and uptake (Kotnik 2013a, b; Weaver 2013). In this paper, we approach the topic of abiotic HGT mechanisms more broadly, positing that three physical methods used for artificial genetic transformation—freeze-and-thaw, microbeads-agitation, and electroporation-based transformation—all have prominent analogues in nature: freeze-and-thaw cycles in polar waters, agitation by sand in waters at riverbeds and foreshores, and lightning-triggered electroporation in all aqueous habitats accessible to lightning strokes. We outline quantitative estimation of the efficiency of these mechanisms as agents of genetic transformation. Clearly, uncertainties in such estimates are large, in some cases spanning many orders of magnitude, and reducing them will require many parameters to be evaluated and re-evaluated, but this does not rule out a useful plausibility argument.

Laboratory HGT Techniques are All Abiotic

To replicate genes artificially, laboratory techniques rely on DNA polymerase—the same biomolecule that performs this process in the natural environment; in contrast, to transfer genes artificially, none of the laboratory techniques utilizes the enzymes, membrane receptors, and/or other biomolecules employed in natural HGT; instead, they all rely on rather elementary mechanisms of restricted and temporary membrane permeabilization. Of the five techniques presented below, the fourth one—electroporation-based transformation (electrotransformation)—is prevalent today due to its by far the highest efficiency and broadest applicability, but we describe them in their chronological order of laboratory introduction, as this allows also outline how some deficiencies of the earlier techniques were overcome by the ones developed later.

Chemotransformation

Chronologically, the first technique of artificial gene transfer was chemical, based on exposures to highly supraphysiological ($\sim$100 mM) concentrations of divalent ions—initially Ca^{2+} (Mandel and Higa 1970; Cohen et al. 1972), and later combinations of Ca^{2+}, Mg^{2+}, and/or Mn^{2+} (Lederberg and Cohen 1974); these disrupt the membrane, and simultaneously facilitate the contact between extracellular DNA and the membrane (Weston et al. 1981; Trump and Berezesky 1995). Still, in microorganisms enveloped also by a cell wall, unless the latter is disrupted by complementary chemicals such as detergents, the efficiency of chemotransformation is limited to at most $\sim 10^3$ transformants (i.e., viable microorganisms with expression and heritability of the transferred genes) per μg DNA; moreover, the effects of ions' disruptive action accumulate until these ions are removed or highly diluted, which makes high rates of cell death almost impossible to avoid (Aune and Aachmann 2010). Thus, in search of a mechanism whose permeabilizing action can be halted more abruptly and thus better controlled, researchers soon turned to physical methods.

Freeze-and-Thaw Transformation

In the first attempts to replace a chemical exposure by a physical mechanism of permeabilization, researchers utilized cycles of freezing and thawing. The first report used freezing at −70 °C and thawing at 37 °C (Dityatkin et al. 1972), but later studies revealed that the efficiency of transformation is practically unaffected if the temperature used for freezing is decreased to −196 °C, or increased to −20 °C (Merrick et al. 1987). This method was shown to work with diverse bacteria (Holsters et al. 1978; Weiss and Falkow 1982; Merrick et al. 1987), but its simplicity was overshadowed by its low efficiency, which, unless augmented by permeabilizing chemicals (Merrick et al. 1987; Stepanov et al. 1990; Zibat 2001), is at most $\sim 10^3$ transformants per μg DNA, i.e., within the same order of

magnitude as chemotransformation alone. Furthermore, while both cooling and heating can generally be halted more rapidly than chemical exposure, they are based on heat transfer through the sample, so halting the action of the cooling/heating sources does not instantaneously halt the temperature changes throughout the medium containing the microorganisms.

Microbeads-Agitation Transformation

The next technique developed for artificial gene transfer was mechanical disruption; this allowed for quick and thus well-controlled triggering and halting of the permeabilization process, but with rather variable permeability induced in individual microorganisms. The initial approach consisted of scraping the cells with a rubber stick, which only works if the cells are attached to a surface (McNeil et al. 1984), but this was quickly supplanted by agitation with small glass beads in a vortex mixer, which also worked with microorganisms in suspension (Costanzo and Fox 1988). Various studies utilized beads with diameters from 0.15 to 1.2 mm, and durations of vortexing or shaking from 5 to 60 s (Kindle 1990; Tam and Lefevre 1993; Hawkins and Nakamura 1999; Coll 2006). Sterilized and filtered sea sand was also tested as an alternative to glass beads and found to be at least as efficient, with best efficiencies obtained with sand grains of 0.8–1.3 mm diameter, followed by glass beads of 0.15–0.21 mm diameter (Hawkins and Nakamura 1999). Unlike chemotransformation and freeze-and-thaw transformation that were predominantly tried with bacteria, the microbeads-agitation technique was mainly used with yeasts and microalgae, but efficiencies were even lower—at most $\sim$300 transformants per μg DNA (Costanzo and Fox 1988; Kindle 1990).

Electrotransformation

The main reason why, despite its simplicity, microbeads-agitation transformation never gained prominence (even with yeast and fungi) was the contemporaneous emergence of the most universally applicable and most controllable technique of artificial genetic transformation known to date: the one based on electroporation—membrane and wall disruption by exposure to short (microseconds to milliseconds) and strong (hundreds to thousands of V/cm) electric pulses delivered to a suspension of microorganisms. Initially, electrotransformation was reported to only be achievable with the cell wall removed prior to the exposure (Shivarova et al. 1983), but as stronger pulse generators were developed, electrotransformation was soon also achieved in microorganisms with an intact wall, ranging from yeasts (Hashimoto et al. 1985) to bacteria (Chassy and Flickinger 1987), archaea (Micheletti et al. 1991), and microalgae (Brown et al. 1991). Compared to the earlier techniques, electrotransformation is thus applicable to the broadest range of microorganisms, but what was likely decisive for its rapid rise to prevalence was its far higher efficiency: up to $\sim 10^{10}$ transformants per μg DNA for Gram-negative bacteria, up to $\sim 10^{7}$ for Gram-positive bacteria and archaea (as they have a thicker wall), and up to $\sim 10^{6}$ for microalgae and yeasts (as they have both a wall and a nuclear membrane). This reflects the facts that (Nickoloff 1995; Lee et al. 2000)

(i) with electroporation, as the electric field ceases, its permeabilizing action also ceases;
(ii) electroporation is mostly reversible at moderate electric field strengths (limited and temporary membrane permeabilization, DNA uptake, retained viability), and irreversible at higher strengths (extensive membrane disruption, DNA leakage, loss of viability); in lab protocols, this enables selective exposure to the conditions either for transformation, or for DNA extraction;
(iii) the start and the end of the exposure, as well as the time courses and amplitudes of the electric field and the electric current, are well defined and, in the lab protocols, can also be preset to a high accuracy; with voltage-pulse generators, the time course and spatial distribution of the field are determined by the geometry (shape and position) of the electrodes and the voltage delivered to them, while with current-pulse generators, the field induced by a given current in the suspension between the electrodes is inversely proportional to the suspension's electrical conductivity, and to retain a fixed field in suspensions with differing conductivities, the current must be adjusted proportionally; and finally
(iv) in the lab protocols, by means of electrode design, the electric field can be made either almost uniform (e.g., for optimal yield of transformed organisms) or suitably variable (e.g., to yield mostly irreversibly porated DNA donor organisms in one region of the exposure chamber, and reversibly porated DNA recipient organisms in another region).

Pressure Shock Transformation

As electroporation gained ground due to its highest efficiency and most generally applicability as a method of genetic transformation, and perhaps also based on reasoning by analogy, several studies explored gene transfer by brief but intense pulses of mechanical pressure—pressure shock transformation, also termed sonoporation, as pressure waves in the kHz frequency range represent sound

(audible to humans up to ~20 kHz, and ultrasound at higher frequencies). In the first such report, mammalian eukaryotic cells were used, resulting in gene expression without heritability (Lauer et al. 1997), but later the approach was also demonstrated to achieve transformation in bacteria (Jagadeesh et al. 2004; Han et al. 2007; Loske et al. 2011) and fungi (Magaña-Ortiz et al. 2013). In most studies, the shock waves were delivered by generators designed for clinical lithotripsy (breaking of kidney stones by ultrasound), so the wave parameters were limited to the in-built values and/or ranges: pulse durations of several μs and peak pressure amplitudes of tens of MPa (Rivera et al. 2014); at least in bacteria, delivery of a single such shock wave may be insufficient, as one study reported that the exposure effects only became significant if the shock waves were delivered in two or more batches (Alvarez et al. 2008). Still, in one study with a custom-developed shock-wave generator, the authors reported optimal efficiency with ultrasound waves delivered at a 1 MHz frequency in a much longer exposure (90–450 s) at a much lower peak pressure amplitude (~1 MPa) (Han et al. 2007). But in general, with efficiencies up to $\sim 10^5$ transformants per μg DNA, pressure shock transformation is markedly inferior to electrotransformation and never gained more than sporadic use.

Do Abiotic HGT Mechanisms Act in Nature?

While naturally occurring concentrations of Ca^{2+} may not suffice for chemopermeabilization in any habitat (even in seawater they rarely exceed 12 mM), natural conditions not unlike those applied in the physical techniques of transformation are easily imagined.

Freeze–Thaw Cycles

Temperature fluctuations resulting in alternations of freezing and thawing occur in polar seas, as well as in the active upper layer of the permafrost in the polar and mountainous regions of the continents. Limiting our considerations to aquatic habitats in which DNA diffuses the most easily, temperature fluctuations resulting in alternations of freezing and thawing occur in polar seas in rather regular annual cycles, with the sea ice surface area fluctuating in the Northern Hemisphere by ~10 million km^2, and in the Southern Hemisphere by ~15 million km^2 (NOAA 2015); thus, the cumulative annual fluctuation of the sea ice surface area in both hemispheres, even if shorter-term fluctuations are disregarded, amounts to ~25 million $km^2 = \sim 2.5 \times 10^{13}$ m^2.

The sea ice has an average thickness of 2–3 m in the Northern Hemisphere, and 1–2 m in the Southern Hemisphere (NSIDC 2015), and the typical thickness of the first-year sea ice is ~1 m (Nakawo and Sinha 1981; Tison et al. 2008). Thus, the volume of sea water frozen and thawed is at least $\sim 2.5 \times 10^{13}$ $m^2 \times \sim 1$ m $= \sim 2.5 \times 10^{13}$ m^3 per year, even disregarding the contribution to this volume of those areas where the sea ice thickness fluctuates without vanishing. With seawater typically containing $>10^{11}$ microorganisms per m^3 (Whitman et al. 1998, Table 1), it follows that even with the regions of permafrost disregarded, the freeze-and-thaw cycles affect at least $\sim 10^{24}$ microorganisms per year. We note here that this estimate does not imply the number of transformants, as for this the number of microorganisms experiencing freezing and thawing would have to be multiplied by the ratio of those transformed by such a process; we will return to this later, in the section titled "How important are abiotic HGT mechanisms relative to each other?"

Agitation by Sand

Restricting the analysis again to aqueous environments, the action of water-driven movement of silt, sand, and gravel (henceforth all referred to as "sand," for brevity) on microorganisms inhabiting the eulittoral (foreshore) of seas and lakes, as well as in riverbeds, clearly resembles the effects to which they are exposed in artificial genetic transformation by agitation with microbeads in a vortex mixer or a shaker—particularly considering that, as already mentioned above, this lab technique works just as efficiently if sea sand is used instead of glass beads. The famous coastline length paradox can be disregarded in such an analysis, as it is the volume of agitated water that matters, and not its edge length. Even at a coarse resolution, the global sea coastline length is estimated at $\sim 10^9$ m (Schwartz 2005), and while the fraction of this length with the eulittoral covered by sand does not seem to have been documented, it seems safe to assume that it represents at least a tenth of the total length, i.e., at least $\sim 10^8$ m. Assuming very conservatively that sand only agitates microorganisms in 1 L of water per 1 m of coastline, this still implies that sand agitation occurs continuously in a volume of at least $\sim 10^5$ m^3—even disregarding all lakes and rivers due to the lack of reliable estimates of cumulative lake coastline length and riverbed area. With a further conservative estimate that it takes a full day for most of the exposed microorganisms to leave the volume of exposure and be replaced in this same volume by other microorganisms in similar numbers (by active motion and/or diffusion of the organisms, and/or by diffusion of the water containing them), we obtain an estimate of an effective seawater volume of at least $\sim 10^8$ m^3 exposed per year, which at $>10^{11}$ microorganisms per m^3 implies that the sand agitation affects at least $\sim 10^{19}$ microorganisms

per year. Again, without taking into account the ratio of transformants generated by such agitation, this says little about the importance of the mechanisms, and we return to this later. Moreover, we note that if the rates at which the microorganisms are leaving the volume of exposure are too slow, this might result in overexposure, as studies show that with vigorous exposures lasting more than 20 s, viability starts to decrease with increase of the exposure duration (Hawkins and Nakamura 1999). This could further diminish the importance of sand-agitation compared to freeze–thaw cycles in natural abiotic HGT.

Lightning-Triggered Electroporation

Focusing again on aqueous habitats, in the context of electroporation, they must be treated according to their electrical conductivity, and in this aspect, seawater differs from freshwater by at least an order of magnitude ($\sim$20–50 mS/cm for seawater, $\sim$1–3 mS/cm for freshwater lakes, $\sim$0.1–0.5 mS/cm for rivers). As seawater covers a surface area more than two orders of magnitude larger than freshwater, we first turn to saline environments, but we then show that freshwater habitats, where lightning strokes are more frequent and subject a considerably larger volume to conditions for electroporation, may well be a more important contributor to the overall number of microorganisms subjected to electroporation adequate for genetic electrotransformation.

Even after the air ionization is completed and the lightning's path through the air is fully established, the electrical resistance of this path (typically several km long) dominates over the resistance of the ground through which the current then continues its propagation; as a result, the electric current of lightning strokes is largely independent of the local composition of the ground it enters (be it highly resistive dry soil or sand, moderately resistive freshwater, or highly conductive seawater), with the median peak electric current of $\sim$30 kA (Berger et al. 1975; Anderson and Eriksson 1980; Chowdhuri et al. 2005). In aqueous habitats, it is furthermore reasonable to assume that the stroke's current spreads out roughly radially outwards and downwards from its point of entry, so that the electric current density and the electric field strength it induces decrease roughly inversely proportionally to the square of the distance from this point. Assuming for seawater its average electrical conductivity of 40 mS/cm (Kaye and Laby 1995), a radially flowing current of 30 kA induces an electric field of 9 kV/cm (sufficient for reversible electroporation of most microorganisms) at a radial distance of $\sim$3.6 cm, and hence, electroporation can occur in a hemispherical volume of at least $\sim$100 cm^3 [for the formulae on which these calculations are based, as well as for their derivation, see e.g., pp. 362–363 in (Kotnik 2013a)]. Similarly, an electric field of 30 kV/cm (sufficient for irreversible electroporation of most microorganisms) is induced at a radial distance of $\sim$2 cm, so that within the abovementioned $\sim$100 cm^3 where electroporation can occur, in the inner $\sim$20 cm^3, it is predominantly irreversible (i.e., with high probability of cell death), and in the remaining $\sim$80 cm^3 largely reversible. With $\sim 3 \times 10^9$ cloud-to-ground strokes per year, and with $\sim$1 % of them striking the seas, this corresponds to $\sim$2400 m^3 of seawater per year subjected to conditions for reversible electroporation, which at $>10^{11}$ microorganisms per m^3 implies that at least $\sim 10^{14}$ microorganisms per year are subjected in seawater to conditions suitable for electrotransformation.

In freshwater lakes, due to their much lower electrical conductivity, the volumes subjected to irreversible and reversible electroporation at the same peak electric current are about three orders of magnitude larger: assuming an electrical conductivity of 2 mS/cm (Talling and Talling 1965), 9 kV/cm is exceeded in $\sim$9000 cm^3, and 30 kV/cm in $\sim$1500 cm^3. Moreover, about $\sim 3 \times 10^9$ cloud-to-ground strokes ($\sim$99 % of all such strokes) strike continents and islands. Freshwater lakes cover more than 0.5 % of the total continents' surface area (the continents cover $\sim 1.5 \times 10^8$ km^2, of which the 30 largest freshwater lakes alone cover $\sim 6.2 \times 10^5$ km^2, or $\sim$0.41 %) under the assumption that lightning strokes are roughly as densely distributed over the land as over the lakes, corresponding to at least $\sim 1.5 \times 10^7$ cloud-to-ground strokes striking the lakes, with a volume of $\sim 10^6$ m^3 of freshwater per year subjected to conditions for reversible electroporation, which at $>10^{11}$ microorganisms per m^3 (this lower-bound estimate is valid both for seawater and freshwater, see Table 1 in Whitman et al. 1998) implies that at least $\sim 10^{17}$ microorganisms per year are subjected in freshwater to conditions suitable for electrotransformation.

To summarize, these rough estimates suggest that $\sim 10^{17}$ microorganisms per year are subjected to conditions suitable for electrotransformation, most of them in freshwater habitats.

How Important are Abiotic HGT Mechanisms Relative to Each Other?

The above considerations suggest that under contemporary conditions, per year, at least 10^{24} microorganisms are affected by a freeze-and-thaw cycle, at least 10^{19} are agitated by sand, and at least 10^{17} are subjected to conditions suitable for electrotransformation. Still, the ratios of these numbers do not imply the relative importance of the three mechanisms; for this, each number would have to be multiplied by the particular mechanism's efficiency. For a definitive answer, these efficiencies would have to be known

at naturally occurring concentrations of environmental DNA suitable for transformation, and to complicate matters further, these concentrations are combinations of DNA released by the mechanism under consideration and DNA released due to other causes of cell death. Thus, reliable answers will certainly require substantial measurements and experiments.

Still, a very rough, order-of-magnitude assessment can be made based on the ratios of highest efficiencies achievable under optimized laboratory conditions stated in the preceding sections: electrotransformation yields up to $\sim 10^{10}$ transformants per μg DNA for Gram-negative bacteria, up to $\sim 10^{7}$ for Gram-positive bacteria and archaea, and up to $\sim 10^{6}$ for microalgae and yeasts; freeze-and-thaw transformation yields up to $\sim 10^{3}$ transformants per μg DNA for bacteria but has not been documented in archaea, microalgae, or yeasts; and sand-agitation transformation yields up to ~ 300 transformants per μg DNA in microalgae and yeasts but has not been reported in bacteria or archaea.

Assuming that electrotransformation is indeed by a factor of $\sim 10^{7}$ more efficient than freeze-and-thaw for Gram-negative bacteria, this may well compensate for the $\sim 10^{7}$ times more such bacteria exposed to conditions suitable for freeze-and-thaw transformation than to those for electrotransformation, resulting in the number of electrotransformants similar or even higher than the number of freeze-and-thaw transformants—at least among Gram-negative bacteria. Similarly, assuming that electrotransformation is by a factor of $\sim 10^{3}$ more efficient than sand-agitation for microalgae and yeasts, this may even overcompensate for the $\sim 10^{2}$ times more microorganisms exposed to conditions suitable for sand-agitation transformation than to those for electrotransformation, resulting in more electrotransformants than sand-agitation transformants—at least among microalgae and yeasts. For Gram-positive bacteria and archaea, such compensating effects seem less likely.

The above assessment is limited to contemporary conditions and current microorganisms. Clearly, throughout evolution, the conditions have differed considerably (e.g., for freeze-and-thaw cycles during the ice ages, and for lightning stroke rates during periods with intense volcanic activity). Also, the microorganisms may have differed importantly in their ability for biotic HGT, in the natural permeability of their walls and membranes to DNA (it may have been much higher in protocells and primitive bacteria), and perhaps also in their susceptibility to the abiotic permeabilization mechanisms treated above. Any assessment of the relative importance of abiotic HGT during evolution will thus have to be based on extensive quantitative knowledge of the geological and biological history of Earth—a daunting task, but one with potentially paramount implications for understanding of evolution.

How Important is Abiotic HGT Compared to Biotic HGT?

This is a question that we are unable to answer—even in rough estimates. As outlined in the introductory section, even among biotic HGT mechanisms, assertions on their relative importance vary wildly, with some prominent experts claiming that competence, conjugation, and transduction can explain virtually all known HGT in prokaryotes (Syvanen 2012), and other contemporary studies positing that GTAs are the predominant mechanism of HGT in the oceans (Kristensen et al. 2010; McDaniel et al. 2010; Lang et al. 2012). Thus, perhaps the only aspect that the existing evidence suggests rather clearly is that in the sense of attainable phylogenetic distance between the DNA donor and recipient microorganisms, electrotransformation acts not only more broadly than other abiotic HGT mechanisms but in general also more broadly than any of the biotic mechanisms. Other than this, to assess what fractions of the natural HGT are due to each individual mechanism, and which HGT mechanism dominated before any of the biotic mechanisms had evolved, will likely require substantial progress in our knowledge not only of abiotic but also of biotic HGT.

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