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Prokaryotic diversity, electrified DNA, lightning waveforms, abiotic gene transfer, and the Drake equation: Assessing the hypothesis of lightning-driven evolution

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In a review article in this journal [1], I discussed the feasibility of lightning-triggered electroporation and electrofusion acting as purely (bio)physical mechanisms of horizontal gene transfer and thus contributing to biological evolution, particularly among the prokaryotes. I would like to thank the authors of all commentaries on this article [2–6], and here I try to address the most important issues raised in them.

Golberg [2] stresses that for different bacteria, the range of electric fields strengths causing reversible electroporation with ability for DNA uptake generally differs, and so does the range of electric field strengths causing irreversible electroporation with DNA release. This is true for prokaryotic organisms in general (both bacteria and archaea), and consequently, in the area where some prokaryotic species are electroporated irreversibly, others may be electroporated reversibly, avoiding the need for DNA to travel from the area where it is released from an irreversibly porated prokaryote to the area where it can enter a reversibly porated prokaryote and transform it. As I tried to emphasize in the last paragraph of Section 3.1 in my review article [1], the ranges of reversible and irreversible electroporation overlap even for prokaryotic organisms belonging to a single strain, because individual organisms vary in their size (due to being in different stages of the division cycle) and in their orientation with respect to the field direction, and also because pore formation is to some extent stochastic. But indeed, for two randomly sampled organisms belonging to different strains, there will – at least on average – be larger differences in their shape, size, as well as in composition of their wall and membrane, than if the two organisms being compared are of the same strain. Furthermore, the more distant the two strains are phylogenetically, the more pronounced – again, at least on average – most of these differences will be. With increasing differences between the donor and the acceptor, the availability of DNA released from the

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donors to electrotransformable acceptors will therefore generally increase, at least partially compensating for possible decrease in transformation efficiency between genetically distant species.

In addition, Golberg points to a recent study implying that electric field pulses with durations and amplitudes sufficient for electroporation can also induce nicks (single-strand breaks) in DNA [7], suggesting that this might lead to enhanced DNA integration into the host genome, thus further increasing the feasibility of lightning-triggered horizontal gene transfer. Since these results were obtained with naked (freely dissolved) DNA, they may perhaps not directly imply that a single lightning stroke can cause both the release of a DNA molecule from the interior of an irreversibly electroporated prokaryote and the nicking of this same DNA molecule, but multiple lightning strokes are also abundant – roughly every second negative lightning flash consists of two or more strokes (see e.g. Table III in [8]) proceeding along the same path.

Liberti, Apollonio, Merla, and d'Inzeo [3] stress that for experimental assessment of the hypothesis of lightning-driven horizontal gene transfer, the setup and exposure protocol have to be soundly designed, particularly from the aspect of emulating the electrical properties of the lightning as realistically as possible. In a manuscript currently under review, with my PhD student Igor Marjanovič we tried to focus on this aspect, developing a prototype exposure system that provides easy sample insertion and removal, protection from airborne particles, observability during the experiment, easy discharge generator connection, accurate discharge origin positioning, and discharge delivery into the sample either through an electric arc with adjustable air gap width or through direct contact [9].

A concern is often expressed that due to their considerable downscaling in total electric current of the discharge as compared to natural lightnings, such exposure systems can not realistically emulate effects of natural lightnings. But the crucial point in this aspect is that in lightnings striking a natural environment, the electric current dissipates roughly radially downward and outward from its point of entry, so that the electric current density and the electric field induced by it are roughly inversely proportional to the square of the distance from this point. For example, considering an electric current of a lightning stroke with a time course $I(t)$, the electric current density and electric field strength induced by this current at a distance X from its point of entry into a given medium are the same as those caused by an electric current with a time course $I(t)/10\,000$ at a distance $X/100$ from its point of entry into the same medium.

Peak currents of lightning strokes are in the range of tens to hundreds of kiloamperes, and by injecting a current with the same waveform but downscaled in magnitude by a factor of 10 000 (i.e., in the range of several amperes, which is achievable by a broad range of commercially available high-voltage pulse generators and/or amplifiers), the effects caused e.g. at 5 millimeters from its entry into a sample of a given natural environment emulate rather realistically the effects caused by a natural lightning stroke at approximately 50 centimeters from its point of entry into this natural environment. As we elaborate in our abovementioned manuscript, three-dimensional radial dissipation of the current in an exposure system can be ensured by shaping the system's receiving electrode as a conductive hemispherical bucket, filling this bucket with a medium containing the organisms to be exposed (for realistic studies, the medium has to be their natural habitat, or at least its adequate emulation), and injecting the current from the emitting electrode centrally into the surface of the medium through either direct contact or an electric arc.

Certainly, the very highest current densities of lightning strokes and electric fields induced by them do not occur in downscaled exposure systems, but those extreme conditions damage all living matter irreversibly and lethally, and moreover through mechanisms of heating and/or electrical breakdown, which are rather elementary and well understood.

The crucial remaining issue is then to ensure that the waveform of the electric current injected into the investigated sample by an exposure system is sufficiently similar to those of lightning strokes. The left panel of Fig. 1 shows a typical time course of the total electric current in the most common, first negative lightning stroke discharged into the ground, obtained by taking the normalized lightning current waveform from Fig. 12 of Ref. [10], and fitting it to the median statistical parameter values of such a current (30 kA peak value and 5 μs zero-to-peak time) from Table I of Ref. [8]. For comparison, the right panel of Fig. 1 shows the time course of the total electric current generated by a 0.25 μF capacitor precharged to 400 V and discharged centrally into a disk-shaped sample of agar (common medium for culturing of bacteria) with 86 mm diameter and 1.72 mm thickness, with one contact of the capacitor connected to the receiving electrode shaped as a ring encircling the sample, and the other contact brought into point-like contact with the center of the sample via a fast semiconductor switch (switching time below 0.1 μs) [9].

Disregarding the differences in scale, the comparison shows a relatively close similarity, reflecting the fact that polarization between a cloud and the ground resembles that of a parallel plate capacitor. This suggests that capacitor-discharge generators, with suitably adjustable capacitance and peak discharge voltage, may be quite useful

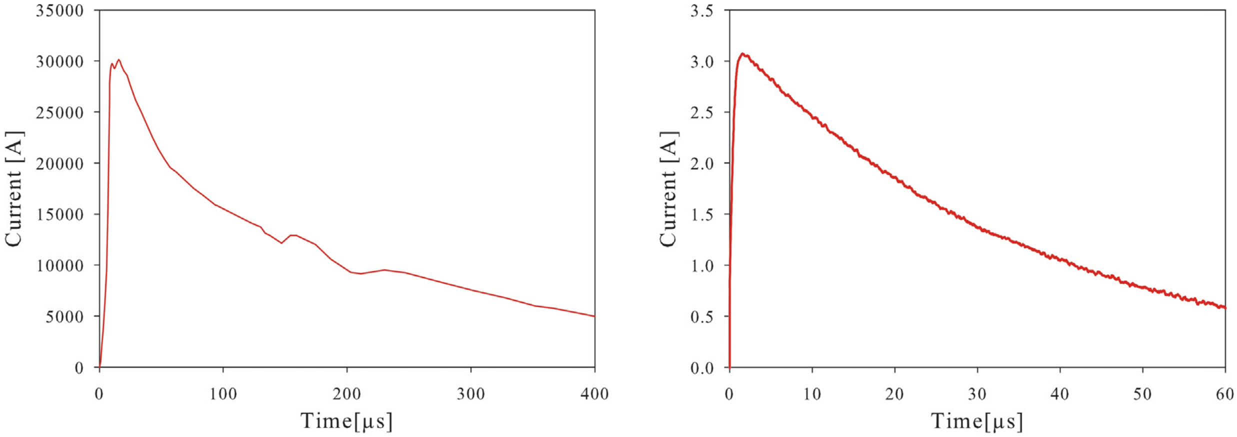

Fig. 1. Time courses of the total electric current generated by a lightning stroke and a parallel plate capacitor discharge. Left: A natural negative first lightning stroke discharged into the ground, with the normalized waveform taken from Fig. 12 of Ref. [10], and with waveform's peak current and zero-to-peak risetime fitted to the median statistical values as given in Table I of Ref. [8]. Right: A 0.25 μF capacitor charged to 400 V and discharged through an agar plate. Note the differing current and time scales in the two panels.

in emulating lightning strikes (as the time constant of the discharge is linearly proportional to the capacitance of the system, the difference in time scales of the two discharges compared in Fig. 1 could thus be bridged by using in the artificial discharge a ∼1.66 μF capacitor instead of the 0.25 μF one).

The observed differences, particularly the irregularities in the time course of the lightning strokes' electric current (see the left panel of Fig. 1), largely reflect the facts that the cloud-and-ground system is neither fully parallel nor entirely planar, the charge is not completely uniformly distributed over the polarized surfaces, and lightnings propagate in stages and through different layers of air. If one would want to emulate the lightning's current as realistically as possible, this would raise the requirements for the current generator, as instead of a simple capacitor discharge delivered through a sufficiently fast switch, a programmable function generator would have to be used. In such an approach, the data describing the sampled time course of the particular stroke to be emulated have to be suitably downscaled in magnitude, uploaded into the programmable generator, its output current amplified by a suitably powerful and fast current amplifier [11], and the amplified current injected into the sample. This approach would also allow to emulate closely the components detected in the frequency spectra of lightnings [12,13], as proposed by Liberti, Apollonio, Merla, and d'Inzeo [3].

Rubinsky [4] suggests that there are two additional plausible purely physical mechanisms of horizontal gene transfer. One of them is also based on electroporation in aqueous habitats, only not at their surface and due to lightnings, but at their bottom and due to the fact that relatively modest electrical charging, when occurring between two parallel surfaces very close together, can result in a rather strong electric field in the space separating these surfaces. Thus, polarization in materials with sheet-like microstructure, which is found in many clays and some other minerals, could cause electroporation of organisms inside – or bridging laterally – the space between the adjacent sheets [14]. As the inter-sheet distance in most clays is below a micrometer, this seems prohibitively close for any microorganism to squeeze inside, but as Rubinsky [4] himself points out, an organism coming into direct contact with the edges of two adjacent sheets in the manner as to bridge the inter-sheet gap by its membrane, could still be electroporated in that area of the membrane. In the second mechanism proposed by Rubinsky, the pores allowing for DNA exit and/or entry occur not due to electric fields, but due to membranes being pierced by ice crystals [15].

Indeed, while evolutionary biology currently acknowledges only the three "standard" and well-researched biochemical mechanisms of horizontal gene transfer (bacterial conjugation, natural bacterial competence for DNA uptake, and viral transduction), physical mechanisms of horizontal gene transfer may abound as well. And unlike the biochemical mechanisms, which are all rather intricate and must have formed during certain stages of biotic evolution, the physical mechanisms can explain how horizontal gene transfer could have proceeded since the very dawn of cell-based life.

Teissié [5] notes that in addition to the electric current, a lightning also generates a mechanical and a thermal shock wave that propagate through the struck environment, and he stresses that such shock waves have been shown to affect the microorganisms in aqueous habitats, where they can cause their destruction [16], and under some conditions their transformation [17]. Like the extended overlapping suggested by Golberg [2], the additional shock-wave effects could also perhaps enhance gene transfer between microorganisms in aqueous habitats, but a thorough assessment of the possibly supra-additive effects of electric fields accompanied by mechanical and/or thermal shock waves will likely require considerable theoretical and experimental effort. Teissié [5] also points out that possible relevance of electrofusion in evolution was already contemplated thirty years ago, in two papers by the group of Ulrich Zimmermann [18, 19]. It is embarrassing to admit that I have been unaware of these two papers, but it is a fact, and stressing that I made every effort to perform a thorough literature search can not much alleviate my regret that I missed these two papers. Weaver [6] cites a third paper of the same group, published on the same topic in the same period [20], reinforcing my discomfort for not being able to find any of these papers myself.

Weaver [6] also raises the question of realistic lightning waveforms, which I tried to address above (see Fig. 1 and the discussion in the paragraphs surrounding it), and most importantly he proposes a quantitative approach for estimating the importance of lightning-triggered horizontal gene transfer in evolution. He bases his proposed formula on the famous Drake equation [21] that expresses the estimated number of currently existing intelligent civilizations in our galaxy as the product of the rate of star formation and the conditional probabilities of nested events required for an intelligent civilization to develop and exist at the present time.

In Weaver's proposed recasting of the Drake equation, the estimated number of evolutionarily significant gene transfers triggered by lightnings is expressed as the product of the rate of lightning strokes per unit time, the number of prokaryotes electroporated by a single lightning stroke, the fraction of electroporated cells that undergo evolutionarily significant gene transfer, and the time span during which such gene transfer has been ongoing.

Weaver concludes that the most difficult variable to evaluate in this formula is the fraction of electroporated prokaryotic organisms that undergo evolutionarily significant gene transfer, but also stresses that for the total number of evolutionarily significant gene transfers to be negligible, this fraction would have to be extremely low – below one occurrence of evolutionarily significant gene transfer per 10^{25} occurrences of electroporation. While this fraction clearly depends on the concentration of DNA available in the vicinity of electroporated prokaryotes (e.g. with no extracellular DNA it would be zero), irreversible electroporation itself causes DNA release. Furthermore, transformation by means of natural competence has been detected in a number of aquatic bacteria (see e.g. Table I in [22]), and this fact implies that at least in some aqueous habitats, the extracellular concentration of DNA present naturally (due mostly to release from dead microorganisms) suffices for significant DNA uptake. Therefore, although it is clear that extensive – and very interesting! – research is still ahead, it is difficult to disagree with the general conclusion made by Weaver: unless there is some yet unknown fundamental mechanism hindering it dramatically, lightning-triggered horizontal gene transfer and its contributions to the biological evolution can not be negligible.

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