Cell Fusion by Simulated Atmospheric Discharges: Further Support for the Hypothesis of Involvement of Electrofusion in Evolution

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Electrofusion of mesophyll cell protoplasts of *Avena sativa* was induced by simulated atmospheric discharges. It is shown both experimentally and theoretically that the potential differences which occur at quite long distances from the point of lightning stroke are large enough to induce fusion. Besides electromagnetic waves which are emitted during lightning (G. Küppers and U. Zimmermann, FEBS Lett. **164,** 323 (1983)) cell fusion may have also occurred directly by means of the voltage built-up on the earth during evolution in response to a lightning stroke.

Introduction

We recently reported that electrofusion of cells can be achieved by the emission of electromagnetic waves during a spark discharge initiated a significant distance away from the fusion chamber [1, 2]. The finding that the Pt-wires of the fusion chamber could be replaced by ore-containing pieces of rock suggested that electrofusion may have represented an important step in the evolutionary process and that new species may have arisen in this way by means of electromagnetic waves emitted by lightning during thunderstorms [1–3]. It is generally accepted that there were periods of great thunderstorm activity during evolution.

In this communication we demonstrate by means of artificial lightning discharges that cell fusion is elicited not only by the electromagnetic waves emitted during a lightning discharge but that voltage differences arising on the earth's surface from travelling electrical waves may also induce cell fusion at a sufficient distance away from the point of a lightning stroke. The intensity of the electrical field is sufficiently high both to establish the required close membrane contact between cells in a suspension of high density (by dielectrophoresis) and to induce a reversible electrical breakdown in the membrane contact zone, which results in the fusion of two adhering cells [3–9].

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Theoretical background

Lightning arises from charge separation within large cumulo-nimbus clouds [10, 11]. Various discharge phenomena may be distinguished depending on the polarity of the cloud discharge and on the direction of spread. In the most common form of lightning the clouds are negatively charged (on the lower side), and the lightning is discharged from the cloud to earth (so-called negative cloud to earth lightning in 88% of all cases). The current impulse on the ground resulting from a lightning discharge occurs in the following typical forms (Fig. 1). The lightning current amplitudes vary considerably and may assume values between 3 and 400 kA. In about 90% of cases the value exceeds 10 kA, in 10% 80 kA and in 1% 200 kA [11–13].

Lightning discharge represents an impressed current, *i.e.* the impedance of the ground does not influence the course of the lightning current $J_B(t)$ at the site of the lightning strike. In the ground and along the surface field intensities $E(r, t)$ and poten-

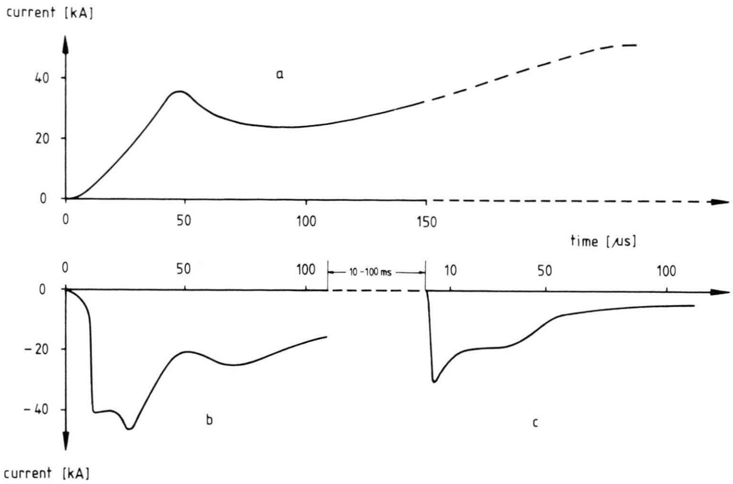

Fig. 1. Typical variation of current shape during the different forms of lightning. In (a) is shown a positive stroke, which is infrequent but lasts roughly 10 times longer than the most common (88% of all cases) discharge, the negative stroke (b). The negative stroke may be accompanied by up to 40 subsequential strokes each having the form shown in (c).

tials $\varphi(r, t)$ arise which have the following dependence on the current density $j_B(r, t)$ and the specific resistance ϱ of the ground:

$$E(r, t) = j_B(r, t) \cdot \varrho\,; \tag{1}$$

$$\varphi(r, t) = -\int_{\infty}^{r} E(r, t) \cdot dr\,, \tag{2}$$

where r is the distance from, and t the time after, the lightning stroke. From the point of the lightning stroke the current density distribution and the associated field intensity distribution propagate at approximately the speed of light in the form of a travelling electrical wave [14]. Inhomogeneities in the ground lead to refraction and reflection. The impedance of the ground also causes losses and damping. These effects and the reduction in amplitude with increasing distance cause the travelling wave to be deformed. Some distance away from the point of the stroke both the rate of increase of current and amplitude are reduced. The wave becomes broader with time (dispersion), but the charge flowing through it is preserved. As a result a potential difference U is generated for a short period of time between two points on the ground at different distances r from the point of the stroke. At a point-to-point distance, $\Delta r = 1$ m, this potential difference represents the step voltage occasionally dangerous to humans [11]. It represents the voltage received by a human *via* his feet in one step on the ground.

Material and Methods

In contrast to earlier experiments with spark coils and emitters in which cell fusion was initiated by electromagnetic waves [1, 2], the experiments described in this communication utilised the phenomenon of step voltage for cell fusion.

In order to investigate the fusion effects of field distributions as observed in natural atmospheric discharges on the earth's surface, experiments were carried out with a lightning impulse voltage generator. Fig. 2 shows a schematic diagram of the lightning generator. With this apparatus it was possible to generate voltage distributions such as those arising on the ground by the action of at-

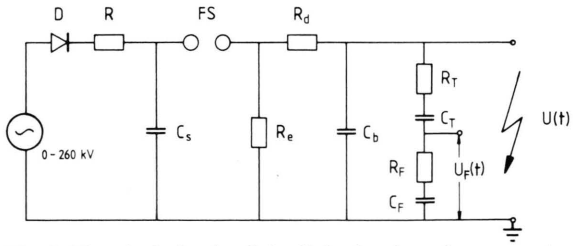

Fig. 2. Electrical circuit of the lightning impulse generator used. U(t) up to 1.5 MV, lightning impulse voltage; $U_F(t)$, voltage tapped of for fusion; D, rectifier diode; R, charging resistor; FS, switching spark gap; $C_s = 40$ nF, impulse capacitor; $R_e = 1830\ \Omega$, discharge resistor; $R_d = 900\ \Omega$, damping resistor; $C_b = 50$ pF, load capacitor; $R_T = 920$, divider resistor; $C_T = 350$ pF, divider capacitor; $R_F = 0.3-0.7\ \Omega$, divider resistor; $C_F = 500-1000$ nF, divider capacitor.

mospheric discharge (travelling waves). The standard lightning impulse is a standardised voltage form used for voltage testing, which corresponds to the most commonly occurring impulse form. This voltag course has a front time of $T_s = 1.2$ µs and a time to half value of $T_r = 50$ µs. In the experiments described here a lightning impulse voltage of negative polarity was used, corresponding to the most commonly occurring form of lightning (see above).

For cell fusion a chamber was used which consisted of a microslide, two adjacent areas of which had been coated with gold. The distance between the two conducting areas was 150 µm.

Plant protoplasts, prepared from *Avena sativa* leaves according to Hampp and Ziegler [15] and suspended in 0.5 M mannitol solution, were pipetted into the gap between the two metal surfaces. Within a few minutes the cells sank to the bottom of the chamber. Because of the high suspension density, the distance between the cells was relatively small (see Fig. 3a).

Results

In the first set of experiments protoplasts of *Avena sativa* were fused by means of a suitable step voltage taken from the floor of the laboratory containing a lightning impulse generator. However, measurements of the voltage distribution on the floor of the laboratory with the aid of an oscilloscope showed that the theoretically predicted voltage course during a lightning discharge was greatly distored by resonances in the laboratory. These resonances were determined by conditions inherent in the building, and we were therefore unable to remove them. The measured voltage distributions no longer corresponded to the distributions occurring in nature – at least under present-day atmospheric conditions. For this reason fusion experiments were carried out with simulated lightning impulse voltages. A standard lightning impulse was used which corresponded to the most common natural form of lightning current. With the aid of a high-voltage divider at the output of the lightning generator (Fig. 2) it is possible to obtain a voltage pulse form which corresponds to the natural voltage courses occurring in the ground during a lightning discharge. The amplitude of this voltage pulse could be reduced as desired. One pre-requisite of this experiment was that there must be no total discharge at the high voltage electrodes of the generator, which would drastically reduce the voltage at the divider. With this arrangement it was possible to achieve a very accurate simulation of travelling waves in the ground over a range of distances, *i.e.* with variable spatial damping. The voltage obtained from the high-voltage divider was conducted *via* co-axial cable into a measuring chamber which was screened by a Faraday cage and contained the fusion chamber. Screening with a Faraday cage was necessary so that fusion by the action of electromagnetic waves as described earlier [1] could be ruled out. In the measuring chamber the fusion process could be observed under the microscope. Fig. 3 shows the fusion of protoplasts of *Avena sativa*. Both suspension density and field intensity were sufficiently high for dielectrophoresis to proceed during the voltage pulse and, in turn, for intimate membrane contact to be established between a few cells, followed by a dielectric breakdown in the membrane contact zone and subsequent fusion of the cells.

The rounding-up process of the fused cells took about 2 min and was thus comparable with the time taken in conventional electrofusion techniques [4–9] and in fusion induced by electromagnetic waves [1, 2].

However, the field intensities of the stimulated lightning impulses leading to optimal fusion were about 8 times higher than those used in conventional electrofusion processes (about $800\,\mathrm{kV \cdot m^{-1}}$ compared with about $100\,\mathrm{kV \cdot m^{-1}}$) [6]. The difference in field strength may be attributable to the shape of the field pulse (Fig. 4). In contrast to the square pulse in conventional electrofusion, which has a duration of 20 to 50 µs, the maximal value of the field intensity of $800\,\mathrm{kV \cdot m^{-1}}$ was reached within about 1 µs in stimulated lightning discharges; the field intensity then declined exponentially with a time to half value of 50 µs. The pulse shape thus approximately corresponded to that generated by a discharged chamber such as the one used for electrical breakdown experiments [6]. The relaxation time of the membrane charging process in non-electrolyte solutions is of the order of a few µs [16] so that the stationary voltage level across the membrane which would correspond to a field intensity of $800\,\mathrm{kV \cdot m^{-1}}$ is not achieved. However, even when the declining field intensity and the pulse length

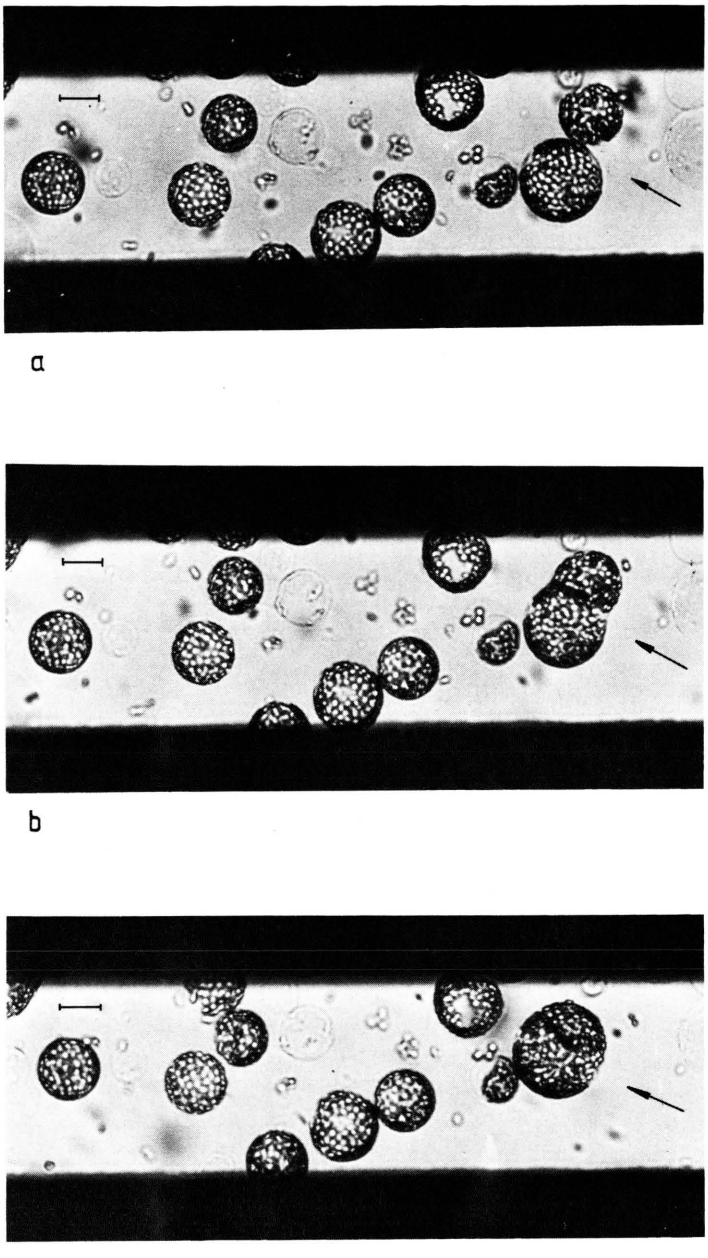

Fig. 3a–c. Fusion of plant protoplasts prepared from the mesophyll tissue of leaves of *Avena sativa.* The cells were first suspended in 0.5 M mannitol solution between two conducting metal areas (a). Cell fusion was induced (b, c, see arrows) by application of a field pulse which simulated that occurring in the ground in response to a lightning stroke. Bar = 25 μm.

dependence of the breakdown voltage [17] are taken into consideration, the critical breakdown voltage is certainly reached after about 10 μs. However, this statement probably only holds for membrane sites oriented in field direction [4, 7, 8, 16]. For membrane sites oriented at an angle to the field direction, the field intensity required to reach the electrical breakdown voltage in these sites is considerably higher. In fact, as demonstrated elsewhere [4, 7], the field intensity may take on infinitely high values as the angle approaches 90°. As shown in Fig. 3, there is a preference for fusing cells oriented at a certain angle to the field direction. In contrast to conventional electrofusion, there is no orientation of the cells along the field lines under these conditions. Nevertheless, during the first few μs after the onset of the simulated lightning impulse there will certainly be movement of the cells towards each other because of the generation of dipoles within the cells, and this will lead to intimate membrane contact.

Since the field intensity is sufficiently high to induce pore formation over wide areas of the membrane, there is immediate fusion.

Furthermore, it has to be considered that it is very difficult to indicate the exact field strengths actually required for cell fusion under these conditions from the following reason. The metal areas of the fusion chamber were connected to the voltage generator. Theoretically it can be shown that the electric field is nearly uniform in the middle of the gap, however, it increases dramatically as the edges of the metal plates are approached. Introduction of cells having a mean diameter of 35 μm into the gap leads to a disturbance of the field lines. Therefore, it is very difficult to give the exact field strength in the gap which leads to cell fusion. The values given here were calculated on the assumption of a homogeneous, undisturbed field.

These qualitative considerations demonstrate that the high field strengths calculated to be present during all fusion by lightning discharge are probably compatible with the field strengths leading to cell fusion in conventional electrofusion.

Discussion

The results reported here demonstrate that lightning discharges can lead to cell fusion, provided that the suspension density is high enough. The experimental arrangement resembles that of the discharge chamber first successfully used in 1978 [18] to fuse human erythrocytes in high suspension densities. The experiments described here show that the voltages occurring on the surface of the earth are sufficiently high to initiate fusion. However, these experiments do not rule out the possibility that electromagnetic waves, such as those emitted during a lightning discharge, may also have played a role in the origin of new cell species during evolution as shown previously [1, 2]. In our opinion the experiments described here demonstrate that the probability of cell fusion during the evolutionary process was very high because various electrical mechanisms were available for the fusion process. This concept will be further expanded in the following discussion by estimates designed to demonstrate how far away from the point of a lightning stroke cells may be expected to have undergone electrical fusion. The lightning stroke generates an electrical travelling wave which moves away in a hemispherical manner (radius r) with a speed substantially that of a electromagnetic wave in free space (*i.e.* "the speed of light"). This means that within a few hundred metres of the point of the stroke, the duration of the stroke is much larger than the time of travel. Hence

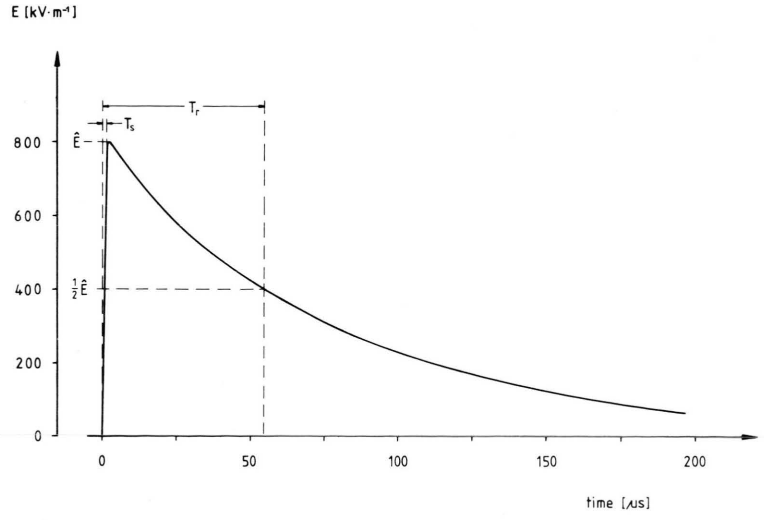

Fig. 4. Time course of the field pulse used for cell fusion. The peak field strength ($\hat{E}$) is 800 kV/m, the front time (*Ts*) is 1.2 μs and the time to half value (*Tr*) is 54 μs.

the only effect of changing the distance within this radius is to change the amplitude, and not the shape, of the wave. The dispersion referred to above only becomes significant at much greater distances. Assuming a homogeneous material, the current density of the lightning current in the ground declines with increasing area spread A according to the following equation:

$$j_B = \frac{J_B}{A}. \tag{3}$$

The area A corresponds to the surface area of a hemisphere. It thus follows that:

$$j_B = \frac{J_B}{2 \cdot \pi \cdot r^2}. \tag{4}$$

The field strength (for a homogeneous material) is thus:

$$E = j_B \cdot \varrho. \tag{5}$$

The field strength E at a certain point in a distance r from the point of the stroke is thus calculated from Eqns. (4) and (5) to be:

$$E = \frac{J_B \cdot \varrho}{2 \cdot \pi \cdot r^2}. \tag{6}$$

When using these equations it is assumed that the ground is homogeneous, *i.e.* that there are no variations in the electrical resistance and dielectric constant. This is of course a very rough assumption. The calculated course of the field intensity for a certain lightning current J_B and a specific resistance ϱ of the ground using Eq. (6) (Fig. 5) thus represents only an approximate value; locally, the field intensities may be substantially higher (see below). The current amplitude of a relatively strong lightning under present-day atmospheric conditions is about 100 kA. The electrical resistance of the ground (gravel, limestone, rocky soil) is about 1000 Ωm [11]. As demonstrated experimentally, the field strength inducing fusion is in the order of 800 kV · m^{-1}. As shown in Fig. 5 this field strength is still given at a distance of 4.5 m.

At first sight this distance appears relatively small, considering the heat development associated with a lightning stroke, which could destroy the cells.

However, the following considerations demonstrate that under certain natural conditions the required potential differences may be established at much greater distances from the point of the stroke. Higher field strength in larger distances from the point of the lightning stroke can be built up, if one assumes that conducting materials, *e.g.* ore-containing rock, are present, whose shortest distance d to one another is less than the distance between the points where the rocks contact the ground. There is a potential difference U between the rocks which can lead to a high field strength at the narrowest gap between the rocks.

$$E = \frac{\varphi_1 - \varphi_2}{d} = \frac{U}{d}. \tag{7}$$

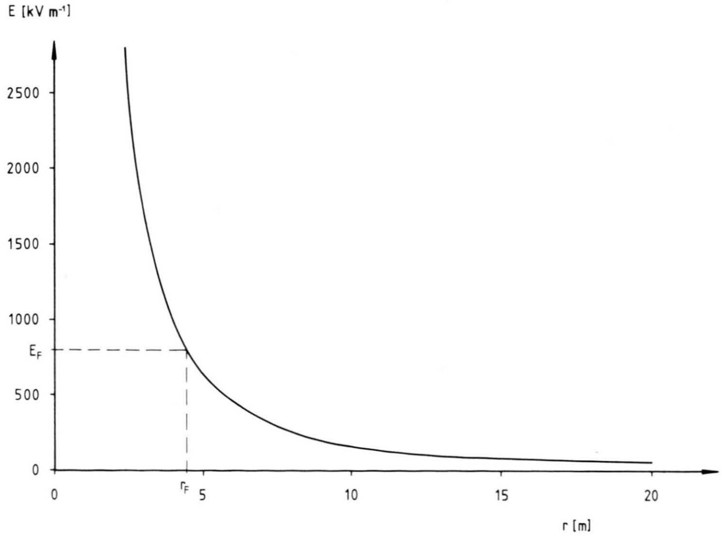

Fig. 5. Field strength E in the ground as a function of distance r from the point of a lightning stroke. The data set was calculated using Eq. (6) after assuming the following values: stroke peak current, 100 kA; average specific resistance of the ground, 1000 Ωm. E_F is the field strength required to give cell fusion; r_F is the distance at which $E = E_F$.

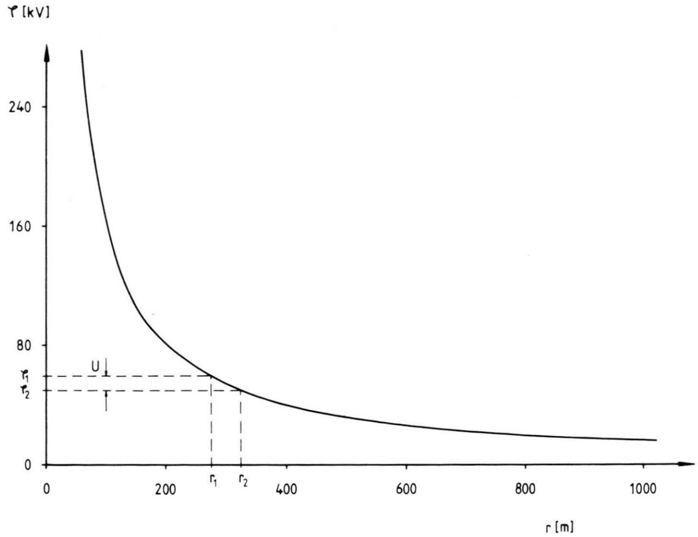

Fig. 6. Potential φ of the ground as a function of distance r from the point of a lightning stroke. The data set was calculated using Eq. (8) after assuming the same values as in Fig. 5. Between two points on the ground at distances r_1 and r_2 from the point of the stroke the potential difference is $\varphi_1 - \varphi_2$. When $r_2 - r_1 = 1$ m, this potential difference is the step voltage U.

The dependence of the potential φ as a function of the distance from the point of the stroke is obtained *via* Eq. (2) and the integration of Eq. (6) over dr:

$$\varphi = \frac{J_B \cdot \varrho}{2 \cdot \pi \cdot r}. \tag{8}$$

The course of the potential is shown in Fig. 6.

If we regard r_1 as the distance of an ore-containing piece of rock from the point of the lightning stroke and r_2 as the distance of a second piece of ore-containing rock, we obtain, by substituting Eq. (7) into Eq. (8), the following equation for the field strength in the gap between the two pieces of rock for the shortest distance d between the rocks:

$$E = \frac{J_B \cdot \varrho}{2 \cdot \pi \cdot d} \cdot \left(\frac{1}{r_1} - \frac{1}{r_2}\right). \tag{9}$$

The critical field strength may be reached when d is very much smaller than $r_2 - r_1$.

Equation (9) is only valid when the resistance in the gap between the two pieces of rock is considerably greater than the resistance of the two rock pieces, *i.e.* when the gap is filled with air, sand or rain water. If this condition is not fulfilled, *i.e.* if the rocks have a higher resistance or if the gap contains more highly conducting salt water, the voltage is merely reduced as in an electrical voltage divider [1].

Assuming that the resistance in the gap is substantially greater than the resistance of the rocks, and taking for $\Delta r = 10$ cm, *i.e.* $r_2 = r_1 + 0.1$ m, $d = 0.02$ cm, for a lightning current $J_B = 100$ kA, an assumed resistance of $\varrho = 1000\ \Omega$m, and a critical field strength of 800 kV $\cdot$ m^{-1}, we obtain a distance r_F of 100 m from the point of the stroke, at which fusion can still take place. For this calculation we also assumed that $r_1 \approx r_2 = r_F$. For a distance of $\Delta r = 1$ m between the points where the rocks contact the ground this distance is increased to 315 m.

These estimations show that cell fusion due to a lightning stroke is still possible at great distances from the point of the stroke, if fusion is initiated by travelling electrical waves.

If the possibility that fusion can also be induced by electro-magnetic waves [1, 2] is also included, we think that there cannot be any doubt that such mechanisms will have to be taken into consideration in any future discussion of the origin of species. Needless to say, investigations of the viability of cells formed in this way and analyses of the chromosomes will be necessary in order to lend further support to the hypotheses put forward here.

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