ORIGINAL ARTICLE

Elastic constant of *Dendrobium* protoplasts in AC electric fields

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Abstract

Wanichapichart, P., Maswiwat, K., and Kanchanapoom, K.
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Songklanakarin J. Sci. Technol., 2002, 24(Suppl.) : 799-806

This work reports elongation of *Dendrobium* protoplasts in an ac electric field between two cylindrical electrodes. A protoplast firstly was translated towards an electrode by dielectrophoretic force in 17 kV.m^{-1} field strength at 1 MHz, and secondly it was elongated due to an interaction between an induced electric dipole (μ) and the electric field (E). Protoplast elongation was observed by varying both the field strength at 30, 45, 60, and 85 kV.m^{-1} and field frequency at 0.5, 1, 5, and 10 MHz. For a given field frequency and field strength, a parameter a/b (major/minor axis) was measured as the protoplast elongation. Two-step elongation and restoration phases were observed. The former was completed within 2 minutes of field exposure, and the latter was completed within 15 seconds regardless of the field exposure time between 3 and 20 minutes. The evidence of a complete restoration indicated that the elasticity of the protoplast membrane obeyed Hooke's law. This study also found that elastic constant k of the membrane varied non-linearly with the field strength. It was found to be from 0.04 to 0.08 mN.m^{-1}, dependent on the field frequency.

Key words : dielectrophoresis, elastic constant, elongation, alternating electric field, protoplasts

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Received, 24 February 2003 Accepted, 18 April 2003

As is known, cell polarization occurs when a cell is placed in a low conducting medium under a non-uniform electric field. Under Maxwell-Wagner frequency range (10^3-10^7 Hz), an electric dipole moment of the cell interior is induced with its magnitude and direction depending on the applied electric field and frequency. For an appropriate field frequency, a uni-axial force which is a result of a vector product between the dipole moment and the gradient of the applied field attracts the cell towards an electrode surface. When the field strength is further increased, the cell would experience a greater force, which stretches the cell in the direction of the electric field. This mechanical stress introduced to a cell by ac electric field has been studied in human red blood cells (Engelhardt and Sackmann, 1998), and urchin eggs (Marszalek and Tsong, 1995). These studies reported the dependence of cell elongation on field frequency, field strength, medium temperature, and medium conductivity. The applied electric field used to induce the dipole varied from 18 to 110 kV.m^{-1}. These studies assumed that (1) a cell was homogeneous, (2) cell polarization effect was mainly at the thin membrane, and (3) the variation of force acting on the cell during the elongation was negligible.

Theoretical approach for a bioelectrorheological model has been studied extensively (Poznanski *et al.*, 1992) using fungi; *Neurospora crassa* cells. Their work explained viscoelastic deformation and electrodestruction of cellular membranes with a model including the membrane, surface tension and the cell interior. It was suggested that the deformation and cell elasticity could be used to distinguish cell age, health, and physiological state of the membrane.

In our previous studies (Wanichapichart *et al.*, 2002), two identical, cylindrical electrodes were used to induce cell translation in an ac field. Plankton cells and mesophyll protoplasts were studied to reveal their dielectric properties. The studies reported protoplast deformation, which was only observed in *Dendrobium* sp. The protoplast of *Lilium longiflorum* seemed to be very brittle and membrane rupture occurred without elongation. It is therefore of interest to investigate elastic constant of *Dendrobium* protoplasts under various electric field strength and frequency to provide a fundamental information for biotechnological work. On estimation of membrane elastic constant, the real part of dielectric properties of the protoplast, reported in our previous study, was used.

Theoretical approach

In the present study, *Dendrobium* protoplasts are considered as spherical cells - each with a single dielectric shell of cell membrane (Figure 1c). The cell membrane separates the cell interior, which is regarded as an electrolyte, from an external suspending medium of low conductivity. When the cell is suspended in an external AC electric field, electric dipoles are induced in the cell. An interaction between the external electric field and the effective electric dipole moment, μ_{eff} (Figure 1b), obtained from the induced dipoles induces a distorting force (F_E), results in an elongation of the cell. A detailed analysis of cell elongation of a single, spherical dielectric shell of cell has been described elsewhere (Mahaworasilpa, 1992). The relation between F_E and Re[f(ω)] is expressed as

$$F_E = -k(2\Delta a) = 8\varepsilon_s r^2 \mathrm{Re}[f(\omega)]E^2 \qquad (1)$$

where k is an elastic constant; Δa, an increment of elongation in the field direction; ε_s, permittivity of the external medium (~$80\varepsilon_o$, where ε_o = 8.85×10^{-12} F.m^{-1}); r, radius of cell; E, an electric field strength; and Re[f(ω)], the real part of f(ω), a complex function as described elsewhere (Mahaworasilpa *et al.*, 1994). The assumed $80\varepsilon_o$ is the dielectric constant of pure water, which is reasonable in this study since only sugar was dissolved in distilled water to make up the external medium of the experimental protoplast.

The elastic constant (k) from Equation (1) can be rewritten as

$$\left(\frac{a}{b}\right)^{\frac{2}{3}} = 1 + \frac{1}{k} 4\varepsilon_s r \mathrm{Re}[f(\omega)]E^2 \qquad (2)$$

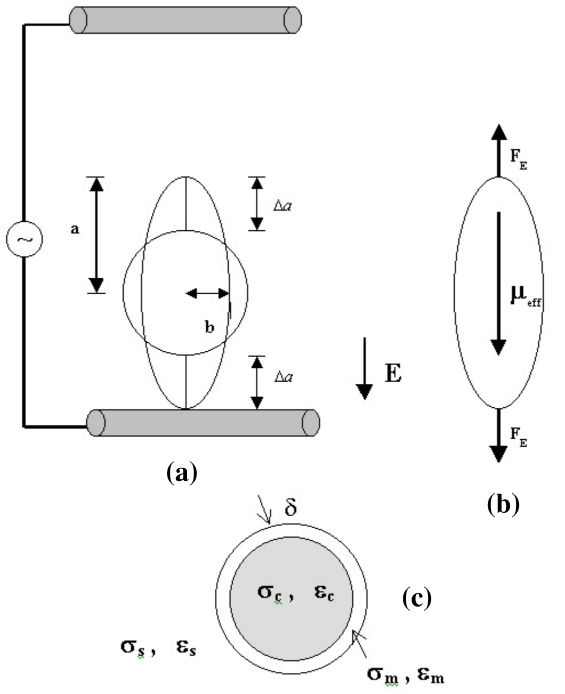


Figure 1. (a) Diagram for protoplast elongation on an electrode surface
(b) Elastic force, F_E, exerting on a cell in both direction parallel to E
(c) A spherical single shell model with dielectric parameters used in this study. The σ_s, σ_m and σ_c are conductivity of external medium, membrane and cytoplasm, respectively. The ε_s, ε_m, and ε_c are permittivities of external medium, membrane and cytoplasm, respectively.

where a is the major axis and b, the minor axis of elongated cell - see Figure 1a. Since k and $(a/b)^{2/3}$ are strictly dependent on cell radius, it is important to carefully select the cells.

Materials and Method

Protoplast preparation

Dendrobium mesophyll was pre-plasmolysed in a 0.7 $kmol.m^{-3}$ mannitol solution for 15 min before being chopped and transferred to an enzyme solution containing 2% Cellulase Onozuka R-10, 1% Driselase and 1% Marcerozyme R-10. These enzymes were dissolved in 0.7 $kmol.m^{-3}$ mannitol at pH 5.7. The leaves-enzyme mixture was shaken in darkness for 4 hours, then sieved through a 141 µm mesh stainless steel screen, yielding a mixture of protoplasts and pellet materials. These protoplasts were separated by centrifugation using the sucrose density gradient method. They were gently collected and washed 2-3 times with 0.5 $kmol.m^{-3}$ mannitol. Only protoplasts with 50 µm diameter were selected for the experiments.

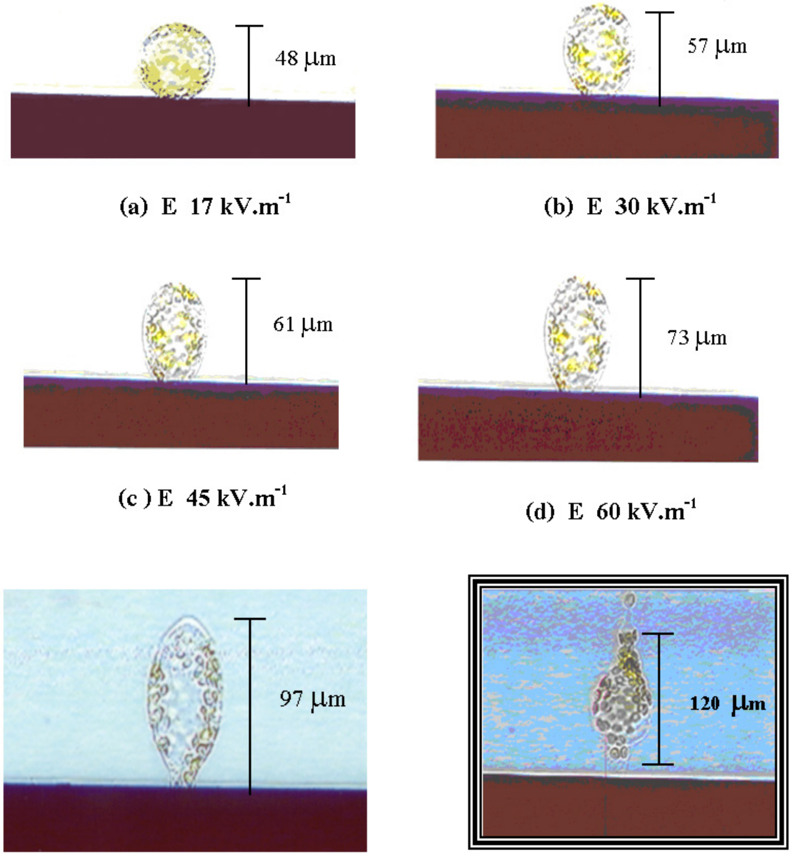
Image processing

Protoplast elongation process in an electric field was recorded by using a CCD camera (Sony SSC-DC18P) and stored in a video recording (Sony SLV-KH7). The width of the electrode, 125 µm, was also recorded to represent a standard scale on a monitor. The processed images were later displayed by 24″ monitoring TV and the protoplast length was measured at 2.4% accuracy under 200x microscope magnification of an inverted microscope (Olympus, LX 700, Japan). Elongation was obtained by measuring the major and the minor axes, a and b respectively, of elongating protoplast and the elongation ratio, a/b, was recorded.

Results and Discussion

Field and frequency effect

After a protoplast was attached to an electrode at 1 MHz in 1 $mS.m^{-1}$ solution, the field was increased from 17 $kV.m^{-1}$ to 85 $kV.m^{-1}$. Pictures 1a-1f shows the distorting force exerting on the cell, causing the cell to elongate. Protoplast elongation increases with the field strength, immediately after the field was switched on. When the field was switched off, the original shape of the protoplast restored simultaneously. There was evidence that longer exposure time (12 min) led to a release of inner organelles at a tapered pole, however, the restoration was still completed after the field was switched off. The leakage of the internal organelles could be due to a higher charge



(a) E 17 kV.m^{-1} **(b) E 30 kV.m^{-1}**

(c) E 45 kV.m^{-1} **(d) E 60 kV.m^{-1}**

(e) E 85 kV.m^{-1} **(f) 0.5 MHz, 85 kV.m^{-1}**

Picture 1. (a-f) Elongations of a protoplast at various electric field strengths. The length was measured after the protoplast has been exposed in the field for 1.5 min. The conductivity of external solution used was 20 mS. m^{-1} at 1 MHz frequency, except in (f).

density at the tapered pole and consequently increasing transmembrane potential. When the field frequency was decreased from 1MHz to 0.5 MHz (see Picture 1f), protoplast rupture took place within 30s. Table 1 shows the critical frequency and tolerant time in the applied field before membrane breakdown taking place.

Further experiment was then carried out to test whether the protoplast membrane obeyed Hooke's law. Table 2 shows that the protoplast was fully restored after each elongation, but the restoring period depended on the field strength only if it experienced the field for 1 min. Under high field strength of 85 kV.m^{-1}, the exposure time for 10 min caused an irreversible membrane breakdown. It should be pointed out that there were two steps of restoration; one at 10 sec after 1 min field exposure and the other 15 sec, independent of the exposure time period from 3-20 min. It was observed that the protoplast took a shorter period of restoration if it was not fully stretched. The results shown in Table 2 are consistent with the report by Engelhardt and Sackmann (1988) in red blood cell that the shear force acting on the membrane is dominated by frequency, but varies accordingly with the field strength.

Table 1. Critical frequency (f_c) for protoplast rupture under different electric field strength. The t_c represents tolerant time before the rupture taking place. All data was recorded under 10 mS.m^{-1} solution conductivity.

E (kV.m^{-1})	Critical frequency, f_c (kHz)	Tolerant time, t_c (s)
30	12.7±2.6	31.7±5.8
45	21.7±3.0	38.3±2.9
60	154.7±11.0	15.0±5.0
85	293.3±11.5	15.3±4.5

Table 2. Comparing restoring time for a protoplast after beingstretched in electric fields under 10 mS.m^{-1} solution conductivity.

Field exposure (min)	Restoring time (s)		
	45 kV.m^{-1}	60 kV.m^{-1}	85 kV.m^{-1}
1	9.1±0.4	10.3±0.3	10.3±0.7
3	14.4±0.5	14.4±0.6	14.8±0.3
5	14.1±0.2	14.8±0.4	14.9±0.3
10	15.2±0.1	14.9±0.4	**Ruptured**
15	14.8±0.3	15.0±0.0	**No observation**
20	14.8±0.7	15.0±0.6	**No observation**

Elongation ratios

Experiments on the effects of field strengths and frequencies on elongation were carried out in 1 and 20 mS.m^{-1} solution. The elongation parameter, a/b, was measured at one-minute interval of field exposure, as shown in Figures 2a, b, c and d. The ratio increases with the field strength and is clearly affected by field frequency and the conductivity of the solution. The increased a/b within the first 2 min shows two elongation phases; the faster rate of elongation occurred within 0.5 min whereas the slower one occurred between 1.5-2 min before the elongation reached its maximum in all cases. The ratio at 0.5 MHz in Figure 2c could not be obtained due to the protoplast rupture. Comparing among these data, elongation under the low field of 30 kV.m^{-1} seems to depend on the frequency. It should be noted that when the field frequency was increased further to 5 and 10 MHz (Figures 2 e, f), only the protoplast in 20 mS.m^{-1} solution was further increased but the maximum a/b appeared at 5 MHz to 1.37±0.01 under the high field strength. However, it should be noted that the maximum a/b obtained in this study was 1.46±0.02 at 1 MHz in 1 mS.m^{-1} solution (Figure 2b). It is interesting to point out that the two step elongation brings about the idea that both the membrane and the cell interior might be induced simultaneously under low conducting medium. Moreover, the two step elongation disappeared at 10 MHz. The dependence of elongation on the exposure time and the applied frequency was also observed in red blood cells in other laboratories (Engelhardt and Sackmann, 1988).

Force on membrane and elastic constant, k

The equation (1) allows one to calculate the elastic force if the elongation (2Δa) and the value of Re[f(ω)] are known at a particular frequency used. The Re[f(ω)] has been obtained by our previous study using dielectrophoretic technique (Wanichapichart *et al.*, 2002). In 1 mS.m^{-1} solution where the maximum elongation took place, the Re[f(ω)] at 0.5, 1.0, 5.0 and 10 MHz was 0.035, 0.035, 0.030 and 0.015, respectively. Table 3 shows that although a/b increases with the field at a fixed frequency, the similarity of a/b at frequency between 5 and 10 MHz brings in some doubt on the difference in the k values. It is important to point out that the k varied non-linearly with increasing field strength and force particularly at 60 kV.m^{-1} for every frequency used. This non-linearly increasing k, and the two steps in both elongation and restoration seem to imply that there is an interfacial boundary between the shell membrane and the cytoskeleton, of which the detail should not be over looked. The cell interior might experience the stretching later since the force needs to overcome cytoplasm viscosity. Poznanski *et al.* (1992) also considered the elastic mechanism of a cell as a combination of these

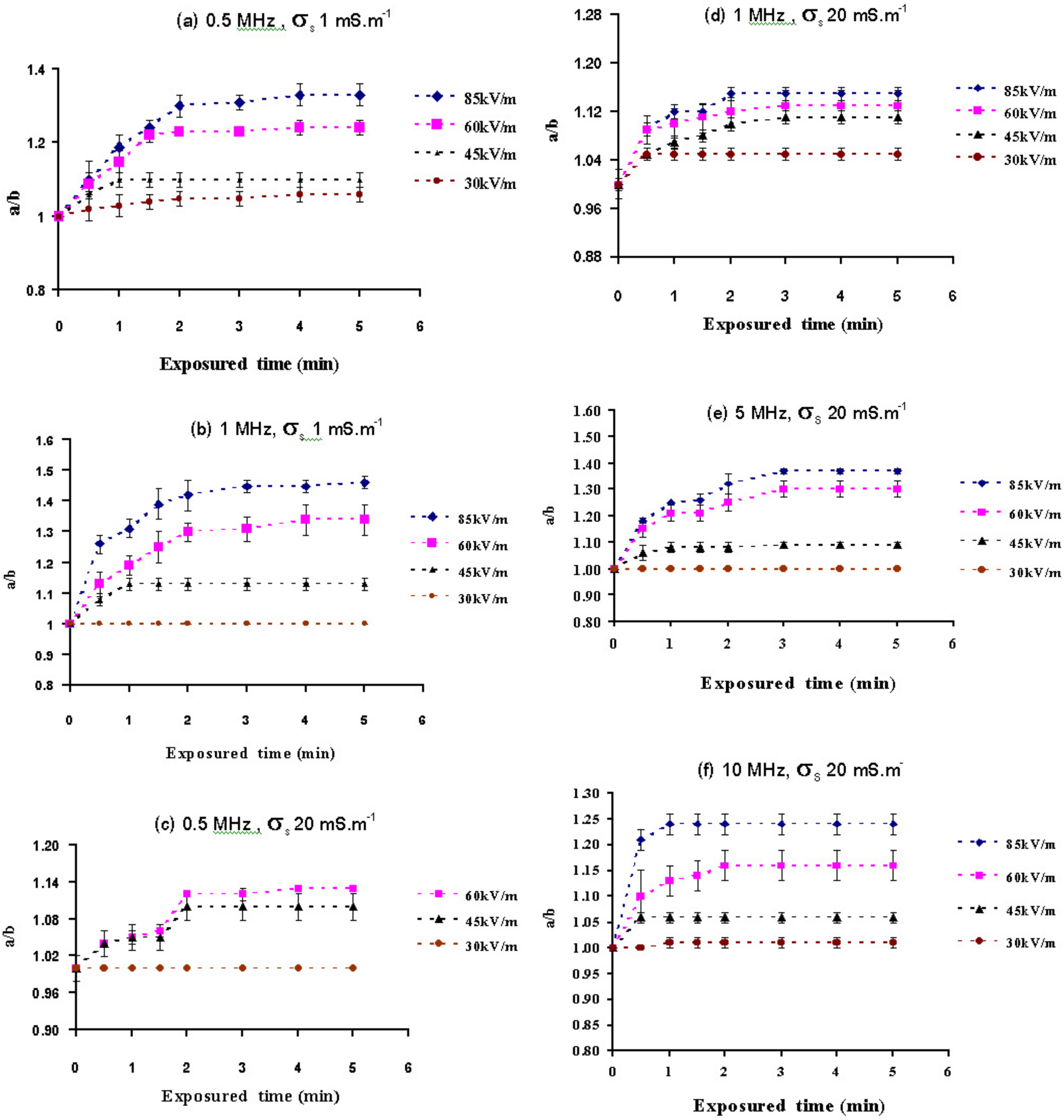


Figure 2. (a-f) Plots of a/b ratios against field exposure time under different applied frequency.

two elastic moduli. On the average, the k constant between 0.5 and 10 MHz of *Dendrobium* protoplasts is 0.06±0.02 mN.m^{-1}, about ten times greater than that of flexible red blood cells (Engelhardt and Sackmann, 1988). Besides, the k constant could be estimated from a plot between $(a/b)^{2/3}$ and E^2 and the relation was expected to be linear (see Equation 2 and Figure 3). Using the slope of the graph and the average Re[f(ω)] of 0.029, the calculated value of k was found to be 0.07 mN.m^{-1}.

Table 3. The ratio a/b was measured after a full elongation of the protoplast was achieved, in 1 mS.m^{-1} solution conductivity. Re[f(ω)] at indicated frequency was obtained from separate experiment (Wanichapichart *et al.*, 2002). The value of k and F_E was obtained from equation (1) and (2).

Frequency (MHz)	Field strength, E (kV.m^{-1})	Re[f(ω)]	a/b	Constant k (× 10^{-4} N.m^{-1})	F (× 10^{-10}N)
0.5	30	0.035	1.05	0.67	1.12
	45		1.10	0.76	2.51
	60		1.23	0.60	4.46
	85		1.31	0.91	8.95
			Average	0.73±0.13	4.26±3.42
1	30	0.035	1.00	0.00	1.12
	45		1.13	0.59	2.51
	60		1.31	0.45	4.46
	85		1.45	0.64	8.95
			Average	0.42±0.29	4.26±3.42
5	30	0.030	1.04	0.72	0.96
	45		1.09	0.73	2.15
	60		1.17	0.69	3.82
	85		1.22	1.08	7.67
			Average	0.80±0.18	3.65±3.92
10	30	0.015	1.05	0.29	0.48
	45		1.10	0.33	1.08
	60		1.20	0.30	1.91
	85		1.23	0.52	3.84
			Average	0.36±0.11	1.83±1.46

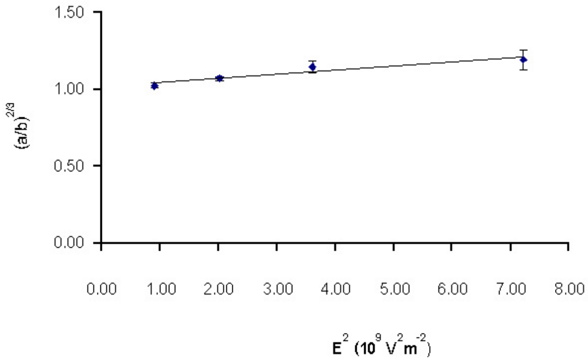


Figure 3. A linear relationship between $(a/b)^{2/3}$ and E^2.

Conclusion

This study shows that elastic limit of *Dendrobium* protoplasts depends on electric field strength, frequency and field exposure time. Within the limitation, the elastic constant of the protoplast obeyed Hook's law. The distorting force varied depending on applied frequency from 0.5 to 10 MHz. The average elastic constant for the protoplasts obtained from the present study was estimated to be from 0.04 to 0.08 mN.m^{-1}, depending on the field frequency. It was found that for a given field strength and frequency, the elongation of these protoplasts was affected by ionic condition of the external medium. Two-step elongation and restoration observed in this study might be related to non-homogeneity of the protoplast.

Acknowledgments

The authors appreciate support from Prince of Songkla University for the present research. Thanks are extended to Dr. Tohsak L. Mahaworasilpa and Prof. I.S.A. Hempenius for valuable suggestions and assistance with the manuscript.

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