

# Kinematographic method of chloroplast movements analysis

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## I n t r o d u c t i o n

All research on phototactic chloroplast movements were hitherto almost entirely limited to a description of such changes in chloroplast arrangement, as were caused by different environmental and inward factors (S e n n 1908). Even when these processes were observed in relation to time, only some temporal chloroplast positions were defined (V o e r k e l 1924, Z u r z y c k a and Z u r z y c k i 1950). Although from this the average rapidity of the phototactic reaction could be obtained no data were forthcoming on the rapidity with which the chloroplasts themselves moved, and on the chloroplast way within the cell. Detailed observations on the dynamism of chloroplast movements and a minute analysis of their ways, movements, rapidity etc. could really elucidate the mechanism of phototactic chloroplast movements. The present work is an attempt to analyse chloroplast movements in *Lemna trisulca* L. with the aid of the kinematographic method of reducing time (Zeitraffung).

## M e t h o d s

The principle of reducing time method depends on photographing the microscopic image on a kinematographic filmroll every several second. The film is then projected with the normal rapidity of 16 images per sec. On the screen an optional acceleration of the photographed process may be thus obtained.

For photographing a reversible 16 mm Isopan ISS film was used. The necessity of using strong light (a 35 W, 6 V microscopic lamp without dim glass, Köhler's arrangement) made possible the filming of only one kind of reaction, i. e. the epistrophe to parastrophe reaction. The move-

ment of chloroplasts onto the side walls was accelerated 160 times (1 photograph was taken every 10 sec.).

A detailed analysis was made by projecting the images one after another on a screen. On a diagram the successive positions of the central point of a chloroplast were marked in each case (K u h l 1949). In this way it was possible to map out exactly the way of each chloroplast in the cell, its rapidity, and changes of the rapidity during the reaction. The method of determining the way and rapidity of chloroplasts during 100 secs (from 10 successive photographs) is given in Fig. 1. The error in estimating the chloroplast position which may arise from a limited precision in projecting the photograph, making the diagram and determining the chloroplast center, may be estimated at approximately  $0,5 \mu$ .

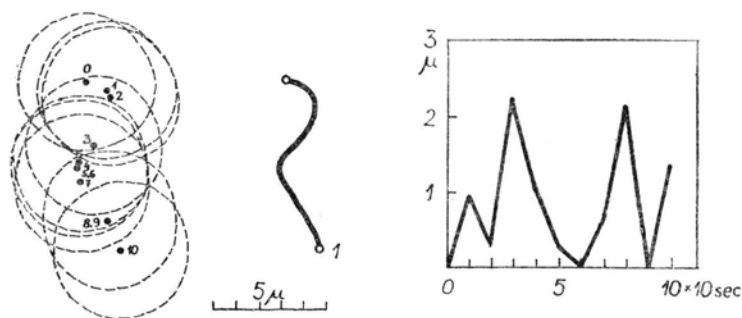


Fig. 1. An example of chloroplast movement analysis during 100 sec.

a) chloroplast outline from 10 successive photographs. Center point of chloroplast marked in. b) chloroplast way. c) rapidity graph.

A detailed analysis is possible only in respect of movements taking place on the upper cell wall, as these occur in a plane perpendicular to the direction of observations. When the chloroplasts move to the side walls it is possible to observe only one component of their movement. The downward movement on the side walls cannot be investigated. In most cases only the movements on the upper cell wall were subjected to analysis. To illustrate the whole of the course of phototactic reactions the percentage change in epistrophe was established for one of the investigated cells only, according to a method described in a previous paper (Zurzycka and Zurzycki 1950).

## Results

When the epistrophe to parastrophe phototactic reaction is observed with a microscope it is difficult to notice the movement of chloroplasts. After some minutes of observation only a change in their arrangement

gement in the cell can be noticed. Quite exceptionally a very slow movement of a chloroplast through usually a short distance can be seen then the movement seems to stop. After 20—30 mins, all chloroplasts can be seen to be on the side walls. Their movements however, are too slow to be observed.

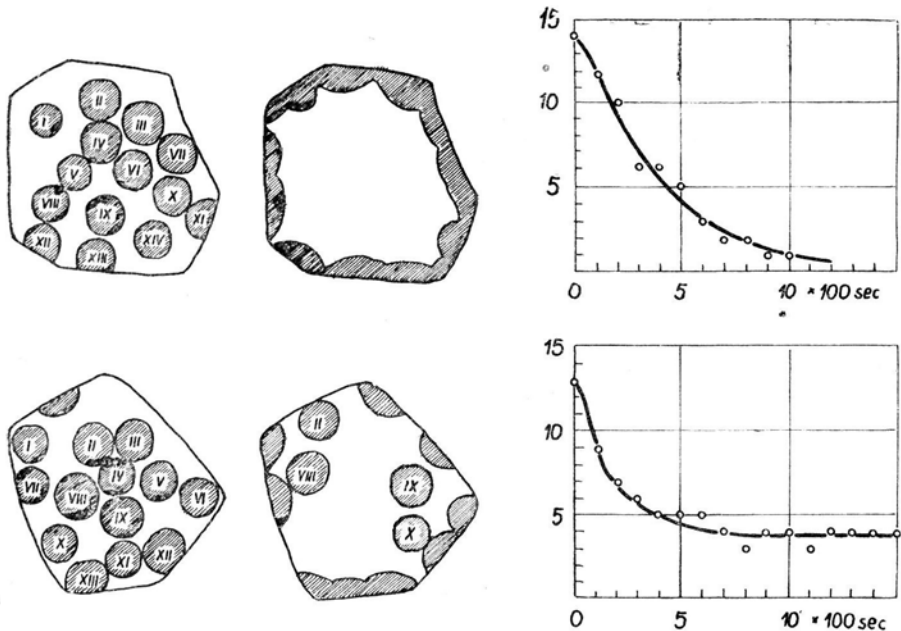


Fig. 2. Initial and final arrangements of chloroplasts in cell 1 (above) and 2 (below), and the course of epistrophe to parastrophe reaction.

The reaction, when filmed accelerated 160 times, shows intensive and complicated movements of all chloroplasts. Immediately after the cell is illuminated all or nearly all epistrophe chloroplasts begin to move. Their movements are fickle and quivering as if they were jerked about in different directions. In each chloroplast separately these movements become progressive, indeterminate in the direction (not necessarily in the direction of the nearest side wall), and follow bow curved or wavy lines. Both these kinds of motion lead, often along extremely complicated ways, to the displacement of chloroplasts onto the side walls. The intensity of these movements is more or less the same throughout the reaction. Chloroplasts after reaching a side wall remain almost motionless, and either stay in one place hardly moving at all, or change their position only slightly. The course of an epistrophe — parastrophe reaction is given on Fig. 2. In cell 1 (spring material) the parastrophe is complete,

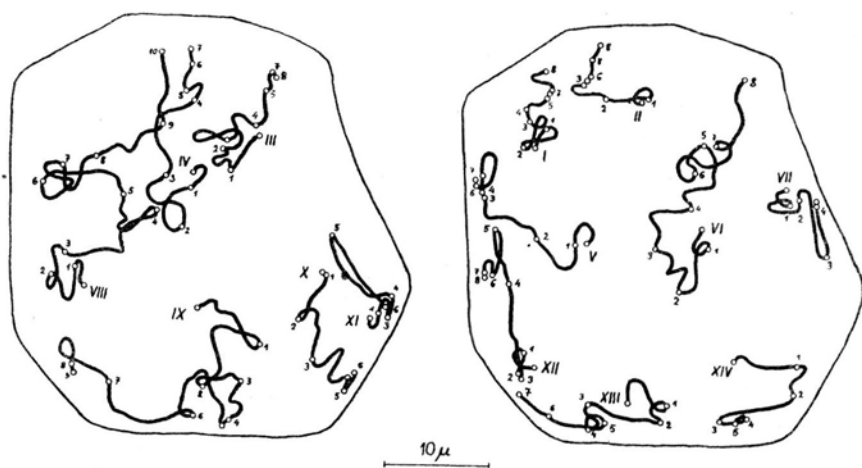


Fig. 3. Cell 1 — chloroplasts ways during epistrophe — parastrophe reaction.

while in cell 2 (autumn material) it is not. Chloroplast arrangements at the beginning and at the end of the reaction, as well as the curve of its course are, in both cells, very characteristic for the epistrophe — parastrophe reaction. The curve illustrating the course of the reaction is very regular considering that it is plotted for one cell only.

Those chloroplasts which are near, to the side walls usually move fairly directly towards them 1 — I, II, 2 — VI, VII, XI, XII). This however is not a general rule and sometimes the chloroplasts move along the side walls in a flat position (1 — II, XII), or towards the center of the cell (2 — X). Those chloroplasts which are not near to the side walls begin to move in indeterminate directions. As a result they sometimes move directly to the nearest side wall (1 -- XIV, 2 — II) and sometimes directly

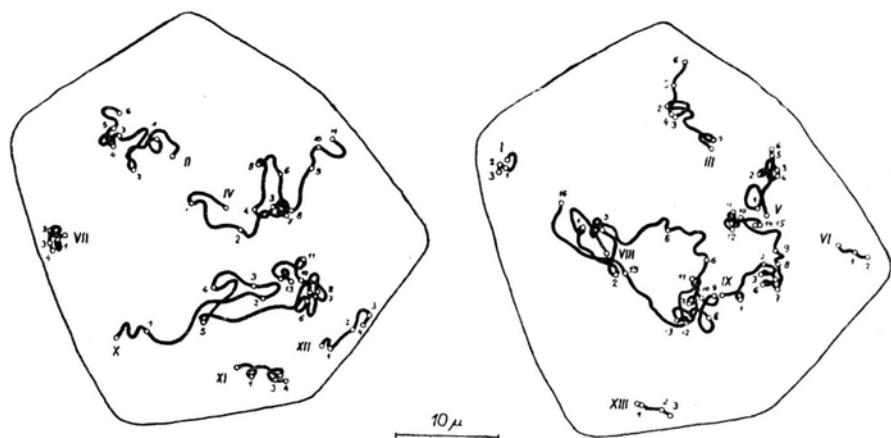


Fig. 4. Cell 2 — chloroplasts ways during epistrophe — parastrophe reaction.

away from it (1 — III, IV). A movement in one direction never lasts long, and during 100 sec. its direction usually changes several times. In consequence the line traced by the center point of the chloroplast is curved, and has many sudden bends and loops. The numerous changes of direction may cause a chloroplast, which has almost reached the side wall, to move to the center of the cell (1 — VIII), or to return near to the place from which it has departed at beginning. The distance travelled by a chloroplast is usually far greater than the distance between the positions of a chloroplast, at the beginning and the end of a phototactic reaction. Chloroplasts when once in a profile position on the side wall, may remain motionless or move only slightly (1 — X, 2 — I, VII), they also may move along the side walls (1 — VII, XIII, 2 — XII) or even return from profile position to a flat one on the upper wall (1 — XI).

The different chloroplasts may move at the same time each one in a different direction. Often, however, mainly when two chloroplasts are close to each other, they move in the same direction. This is not caused by the chloroplasts pushing each other as, very often, they do not touch. The correlativity cannot be thought of as accidental.

The rapidity of the moving chloroplasts varies greatly. From the film only the medium rapidity during each 10 sec. interval can be established. The average rapidity of the chloroplast movement may be estimated at approximately  $0.066 \mu/\text{sec}$ , while the greatest observed rapidity during a 10 sec. interval is  $0.31 \mu/\text{sec}$ . Temporary rapidities may be somewhat greater though probably not much so. The rapidity varies greatly and very suddenly. Often, a chloroplast stationary during one 10 sec. interval moves with almost the maximum rapidity during the next one, only to slow down again during the following interval. Only unceasing changes of rapidity are observed, and no periodic acceleration and retardation of the movements is noticeable — there is no rhythm in the movements. When simultaneous rapidity changes of different chloroplasts are compared a correlativity of these changes is visible. This sometimes refers mainly to neighbouring chloroplasts, but can never be applied to all chloroplasts in a cell. Even when the changes in rapidity take place more or less at the same time the correlativity of ways of neighbouring chloroplasts is always far more pronounced.

When comparing chloroplast movements in spring and autumn leaves no marked differences are visible in rapidity and changes of rapidity. The chloroplast ways however seem to be more complicated in the autumn material.

It is difficult to establish whether chloroplasts revolve at the same time as they move forward, because as seen from above, they are round

and thus their rotation, if there is one, is imperceptible. In one cell, however, it was possible to establish that an elongated chloroplast rotated partly round its axis while moving forward (Fig. 6) In this case there is no relation between rapid progressive movements and rapid rotation.

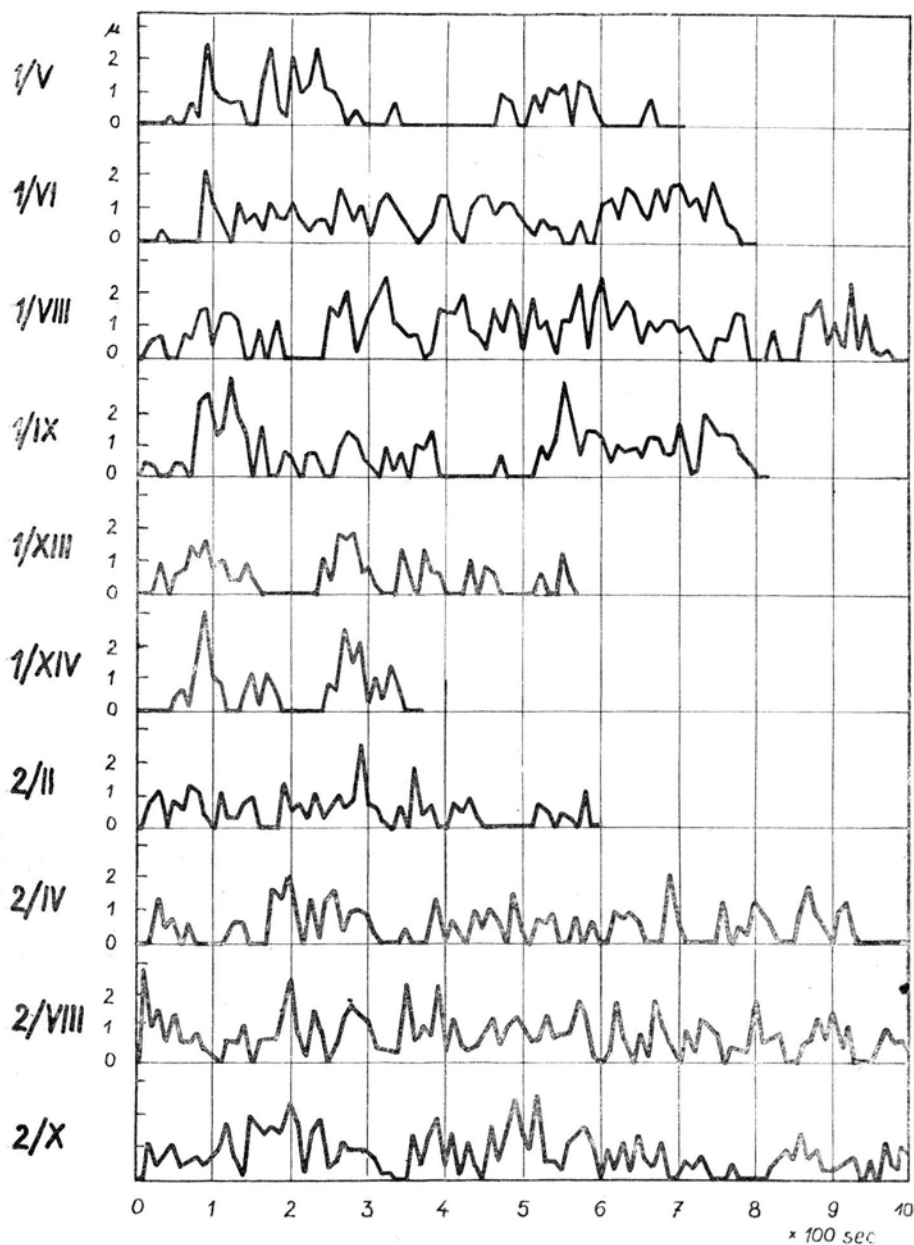


Fig. 5. Rapidity graph of some chloroplasts in the cells 1 and 2.

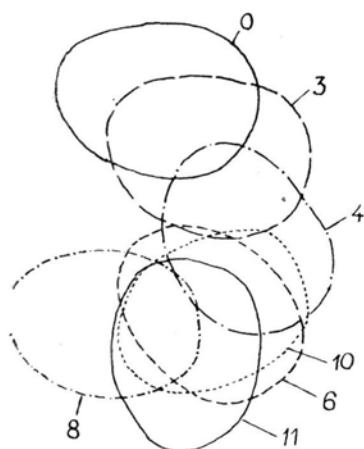


Fig. 6

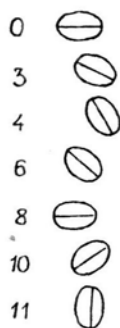


Fig 7

Fig. 6. Rotation of chloroplast round its axis and its transposition. Chloroplast outline drawn every 10 sec. Left — diagram of the rotation of chloroplast.

Fig. 7. Chloroplast turn to a profile position on the upper cell wall. Chloroplast outline drawn every 10 sec.

In one of the 28 reactions which were filmed other revolving movements also took place. However, the cell was from autumn material and perhaps was not quite normal. As the chloroplasts moved to and finally reached an incomplete parastrophe arrangement, some of them turned several times, while still on the upper cell wall, to its side from the horizontal position. The chloroplast remained so for 0,5 to 3 mins. and then returned to the normal horizontal position. It is not known, whether this is a normal though uncommon phenomenon or whether this kind of movement is caused by some defect in the cell.

### Discussion

The method of reducing time has long been applied successfully for solving different problems in cellphysiology. Its application in investigating phototactic chloroplast movements gave also interesting results. The lack of a constant direction of motion as well as the numerous changes of the ways and rapidity make a set of hitherto unknown facts which will have to be considered in the future theory of the mechanism of phototactic movements.

The numerous and irregular changes in the direction and in rapidity of chloroplast movements are similar to though much slower than the creeping movement of protoplasm (Glitschewenug). Nevertheless it is possible that both these processes are in some way related to each other.

TABLE I

The rapidities of chloroplast movements in the phototactic reactions

Material	Rapidity $\mu$ /sec.	Autor	
Mesocarpus	0.014	Senn 1908	The movement of the outer part of chloroplast
Funaria	0.008	Senn 1908	Para — epi. reaction
Striatella	0.120	Senn 1908	The movement to systrophy
Lemna	0.066	This paper	Epi—para. reaction. Average rapidity
Lemna	0.310	This paper	Epi—para. reaction. Maximal rapidity

The average rapidity of phototactic chloroplast movements was given by Senn (1908). He divided the distance travelled by a chloroplast (in a straight line) by the duration of the reaction and obtained values given in Table I. The values obtained in the course of the present work are somewhat higher. This is easily explained if it is considered that chloroplast ways are much longer in reality than Senn assumed.

TABLE II

The rapidities of chloroplast movements in the rotation of protoplasm

Material	Rapidity $\mu$ /sec.	Autor
Nitella	50 — 60	Lambers 1925
Nitella	45 — 50	Umraht 1934
Vallisneria	2 — 17	Jurisc 1925
Elodea	4 — 5	Beikirch 1925
Elodea	5.5 — 6	Zurzycki 1951

The rapidities in Table I are, more or less, in mutual agreement, and differ distinctly from the rapidities of rotary chloroplast movements (Table II).

## SUMMARY

Phototactic chloroplast movements in epistrophe-parastrophe reaction were analysed in *Lemna trisulca*. The method used consisted on reducing time. It was found that:

1. Nearly all chloroplasts which are in the horizontal arrangement are in motion from the very beginning of the reaction.



2. The way of each chloroplast is very complicated and its direction changes constantly.

3. The rapidity of the movement changes constantly and there are no periodic changes or rhythm in the movement. The average rapidity is 0,066 and maximum 0,31  $\mu$ /sec.

4. In some cases temporal correlativity in the direction of movement and rapidity changes may be observed in neighbouring chloroplasts.

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