# The Influence of Temperature on Phototactic Movements of Chloroplasts

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## INTRODUCTION

All researches on phototaxis of chloroplasts were so far concerned mainly with their relation to the colour and intensity of light, (S e n n 1909, V o e r k e l 1934) but the influence of other factors on this process and especially the influence of temperature is very little known.

Investigations on the influence of temperature on phototaxis of chloroplasts concerns two points of importance:

1. the understanding of the influence of temperature on photactic translocations of chloroplasts could lead to conclusions on the mechanism of this movement or the processes which have an effect on it.

2. informations on the rapidity of rections at different temperatures enable an estimation of the error in those experiments in which the temperature was not kept absolutely constant.

Past investigations dealt only with the effect of extreme temperatures on phototatic transpositions of chloroplasts (F r a n k 1872, S e n n 1908, S e n n 1909), and there is no experimental data on the relations between the rapidity of the process and temperatures within normal limits. The reason for this was the difficulty to establish the duration of phototactic reactions. Such reactions develop more or less individually in each cell and it is difficult to establish exactly when they end. It often happens that in neighbouring cells they vary considerably ( V o e r k e l 1934).

This paper presents an attempt to use statistical methods for investigating the four main phototactic movements:

epistrophe to apostrophe
epistrophe to parastrophe
apostrophe to epistrophe
parastrophe to epistrophe

(S e n n 1909) and the influence of temperature on these movements.

## METHODS

The water plant *Lemna trisulca* L., which was grown in an aquarium, was used for the experiments.

The investigation on the phototaxis of chloroplasts in *Lemna trisulca* was divided into four stages:

1. Preparing the needed starting positions of chloroplasts in cells,
2. acclimatisation of the plant to the temperature under investigation without altering the position obtained previously.
3. Measuring the rapidity of translocations of the chloroplasts for different intensities of light and at constant temperature,
4. Computing the rapidities of reactions from data obtained in stage 3.

### 1. Preparing the material.

Several plants were placed in a small basin and were either lighted or darkened so as to obtain the needed position of chloroplasts in cells. The epistrophe was obtained by lighting the plants from a distance of 30 cms. with a 60 W electric lamp during one hour; to obtain the parastrophe the same lamp was used but the distance was 15 cms and the time 30 minutes. The apostrophe was reached by keeping leaves of *Lemna trisulca* in darkness for approximately 12 hours.

### 2. Acclimatizing the plants to the temperature under investigation.

When the needed starting position of the chloroplasts in cells was obtained (epistrophe, parastrophe or apostrophe) the plant was placed on a warmed microscope table where the intensity of light was such as not to alter the previously obtained position of chloroplasts. Then during 15 minutes the temperature was gradually changed and when it reached its final level the leaf was kept in it for 20—30 minutes. During the experiment the microscope was

placed in a thermostat in which the needed temperature was maintained with an accuracy of ± 2°C; also the temperature of the microscope table was regulated with an accuracy of ± 0,2°C. (Zurzycki in press).

3. Measuring the rapidity of transposition of chloroplasts at various temperatures.

When the time of acclimatisation had elapsed the intensity of light was altered and the phototactic reaction was observed.

Epistrophe was induced — in apostrophe to epistrophe, and parastrophe to epistrophe reactions — by illuminating the microscope with a 15 W electric lamp of dim glass placed at a distance of 50 cms from the thermostat aperture. A pane of dim glass placed in front of this aperture further weakend the light.

To induce parastrophe — in the epistrophe to parastrophe reaction — the light came from a 60 W electric lamp of dim glass placed 10 cms away from the thermostat.

When inducing apostrophe — in the epistrophe to apostrophe reaction — the aperture of the thermostat was shut and the leaf was in absolute darkness. Only when measurements were taken the microscope was lit as dimly as possible.

Lange's S60 photoelectric cell which was mounted in the place of the microscope mirror was used to measure the relative intensities of light. These were: 100 (for parastrophe): 10 (for epistrophe): O (for apostrophe).

The counting was done, according to the rapidity of reaction, at intervals of 5, 10, or 20 minutes. In 25 cells chloroplasts in horizontal position (= epistrophe) were counted and for each counting the same 25 cells were as far as possible chosen.

The observations were done at the edge of the leaves with a single mesophyll layer of cells.

A Zeiss microscope with a 10x eyepiece and 40x objective was used for counting.

The change in numbers of chloroplasts in epistrophes was plotted agasinst time on a graph. Observations were done at temperatures of 5, 10, 15, 20, 25, 30 and 35°C. The experiment was repeated four times for each temperature and for each type of reaction. Moreover reactions of every type were observed for three hours each at temperatures of 10, 20 and 30°C.

4. Calculating reaction time.

The duration of the reaction was estimated from the curve which illustrated the proces at the given temperature. It was accepted that the duration of a reaction in the case of epistrophe to apostrophe and the epistrophe to parastrophe reactions was the interval of time from the moment when the first chloroplasts lost their horizontal position to when 50% of their initial number were no longer in this position; on the other hand in the case of apostrophe to epistrophe and parastrophe to epistrophe reactions the interval of time from the moment when half of the position no longer increased. The final number of chloroplasts in epistrophe in respective temperatures was considered to be a hundred percent. Figures 1a. and 1b. illustrate how the durations of the reactions were calculated.

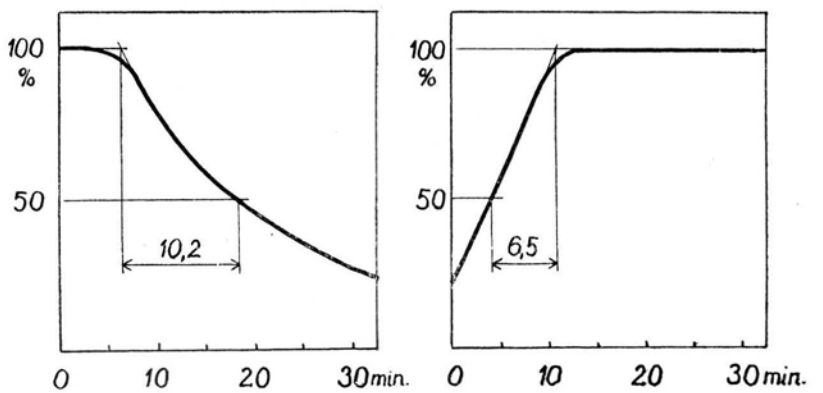


Fig. 1. Diagram for calculating the reaction time in: a) epistrophe to parastrophe, and epistrophe to apostrophe reactions, and b) apostrophe to epistrophe, and parastrophe to epistrophe reactions. For details see text.

## RESULTS.

The statistical method which was employed brought out a considerable uniformity in the movements of chloroplasts. The curves which were plotted are on the whole of a very regular shape and in respect to temperature the obsereed time of reactions coincide rather closely, although the number of chloroplasts in epistrophe varied markedly (in 25 cells there were 130—380 chloroplasts).

Epistrophe to parastrophe reaction.

In all temperatures chloroplasts which on the outset are in epistrophe begin to move towards the lateral sides of the cell imme-

diately the intensity of light is increased. The rapidity of this movement is distincly dependent on temperature. At 5°C. it takes approximately 20 minutes for half of the chloroplasts to disappear from the outer walls of the cell, while at 20°C the same effect is observed in 10, and at 35°C in 5 minutes.

TABLE I.

Rapidity of epistrophe to parastrophe reaction and its relation to temperature.

| Temp. C° | Reaction time min. | | | | Average reaction time | Average rapidity | $Q_5$ |
|---|---|---|---|---|---|---|---|
| 5° | 20,2 | 20,7 | 18,7 | | 19,87±0,58 | 5,03±0,15 | |
| 10° | 14,5 | 15,2 | 16,1 | 14,2 | 15,00±0,50 | 6,66±0,22 | 1,32 |
| 15° | 13,2 | 12,0 | 11,7 | 11,7 | 12,15±0,28 | 8,21±0,36 | 1,24 |
| 20° | 10,1 | 10,0 | 8,4 | 10,3 | 9,70±0,32 | 10,31±0,34 | 1,25 |
| 25° | 6,3 | 7,9 | 9,4 | 7,8 | 7,85±0,57 | 12,74±0,92 | 1,24 |
| 30° | 6,8 | 6,0 | 6,5 | 6,0 | 6,32±0,27 | 15,83±0,68 | 1,24 |
| 35° | 4,7 | 4,9 | 4,7 | 5,5 | 4,95±0,52 | 20,20±2,12 | 1,28 |

The reaction time and also the calculated average time and average rapidity of reactions (inverse of reaction time) are given in Table I. The coefficient of temperature $Q_5$ calculated from the average rapidity of reactions is also tabulated. These coefficients are fairly constant (average 1,26) for all the temperatures, which indicates that within the range of temperatures observed van't Hoff's law can be applied to the epistrophe to parastrophe reaction (fig. 7).

Fig. 2 illustrates the development of the epistrophe to parastrophe reaction during a period of 3 hours at temperatures of 10, 20 and 30°C. The curve shows that at low temperatures (10°C) the percentage of chloroplasts in epistrophe decreases evenly reaching after one to two hours a low figure of several (less than 20) per cent. On the other hand when the temperature is higher, the reaction is at first far more rapid, but it ceases when the percentage of chloroplasts in epistrophe is still relatively high, whereupon there takes place a slight increase in this percentage.

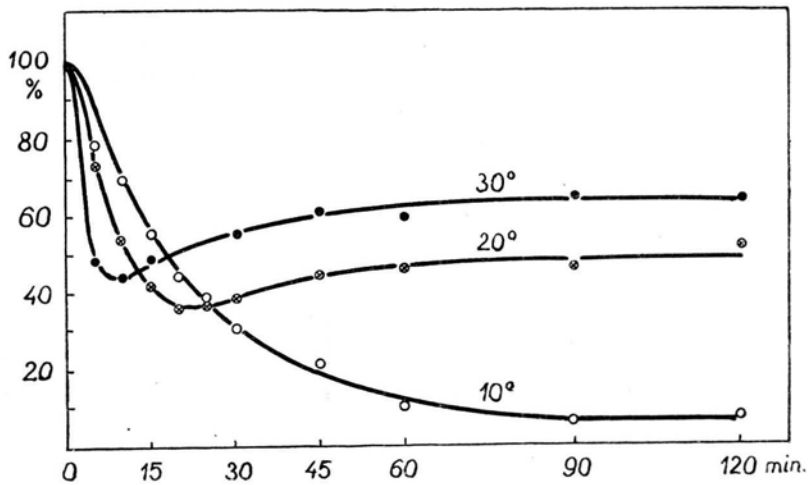


Fig. 2. Development of epistrophe to parastrophe reaction at 10, 20, and 30° C. Abscissa — time in minutes, ordinate — percentage of chloroplasts in epistrophe.

Epistrophe to apostrophe reaction.

It is only at high temperatures that the reaction begins immediately after the leaves are placed in darkness. At medium and low temperatures there is at first no change in the epistrophe and it is not till after some considerable time (up to 90 minutes) that the chloroplasts begin to move. The time which must pass before the reaction starts off was called „the preliminary period of the reaction". The lenght of this period depends on temperature — the lower the temperature the longer the preliminary period of a raction.

Table II besides giving the time of reactions gives also — in brackets — the time of preliminary periods of reactions. Of these the latter was counted from the moment of darkening till, the chloroplasts began to move and the first from this moment onwards.

The time of translocation of chloroplasts is much longer than in the previous reaction. The rapidity of reaction increases with the increase in temperature (Table II) while the coefficients of temperature are also approximately constant (average = 1,47; fig. 7). At 5°C the reaction does not start till after 90 minutes and develope so slowly that even after several hours not more than 20—30% of the chloroplasts have moved. For this reason it is not possible to establish the reaction time on the same basis as in the case of other temperatures.

TABLE II.

Rapidity of epistrophe to apostrophe reaction and its relation to temperature.

| Temp. C° | Reaction time min. (preliminary periods are given in brackets) | | | | Average reaction time | Average rapidity | $Q_5$ |
|---|---|---|---|---|---|---|---|
| 5° | | | | | | | |
| 10° | 88 (81) | 54 (72) | 74 (62) | 82 (68) | 74,50±6,51 | 1,34±0,12 | 1,76 |
| 15° | 35 (22,5) | 33,5 (21) | 35,5 (52) | 66 (18) | 42,50±6,70 | 2,36±0,37 | 1,34 |
| 20° | 34 (37) | 29 (38) | 34 (29) | 29 (20) | 31,50±1,12 | 3,17±0,11 | 1,50 |
| 25° | 21,5 (11) | 19,5 (10,8) | 17,2 (11,3) | 25,6 (10,1) | 20,95±1,50 | 4,77±0,34 | 1,45 |
| 30° | 15,6 (0) | 16,5 (13,6) | 15,4 (0) | 10,3 (0) | 14,45±1,41 | 6,91±0,62 | 1,33 |
| 35° | 15,9 (0) | 5,0 (0) | 11,8(0) | | 10,90±2,71 | 9,17±2,28 | |

The decrease in the percentage of chloroplasts in epistrophe attains eventually a fixed value which remains unchanged for a considerable time. On the whole this percentage is smaller when temperatures are higher and greater in lower temperatures (fig. 3), which is the opposite of what was observed in the case of epistrophe to parastrophe reaction.

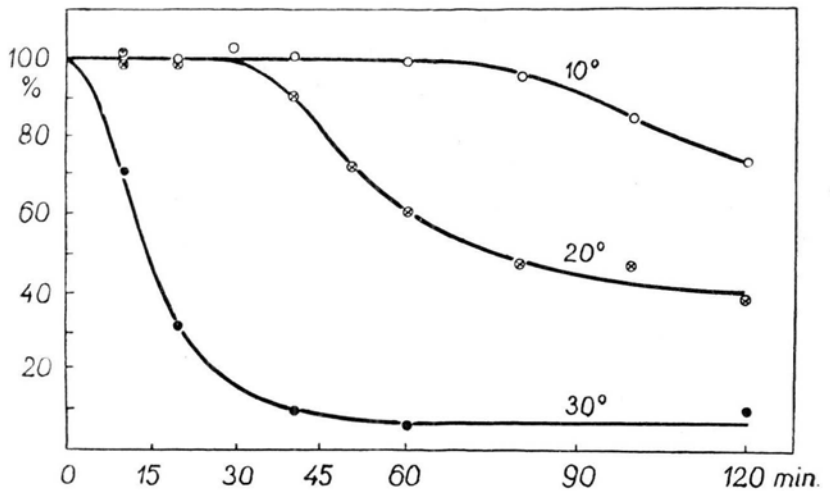


Fig. 3. Development of epistrophe to apostrophe reaction at 10, 20 and 30° C. Details as in fig. 2.

Apostrophe to epistrophe reaction.

This reaction starts off immediately when the leaf is brought into light, however the chloroplasts move at first towards the lower outer walls of the leaf cells (towards light) and only after some delay towards the uper walls where they are counted. The translocation takes place rather rapidly and complete apostrophe is always obtained after 20 minutes. There is no change in this condition for a considerable time (fig. 4).

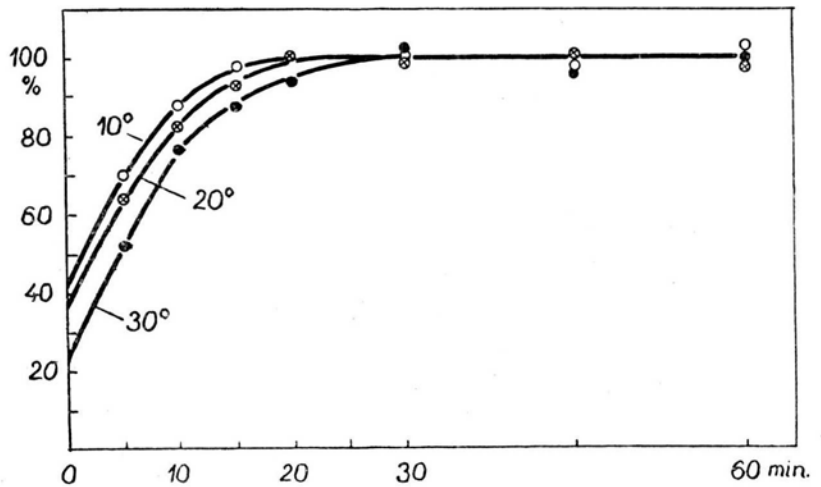


Fig. 4. Development of apostrophe to epistrophe reaction at 10, 20 and 30° C. Details as in fig. 2.

The apostrophe to epistrophe reaction is less regular than the previous ones. There appears to be a considerable discrepancy in the results obtained when the time of reaction is calculated for different temperatures and for different repetitions at the same temperature, also the error of the average value is considerable (Table III). The average velocities at the different temperatures are rather varied, however in wiew of a considerable average error they do not show essential differences within the range of tempraturs observed. On considering the results obtained it seems that this reaction is not dependent on temperature (fig. 7).

In this reaction the epistrophe is not typical at both higher and lower temperatures when the number of chloroplasts in epistrophe reaches an unchanging level, and though the chloroplasts are in a horizontal position almost all remain near the lateral walls of

TABLE III.

Rapidity of aprostrophe to epistrophe reaction and its relation to temperature.

| Temp. C° | Reaction time min. | | | | Average reaction time | Average rapidity | $Q_5$ |
|---|---|---|---|---|---|---|---|
| 5° | 7,2 | 8,2 | 6,4 | 9,5 | 7,82±0,59 | 12,78±0,96 | |
| 10° | 8,4 | 8,5 | 5,7 | 5,6 | 7,05±1,00 | 14,17±2,01 | 1,11 |
| 15° | 6,7 | 9,5 | 7,8 | 5,1 | 7,28±0,79 | 13,73±1,49 | 0,97 |
| 20° | 5,8 | 7,2 | 8,1 | 8,8 | 7,47±0,74 | 13,38±1,33 | 0,98 |
| 25° | 8,8 | 7,0 | 7,3 | 7,8 | 7,82±0,52 | 12,78±0,85 | 0,96 |
| 30° | 8,6 | 8,0 | 6,0 | 7,7 | 7,58±0,45 | 13,18±0,78 | 1,03 |
| 35° | 7,1 | 7,7 | 6,3 | | 7,03±0,37 | 14,21±0,74 | 1,08 |

the cell and the middle of the cell, if viewed from above, is either empty or almost empty. This arrangement is very similar to what V o e r k e l (1934) described as „pseudoparastrophe". As moreover the absolute number of chloroplasts in epistrophe was comparatively low it was thought doubtful whether the epistrophe obtained at extreme temperatures — though unchanging — would correspond quantitatively to the epistrophe obtained in the same cells at medium temperatures. In order to investigate this problem more closely, at the moment when at a given temperature no more change in the epistrophe took place the temperature was either raised or lowered to 20°C and after a new arrangement of chloroplasts had established itself, the chloroplasts in epistrophe were counted once more.

The results of above experiments are illustrated in fig. 5. The number of chloroplasts in epistrophe at 20°C was considered to be a hundred per cent, and relatively the percentage of chloroplasts in complete epistrophe was determined at other temperatures. Within the same cells the number of chloroplasts in epistrophe is far more numerous — often double — at 20°C, than at higher and lower temperatures. Only at 35°C though a typical pseudoparastrophe can be observed there is no further movement of chloroplasts towards the outer walls after the temperature is lowered. As the number of chlo-

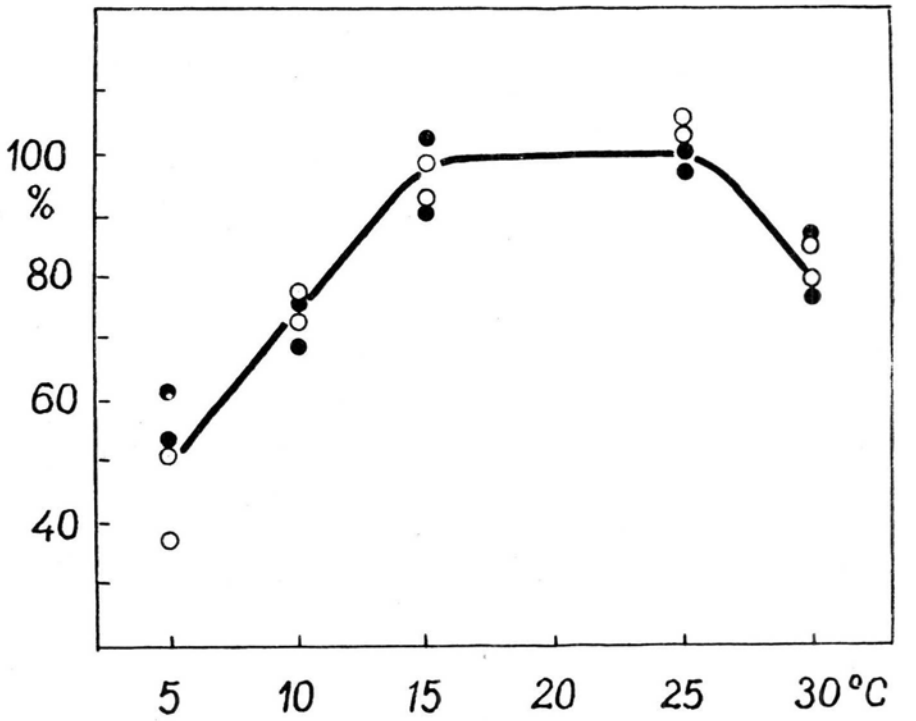


Fig. 5. Percentage of complete epistrophe at temperatures from 5 to 30° C. This percentage wes calculated relatively to the epistrophe at 20°C=100%. Black dots denote apostrophe to epistrophe and white dots parastrophe to epistrophe reactions.

roplast in epistrophe is small it appears that at this temperature also, only a part of the chloroplasts assume a horizontal position, and it seems that such a temperature must damage the cell and so hinder the movement of chloroplasts when the temperature is lowered again.

Parastrophe to epistrophe reaction.

This reaction starts off immediately the intensity of light is decreased, it develops rapidly and is slightly more regular than the last one. In this kind of reaction the chloroplasts move at first towards the upper cell walls (away from light) and this may be the reason why the results are more regular (Table IV).

In this reaction as in the last one the reaction time does not appear to be essentially dependent on temperature. In both higher and lower temperatures the epistrophe is not charactristic and chlo-

TABLE IV.

Rapidity of parastrophe to epistrophe reaction and its relation to temperature.

| Temp. C° | Reaction time min. | | | | Average reaction time | Average rapidity | $Q_5$ |
|---|---|---|---|---|---|---|---|
| 5° | 5,9 | 4,8 | 8,7 | 6,2 | 6,40±0,69 | 15,62±1,68 | |
| 10° | 6,1 | 6,2 | 6,4 | 6,3 | 6,17±0,47 | 16,21±1,24 | 1,04 |
| 15° | 6,4 | 4,8 | 6,5 | 7,2 | 6,22±0,45 | 16,08±1,16 | 0,99 |
| 20° | 4,0 | 6,1 | 6,7 | 6,8 | 5,90±0,55 | 16,95±1,58 | 1,05 |
| 25° | 6,1 | 5,5 | 6,6 | 6,2 | 6,10±0,16 | 16,39±0,43 | 0,96 |
| 30° | 6,8 | 6,6 | 5,6 | 5,8 | 6,20±0,55 | 16,12±1,43 | 0,98 |
| 35° | 6,3 | 5,7 | 7,3 | 6,8 | 6,52±0,32 | 15,33±0,75 | 0,95 |

roplasts in epistrophe are less numerous than at 20°C. In fig. 6 the number of chloroplasts in epistrophe at different temperatures observed is compared to the number of chloroplasts in epistrophe at 20°C. This last number was considered to be a hundred per cent.

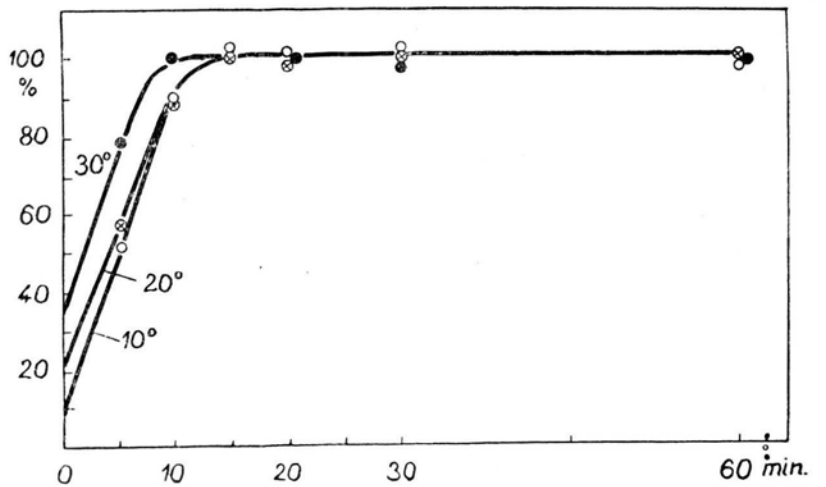


Fig. 6. Development of parastrophe to epistrophe reaction at 10, 20 and 30° C. Details as in fig. 2.

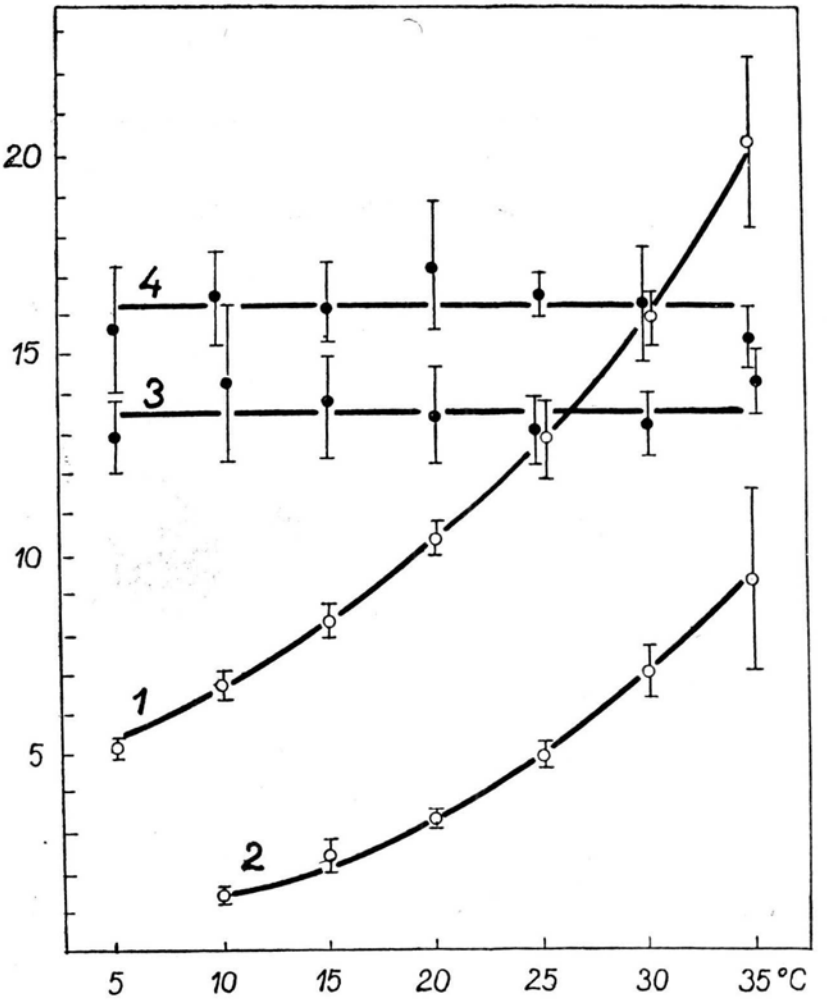


Fig. 7. Dependence of phototactic movements of chloroplasts from temperature. 1 — epistrophe to parastrophe, 2 — epistrophe to apostrophe, 3 — apostrophe to epistrophe, 4 — parastophe to epistrophe. Abscissae — temperatures, ordinates — average rapidities of reactions.

## DISCUSSION.

The main difficulty, when phototactic translocations of chloroplasts are investigated relatively to both light and temperature, is the determination of the reaction time. The two following factors cause the results obtained, when a single cell is observed, to be inaccurate: (i) the capability to react varies in each individual cell,

and (ii) the difficulty to notice when a reaction ends, i. e. the moment when the last chloroplast moves. For this reason if the time of reaction is estimated directly from observations in a microscope the error is very considerable (Voerkel 1934).

The statistical method used in this investigation by considering a number of cells (25) in each experiment, eliminates the individual variability of cell reactions; also by calculating the time of reaction to the moment when still 50% of the chloroplasts are in epistrophe and the reaction is in full development they eliminate the error outcoming from an individual estimation of time, mentioned above. Another reason why such a definition of the reaction time is necessary, especially in the case of *Lemna trisulca,* is that not always all the chloroplasts move towards the lateral walls, and very often, mainly when in apostrophe some remain horizontal.

The four reactions which were investigated can be divided into two groups according to the influence of temperature on their velocity.

1. In one group would be those reactions in which the epistrophe is the initial position and the rapidity of a reaction depends on temperature according to and in agreement with van † Hoff's law.

2. On the other hand in the second group are those reactions in which the initial position of the chloroplasts is vertical (apostrophe and parastrophe) and the rapidity of the reaction is not influenced by temperature.

In research on the wave lengths of light which induce phototactic movements of chloroplasts Zurzycka (in press) has shown that when the epistrophe is the initial position phototaxis is caused by light absorbed by chlorophyll, and that chloroplasts in a vertical position respond to light absorbed by carotene. The results obtained in the present investigation demonstrate that the two reactions are distinctly different not only in regard to the influence of wave lengths of light, but also in regard to temperature.

The epistrophe to parastrophe reaction is the most regular in its development. The epistrophe to apostrophe reaction varies distincly from the previous one, though van't Horr's law can be applied to it. The dissimilarities in the latter case are (i) the existence of a preliminary period, (ii) lesser rapidity of the reaction and (iii) a higher value of van't Hoff's coefficient.

The existence of the preliminary period only in the case of the epistrophe to apostrophe reaction indicates that from the beginning of stimulation (darkening) to the beginning of the reaction a preparatory interval of time is necessary. It is possible that during this interval a substance which starts off the reaction is produced. This process is connected with the metabolism of the cell, and the higher the temperature the quicker it develops.

The lack of a preliminary period in the epistrophe to parastrophe reaction denotes that this reactions is not strictly connected with the metabolism of cells. This is in accordance with the observations of L i n s b a u e r and A b r a m o w i c z (1909) who demonstrated that ether stops completely the epistrophe to apostrophe reaction while no such effect is observed in the case of the epistrophe to parastrophe reaction.

The longer duration of the epistrophe to apostrophe reaction than of the epistrophe to parastrophe reaction described in this paper confirms V o e r k e l's results, who states that in the case of phototactic movements in the cells of *Funaria hygrometrica* at a temperature of ± 20°C the reaction lasts from 18 to 78 minutes and from 5 to 35 minutes respectively.

The marked disparity in van't Hoff's coefficient indicates that the two reactions are of a decidedly different nature.

According to the results obtained those reactions in which the initial position of chloroplasts is vertical (apostrophe to epistrophe and parastrophe to epistrophe) are not dependent on temperature.

The only difference between parastrophe to epistrophe and apostrophe to epistrophe reactions is, that of the two the former develops more rapidly. This can probably be explained by the fact that chloroplasts initially in apostrophe move at first towards the lower walls of the cells (towards light) and only after some time to the upper cell walls where they are counted. On the other hand, when initially in parastrophe, the chloroplasts move at first away from light. In all other respects the influence of temperature on both reactions is identical.

This independance from temperature is very unexpected. Even if we accept that a physico-chemical reaction, inherently independent from temperature — e. g. photochemical — starts off the reaction, we must remember, that a change in temperature causes a different viscosity of protoplasm and changes also the resistance to the movements of chloroplasts within the cell. In both reactions at high as well as at low temperatures the epistrophe observed is not cha-

racteristic (pseudoparastrophe), but it is obtained in the same time as the complete epistrophe at medium temperatures. The reaction is not characteristic in that, only some chloroplasts arrange themselves in a horizontal position — by the walls only —, and the rest do not move from the lateral walls. Such an arrangement of chloroplasts was observed by V o e r k e l (1934) when he exposed the cells of *Funaria hygrometrica* to strong doses of ultraviolet rays. This treatment, he was able to show by centrifugation, caused a considerable increase of viscosity of the protoplasm. It probably can be assumed that the incomplete epistrophe at extreme temperatures is due to the increased viscosity of the protoplasm.

The two curves plotted for the apostrophe to epistrophe and the epistrophe to parastrophe reactions intersect on the graph at a temperature corresponding to 25°C. At this temperature the velocities of the two reactions are approximately equal, which is in agreement with observations made by V o e r k e l (1934) on *Funaria hygrometrica* and by L e w i s (1894) on *Mougeotia.*

Owing to the great influence of temperature on those phototactic reactions in which the epistrophe is the initial position it is of utmost importance in all such experiments for the temperature to be regulated at an unchanging and known level. Neglecting of this condition may introduce considerable errors, as for instance in V o e r k e l's experiments, when temperature varied from 20°C to 28,3°C which could cause a 40% inaccuracy in the estimate of the reaction time.

## SUMMARY OF RESULTS OBTAINED.

1) The statistical method used to estimate the reaction time of phototactic movements of chloroplasts makes a very precise estimation of the duration of the reaction possible.

2) The characteristic points of the influence of temperature on the four main phototactic reaction are:

(i) in epistrophe to parastrophe reactions — a very regular development and a dependence of rapidity from temperature according to van't Hoff's law with a coefficient 1,26.

(ii) in epistrophe to apostrophe reaction — existence of a preliminary period of the reaction and its slow development; the dependence of rapidity from temperature according to van't Hoff's law with a coefficient 1,47.

(iii) in both apostrophe to epistrophe, and parastrophe to epistrophe reactions — a fairly regular development and lack of a marked dependence from temperature.

3) The results obtained indicate that temperature must be very carefully controled when phototactic movements of chloroplasts are investigated.

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