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Daily Cycle of Mitotis in Root Tips of *Hydrocleis nymphoides* (Willd). Buchenau

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Knowledge of the general cycle of nuclear divisions in an organism is quite a useful guide in selecting the appropriate times for collection of material for cytological studies. Thus, to study nucleoli, the best times for collecting the experimental material would be when interphase stages are most frequent in the cycle; and for physiological treatments to effect C-mitosis, the best times for collecting the experimental material might be about just before metaphase stages show up most abundantly in the cycle.

Changes in environmental conditions, such as temperature, acidity, and illumination, may affect the pattern of nuclear divisions. On the other hand, if the pattern of nuclear divisions is found not to be susceptible to changes in environmental conditions, the experimenter would be free to collect his material or to pre-treat it at any time of the day, as opposed to being restricted to specific times only.

The object of the investigation was to find out whether throughout a continuous period of 12 hours there were any significant variations in the relative frequencies of the mitotic stages in the root-tips of an *Hydrocleis nymphoides* cultivation at the University of Ghana, Legon. The cultivation was in a pond in the teaching garden of the Botany Department.

Experiments and results

About 10 root-tips from randomly selected plants at different points in the pond were collected hourly from 6.00 a.m. to 5.00 p.m. Each time, the temperature and pH at different places in the pond water, and the temperature of the atmosphere around the pond, were recorded. On collection of the root-tips, they were fixed immediately in a freshly prepared 1: 3 acetic-alcohol for 12–24 hours. They were then stored in 70% alcohol till needed for slide preparation.

The method of Backman (1935) for chromosome counts was used in the preparation of the slides. The slides were examined immediately after preparation under low power, and the various mitotic stages in a number of different fields were counted.

A second set of hourly root-tip collection was made on a different day when the cultivation had been allowed to resettle; but this time from 6.00 p.m. to 5.00 a.m. The same methods of collection, pH and temperature measurements, fixation, slide preparations and counts of the mitotic stages were followed.

Details of the counts are given by Stephens (1967) but the summaries are in Tables 1 and 2 below:

Table 1. Percentages of various mitotic stages in *H. nymphoides* daily cycle: 6.00 A. M.–500 P. M.

Time	Pond Temp.	Pond PH	Resting stage	Prophase	Metaphase	Anaphase	Telophase
6.00 a.m.	24°C	7.5	66.47	25.90	1.95	3.03	2.64
7.00 a.m.	24	7.5	52.33	37.35	3.02	3.70	3.60
8.00 a.m.	24	7.5	31.75	50.05	5.94	7.80	4.46
9.00 a.m.	25	7.5	37.79	44.32	5.14	6.91	5.83
10.00 a.m.	26	7.5	50.54	38.55	2.68	4.01	4.21
11.00 a.m.	27	7.5	46.05	49.49	2.11	1.01	1.33
12.00 noon	28	0.0	18.26	72.86	2.63	1.79	4.46
1.00 p.m.	28.5	8.0	84.64	12.09	1.74	0.87	0.65
2.00 p.m.	29	8.0	65.31	25.40	1.91	1.21	6.17
3.00 p.m.	28	8.0	61.21	30.06	2.22	1.63	4.88
4.00 p.m.	27	7.5	42.46	49.55	4.04	2.06	1.90
5.00 p.m.	27.5	8.0	38.04	53.03	3.31	2.54	3.09

Table 2. Percentages of various mitotic stages in *H. nymphoides* night cycle: 6.00 P. M.–5.00 A. M.

Time	Pond Temp.	Pond PH	Resting stage	Prophase	Metaphase	Anaphase	Telophase
6.00 p.m.	29.5°C	7.0	43.03	49.32	2.19	2.05	3.41
7.00 p.m.	28	7.0	44.17	47.41	1.13	4.21	3.07
8.00 p.m.	28	8.0	45.31	42.52	3.23	2.64	6.30
9.00 p.m.	28	7.5	48.76	41.85	4.69	2.72	1.97
10.00 p.m.	28	7.5	65.29	27.44	2.34	2.66	2.26
11.00 p.m.	27	8.0	60.14	31.87	2.99	2.84	2.15
12 m'night	28	7.0	68.47	26.10	2.63	1.08	1.7
1.00 a.m.	27	7.5	75.40	18.06	1.65	1.86	3.03
2.00 a.m.	27	8.0	65.35	27.46	2.34	2.67	2.18
3.00 a.m.	26	7.5	46.60	44.42	3.08	1.99	3.90
4.00 a.m.	25	8.0	58.38	34.90	2.22	1.15	3.35
5.00 a.m.	25	7.5	51.11	40.91	3.16	2.22	2.5

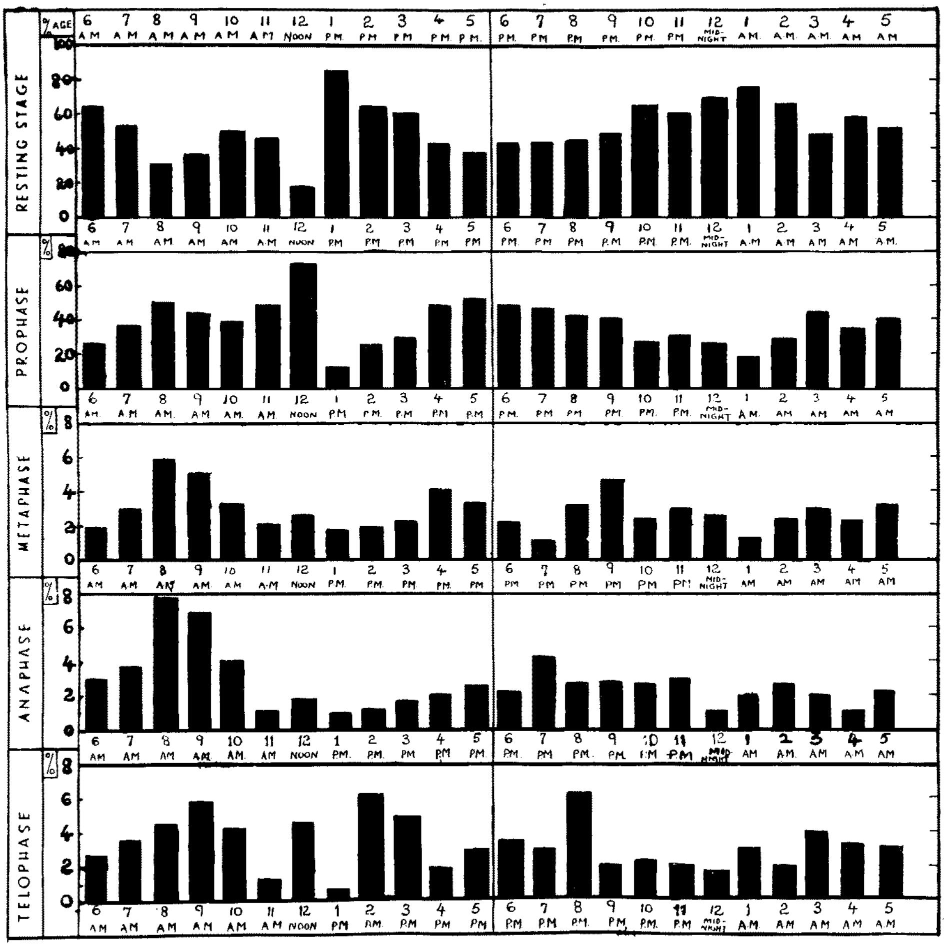
Discussion and conclusions

From Figs. 1 and 2 it is evident that the two sets of collections of root-tips give different patterns of mitotic cycle, which may mean that the mitotic cycle differs from day to night. In both cases, however, interphase and prophase stages are always preponderant; the frequencies of these two stages show a perfect negative correlation (Fig. 2a and b.). And to a large extent in the 6.00 a.m.—5.00 p.m. cycle their frequencies also show a negative correlation with those of the other stages.

In the 6.00 a.m.—5.00 p.m. cycle (Figs 1 and 2), resting stage or interphase shows peaks at 6.00 a.m., 10.00 a.m., and at 1.00 p.m. whilst prophase shows minimal frequencies at these times; further, prophase shows peaks at 8.00 a.m., 12.00 noon, and 5.00 p.m. whilst interphase shows minimal frequencies.

The highest peaks are at 1.00 p.m. for resting stage, 12.00 noon for prophase, 8.00 a.m. for both metaphase and anaphase, and 9.00 a.m. for telophase. Generally, metaphase, anaphase and telophase are quite abundant around 8.00 a.m. and 9.00 a.m. It appears therefore that mitosis in the root-tips of the Legon *H. nymphoides* cultivation generally gives metaphase, anaphase, and telophase stages in high frequencies in the mornings, just when temperatures start rising from the night's minimum. Resting stage and prophase appear most abundantly around noon, when temperatures are quite high. It could be inferred therefore that high tempera-

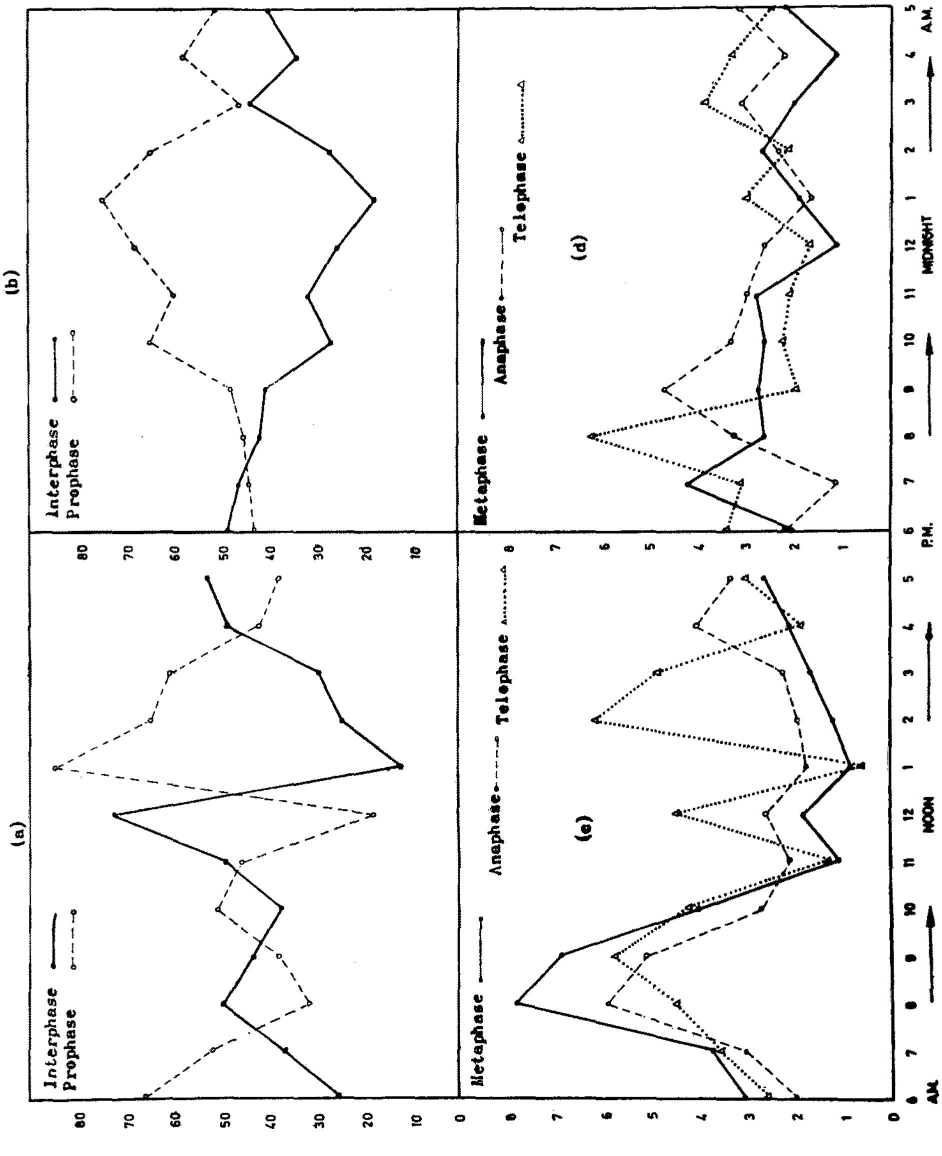
Fig. 1. 12 hour mitotic cycles in root tips-*H. nymphoides*



tures favour resting stage and prophase probably because they are stages of fairly active metabolic activity; and that low temperatures favour metaphase, anaphase, and telophase.

Since telophase also involves some amount of active metabolic synthesis, this may perhaps explain its high frequency at 2.00 p.m. when the temperature was highest.

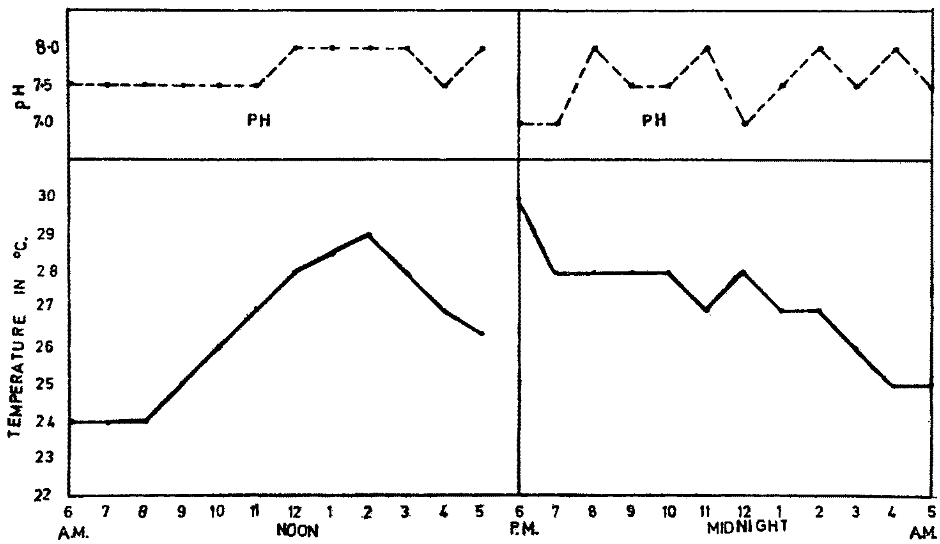
Fig. 2. Percentage frequencies of root tip mitotic stages in *Hydrocleis nymphoides*: 2-12 hour cycles



It is evident throughout the 12 hour cycle that the frequencies of resting stage and prophase are in a clear negative correlation (Fig. 2 a and b), Between 6.00 a.m. and 2.00 p.m. each of these two stages shows a fairly distinct correlation with metaphase, anaphase, and telophase—positive in the case of prophase and negative in the case of interphase.

With the 6.00 p.m.—5.00 a.m. cycle, temperatures were quite high up to about 2.00 a.m. (30°C—27°C). This may perhaps account for the relatively high frequencies of resting stage and prophase. These two stages again show a marked negative correlation in frequencies (Fig. 2 b): from 6.00 p.m. to 1.00 a.m. resting stage shows an increasing trend as prophase decreases steadily in frequencies; these trends are generally reversed after that up to 5.00 a.m. These two stages, however, do not show any correlation with metaphase, anaphase, and telophase in frequencies. In any case, between 6.00 p.m. and about 2.00 a.m. metaphase, anaphase, and telophase show practically the same patterns of cycle (Fig. 2 d).

Fig. 3. Pond water pH and temperature *H. nymphoides* cultivation



Although the two sets of collections were made on different days, it may be inferred that all stages of mitosis occur in the root-tips of *H. nymphoides* throughout the 24 hours of the day, with interphase followed by prophase being preponderant at all times: possibly this is due to their much longer period of duration.

The pH differences do not appear to be correlated with the mitotic cycle; however, it is possible that wide pH differences would affect the mitotic cycle. In any case, the pH and temperature changes were rather too slight as to have had any drastic effects on the mitotic cycles. Thus, the patterns of the mitotic cycle may be due to some inherent periodicity; this however, could be verified by a repetition of the experiments.

With resting stage and telophase, the sharp rise from a minimum frequency to a maximum frequency between noon and 2.00 p.m. is quite significant but cannot be explained. Within the same period that resting stage shows this abrupt rise in frequencies prophase also shows a drastic change in frequencies from a maximum to a minimum. It is possible that these drastic frequency changes were due to sampling error.

With the 6.00 p.m.–5.00 a.m. cycle, the temperature of the pond water decreased continuously throughout the period. The pH of the pond water, however, showed general fluctuations which make it all the less likely to have influenced the mitotic cycle. The highest frequency of anaphase at 7.00 p.m. might have boosted the maximum frequency of telophase an hour later.

It may however be concluded that in *H. nymphoides* whilst all the mitotic stages occur in dividing root-tip cells during both day and night, metaphase, anaphase, and telophase are much more frequent during the day than during the night. Resting stage and prophase are quite preponderant at all times; the two stages always show a distinct negative correlation in frequencies.

It is apparent from Figs. 1 and 2 that although the root-tips for the two sets of experiments were collected on two separate days, the results at the end of the first cycle (5.00 p.m.) merge rather imperceptibly into those at the beginning of the second cycle (6.00 p.m.). This may lend support to the speculation that the mitotic cycle in the Legon *H. nymphoides* cultivation has some underlying inherent pattern although such a pattern may not be too rigid.

Summary

Mitosis in the root-tips of *Hydrocleis nymphoides* occurs throughout day and night with interphase and prophase always predominating and showing a negative correlation with each other. Except for interphase, all the stages are generally more frequent during the day. High temperatures appear to boost interphase and prophase stages whilst low temperatures boost metaphase, anaphase and telophase.

Acknowledgement

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