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Effects of continuous illumination on the rhizoid cluster of *Caulerpa prolifera* [1]

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Under a photoperiodic regime of 12-hr light and 12-hr dark (12L-12D) and continuous illumination (24L-0D), at 24.0±1°C, the rate of elongation of the rhizoid cluster was 8.1 mm/day, and the presumptive site of the cluster initiation was 1.1–1.9 mm from the rhizome tip. However, the plastochron interval under 24L-0D regime was shorter (0.87 day) than under 12L-12D regime (1.18 days). Under both regimes, the plants tended to adjust their presumptive time of cluster initiation to be in the two periods of the day, 0400–0900 hr and 1800–2100 hr. None of the following seemed to correlate with the initiation of a new cluster: a certain number of hours after the initiation of the youngest cluster, a certain distance on the rhizome distal to the youngest cluster, the youngest cluster reaching to a certain length, or a certain value of the rhizoid plastochron index. This adjustment by the plants thus suggested the plants having an ability to perceive the two preferred periods for cluster initiation.

Under a 12-hr light and 12-hr dark (12L-12D) regime, the interval of the rhizoid cluster initiation in *Caulerpa prolifera* was found to be 0.91 day by Chen and Jacobs (*1*). A similar interval, i.e., approximately 1 day, was also reported by Jacobs (*2*). Chen and Jacobs (*1*) found that under a 12L-12D regime the presumptive time of the cluster initiation was mostly in the periods of 0600–0900 hr and 1800–2200 hr, and that the presumptive site of the initiation was 1.3–1.8 mm from the rhizome tip. However Mishra and Kefford (*3*) reported no difference in the rhizoid initiation of *Caulerpa sertularioides* between the light and dark period of a 12L-12D regime. All of these works were carried under a photoperiodic regime similar to that of the natural habitat of *Caulerpa*. Chen (*4*) already reported the effects of continuous illumination (24L-0D) on the elongation of the rhizome of *C. prolifera*. This is the report of the effects of the similar illumination on the initiation and elongation of the rhizoid cluster. The cluster initiation occurred mostly at 0600–0900 hr and 1800–2200 hr as reported by Chen and Jacobs (*1*) could well be due to one of the widely spread time-measuring systems or "clocks" of organisms in their natural habitats (*5*). Thus in the analysis of the present experiment the presumptive time of the initiation under a 12L-12D regime was

Abbreviations: I.C.L., inter-rhizoid cluster length; R.P.I., rhizoid plastochron index.

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compared with that of under a 24L-0D regime to see whether the time is the same under both regimes, and correlations between the initiation time and several morphological events were made to see whether the time correlates with any morphological event.

Materials and methods

The temperature and light intensity (24.0±1°C; 200 ft. c.), the method of culturing experimental plants, and photography of the plants were the same as those described by Chen (*4*). The number of experimental plants used and days in which they were kept under 12L-12D and 24L-0D photoperiodic regimes were identical to Chen (*4*). Numbering rhizoid clusters successively, measuring the lengths of clusters from enlarged photographs, and estimating the rate of cluster elongation were according to Chen and Jacobs (*1*). Estimations of the presumptive time and site of cluster initiation were also similar to those of Chen and Jacobs (*1*). The estimation of the plastochron interval (*4*, *6*) of cluster initiation was done by calculating the interval, in days, of the estimated presumptive time of initiation of two adjacent rhizoid clusters. The frequency distribution of the presumptive times of cluster initiation under each regime (12L-12D or 24L-0D) was represented by a histogram (Fig. 1). Similarly, a histogram (Fig. 2) was made to show the distribution of plastochron intervals under each regime. To determine whether the histograms are single mode or multimode, probit transformation (*7*) was made on these histograms (Fig. 1 and 2). To compare the rate of elongation and the presumptive site of initiation of the cluster under 12L-12D regime against those under 24L-0D regime, the t-test (paired) was employed (Table 1). The same test was also employed to show the difference between plastochron intervals observed under one regime against the other (Table 2). The correlation between two sets of variables, inter-rhizoid cluster lengths (I.C.L.) and plastochron intervals of the plants under each regime, was made, and the correlation coefficient, r ($r^2 = b_{yx} \cdot b_{xy}$, where x = plastochron interval and y = I.C.L.), was calculated. Based on the r-value, a test of significance (t-test) was made (Table 3). To make the resolution of the correlation higher, the plants were divided into two groups, depending on whether the standard error of the rate of rhizome elongation was greater or less than 5% of the mean rate; and each group was further divided into two subgroups, according to the same criterion but pertaining to the plastochron interval (Table 3). Fig. 1 indicates two obvious peaks, 0400–0900 hr and 1800–2100 hr, under both regimes, suggesting that the plants somehow prefer these periods for cluster initiation. To show whether the plants vary their plastochron intervals in order to produce clusters within these periods, the three following sets of histograms were made: 1) both cluster n and $n-1$ initiated within the preferred periods (Fig. 3A and D); 2) the cluster n initiated within but $n-1$ outside of the periods (Fig. 3B and E); 3) both cluster n and $n-1$ initiated outside of the periods (Fig. 3C and F). Successive presumptive times of cluster initiations of two plants, indicated by 1, 2, 3, etc., and their plastochron intervals, in day, between a cluster and the next younger one are illustrated in Fig. 4.

Results

The rate of elongation of the rhizoid cluster under continuous illumination (24L-0D) was not significantly different from the rate under the 12L-12D regime (Table 1; Also see Fig. 2, of Chen (*4*)). Both rates were 4.06 and 4.05 mm/12 hr, or 8.1 mm/day. The presumptive time of the cluster initiation under the 24L-0D regime was also statistically not different from that of the 12L-12D regime (Table 1). Under both regimes the sites were 1.1 to 1.9 mm from the *rhizome* tip. However, the average plastochron interval of the 24L-0D regime (0.87 day) was highly significantly different from that of the 12L-12D regime (1.18 days) (Table 2; Also see Fig. 2 of Chen (*4*), showing that the plotted lines are closer under the 24L-0D regime, i.e., from 8th to 16th day).

Both histograms in Fig. 1 show two periods of high frequency cluster initiation. These periods are at almost the identical time of day for both regimes. Both probit transformations show two relatively linearly ascending segments, one at 0400–0900 hr and the other at 1800–2100 hr, which correspond to those periods of high frequency of the histograms. Both probit plots in Fig. 2 have two straight ascending segments, suggesting that both histograms, one for 12L-12D and the other for 24L-0D, represent two slightly overlapping distributions. The theoretical peaks of the two distributions under the 12L-12D regime are at the vicinities of about 1.0 and 1.5 days; and under the 24L-0D regime, 0.5 and 1.0 day. (Although the significance of the second peak of each histogram in Fig. 2 is based on the frequency of one class being higher than its neighboring classes, the data of Fig. 3A, 3D and Fig. 4 give additional supports of the significance of these two second peaks.)

Table 1

Average rate of rhizoid elongation and presumptive site of rhizoid cluster initiation in each plant under 12 L-12 D and 24 L-0 D regimes

Individual plant	Average rate of elongation (mm/12 hr)			Presumptive site (mm from rhizome tip)		
	(1) 12L-12D	(2) 24L-0D	Column (2) −Column (1)	(3) 12L-12D	(4) 24L-0D	Column (4) −Column (3)
pp 01	3.18±0.16(7)[a]	3.54±0.25(8)[a]	+0.36	1.14	1.28	+0.14
02	3.81±0.32(6)	3.76±0.21(8)	−0.05	1.22	1.10	−0.12
03	3.83±0.23(6)	4.33±0.11(8)	+0.50	1.45	1.43	−0.02
04	4.04±0.20(7)	4.17±0.17(9)	+0.13	1.29	1.66	+0.37
05	4.76±0.36(9)	4.36±0.16(11)	−0.40	1.73	1.82	+0.09
06	4.22±0.35(5)	4.26±0.22(7)	+0.04	1.93	1.80	−0.13
07	3.96±0.21(7)	3.78±0.21(9)	−0.18	1.22	1.28	+0.06
08	4.35±0.19(7)	4.11±0.16(10)	−0.24	1.26	1.68	+0.42
09	4.27±0.29(7)	4.28±0.38(10)	+0.01	1.17	1.59	+0.42
Average	4.05±0.15(9)[a]	4.06±0.10(9)[a]		1.38±0.09(9)[a]	1.52±0.08(9)[a]	
t-Test(paired)	t=0.198[c]; $t_{0.05}$=2.306[b]			t=1.871[c]; $t_{0.05}$=2.306[b]		

[a] Mean, ±standard error, and number of observations in parentheses.

[b] t-Value at 5% level of probability.

[c] Calculated t-value shows no significant difference.

Table 2

Average plastochron interval in each plant under 12 L-12 D and 24 L-0 D regimes

Individual plant	Average plastochron interval (day) (1) 12L-12D	(2) 24L-0D	Column (2) −Column (1)
pp 01	1.23±0.07(6)[a]	1.01±0.02(7)[a]	−0.22
02	1.15±0.06(6)	0.93±0.05(7)	−0.22
03	1.21±0.16(5)	0.95±0.06(7)	−0.26
04	1.18±0.07(6)	0.75±0.07(8)	−0.43
05	0.95±0.05(8)	0.71±0.06(10)	−0.24
06	1.50±0.00(4)	1.13±0.10(6)	−0.37
07	1.18±0.09(6)	0.76±0.07(8)	−0.42
08	1.16±0.08(6)	0.73±0.05(10)	−0.43
09	1.09±0.07(6)	0.86±0.05(9)	−0.23
Average	1.18±0.04(9)[a]	0.87±0.05(9)[a]	
t-Test(paired)		$t=9.791^{c}$; $t_{0.01}=3.355^{b}$	

[a] Mean, ±standard error, and number of observations in parentheses.
[b] t-Value at 1% level of probability.
[c] Calculated t-value shows a highly significant difference.

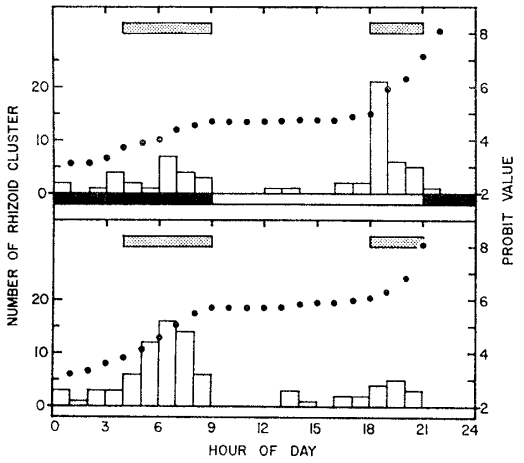

Fig. 1. *Histogram of frequency distribution of the presumptive time of rhizoid cluster initiation in different hours of day; and the probit transformation of the histogram, indicated by solid circles.* Frequencies are represented on the left and probit unit on the right of the graph. Upper one is from the data under 12 L-12 D regime, and lower, 24 L-0 D regime. Notice that in both regimes there are two relatively linearly ascending segments of the probit plot coinciding with two periods of high frequencies in the histogram. These periods are 0400–0900 hr and 1800–2100 hr, indicated by stippled bars.

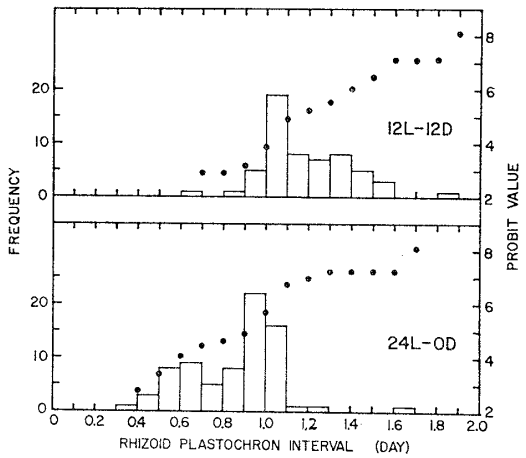

Fig. 2. *Histogram similar to Fig. 1, but pertaining to the distribution of the rhizoid plastochron interval.* Notice that both probit plots have two relatively linearly ascending segments, indicating each histogram being made of two slightly overlapping distributions. The theoretical peaks of the two distributions, which are at the vicinities of the mid-point of the ascending segments, are roughly at 1.0 and 1.5 days under 12 L-12 D regime and 0.5 and 1.0 day under 24 L-0 D regime. (Refer to the text for the significance of the second peak in each histogram.)

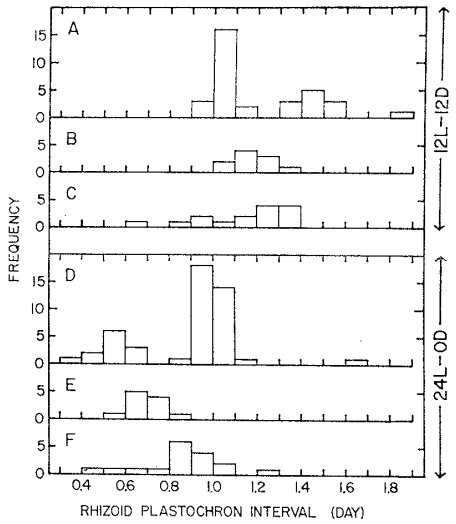

Fig. 3. *Histogram similar to Fig. 2.* Upper and lower 3-histogram sets are respectively the results of grouping the data represented by the upper and lower histograms of Fig. 2. For the criteria of grouping, please see the text. Notice that under 12 L-12 D regime, the position of the distribution of the second histogram (histogram B) is at the area of greater than 1.0 day; while under 24 L-0 D regime, the position of histogram E is less than 1.0 day. Under 12 L-12 D, the position of histogram C tends to be at the place still greater than that of histogram B. On the contrary, under 24 L-0 D regime the position of histogram F is at the area of greater than, instead of less than, that of histogram E.

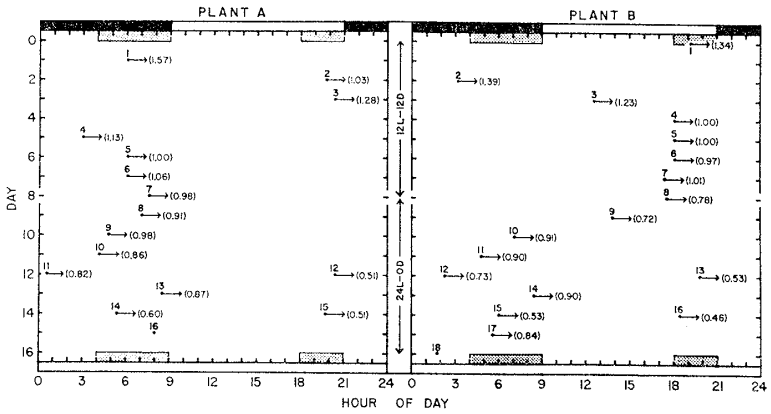

Fig. 4. *Successive presumptive times of cluster initiation of two plants, plant A and B.* From day 0 to 8 is the 12 L-12 D regime and day 8–16, the 24 L-0 D regime. The presumptive times of successive clusters are indicated by the positions of 1, 2, 3 etc., and the plastochron interval, in days, between a given cluster and the next younger one is indicated in parentheses. The two periods preferred by the plants for cluster initiation, 0400–0900 hr and 1800–2100 hr are represented by stippled bars.

The histograms of Fig. 3 suggested 3 phenomena: 1) If the cluster $n-1$ was produced in one of the two preferred periods, under the 12L-12D regime the cluster n tended to be initiated in the same period of one day later or the other preferred period of one and half day later, however under the 24L-0D regime it tended to be produced in the other preferred period, one-half day later, or in the same period, one day later (Fig. 3A and D); 2) If the cluster $n-1$ was produced outside of a preferred period, under the 12L-12D regime, the plants tended to initiate the cluster n in the preferred period of slightly more than one day later, contrarily, under the 24L-0D regime, in the period slightly less than one day later (Fig. 3B and E); 3) If both cluster $n-1$ and n were initiated outside of two preferred periods, the interval varied widely under both regimes, but tended to be longer than one day under the 12L-12D but shorter than one day under the 24L-0D regime (Fig. 3C and F). The described phenomena can also be noticed in Fig. 4, by finding a cluster initiated in one of the shaded areas, the preferred periods, and reading the number in parentheses, the plastochron interval to the next younger cluster; or by finding a cluster outside of the shaded areas and reading the number.

Table 3 shows that when the rhizome elongated at a fairly constant rate (i.e., S.E. of elongation rates being less than 5% of their mean), the variation of I.C.L. was mostly correlated with the variation of the plastochron intervals (three among four correlations showing significant correlation). When the rhizome elongation varies its rate (i.e., S.E. greater than 5% of the mean), the correlation was either significant or not significant. However, in no case was the b_{yx} value very close to zero (i.e., a variable plastochron interval with a constant I.C.L. was not found); actually the b_{yx} values were high in some cases. These facts suggest that an apical area of the rhizome, which is at a certain distance from the most recently formed cluster, was not a signal for initiating a new cluster.

Table 3

Correlation between inter-rhizoid cluster length and plastochron interval

Rate of rhizome elongation	Plastochron interval			
	(S.E. of interval)< (0.05 of mean interval)		(S.E. of interval)> (0.05 of mean interval)	
(S.E. of rate)< (0.05 of mean rate)	A) No plant		B) 12 L-12 D	24 L-0 D
			$r=0.595(2)^{a,b}$ $\begin{pmatrix} b_{yx}=4.791 \\ b_{xy}=0.074 \end{pmatrix}$	$r=0.777(2)^{a,c}$ $\begin{pmatrix} b_{yx}=10.070 \\ b_{xy}=0.060 \end{pmatrix}$
			$0.168(1)^{a}$ $\begin{pmatrix} b_{yx}=-1.287 \\ b_{xy}=-0.022 \end{pmatrix}$	$0.775(2)^{a,c}$ $\begin{pmatrix} b_{yx}=8.003 \\ b_{xy}=0.075 \end{pmatrix}$
(S.E. of rate)> (0.05 of mean rate)	C) 12 L-12 D	24 L-0 D	D) 12 L-12 D	24 L-0 D
	$r=0.490(1)^{a}$ $\begin{pmatrix} b_{yx}=-80.000 \\ b_{xy}=-0.031 \end{pmatrix}$	$r=0.264(2)^{a}$ $\begin{pmatrix} b_{yx}=3.333 \\ b_{xy}=0.021 \end{pmatrix}$	$r=0.622(1)^{a}$ $\begin{pmatrix} b_{yx}=9.003 \\ b_{xy}=0.043 \end{pmatrix}$	$r=0.808(1)^{a,c}$ $\begin{pmatrix} b_{yx}=9.474 \\ b_{xy}=0.069 \end{pmatrix}$
			$0.271(4)^{a}$ $\begin{pmatrix} b_{yx}=-1.994 \\ b_{xy}=-0.037 \end{pmatrix}$	$0.655(2)^{a,b}$ $\begin{pmatrix} b_{yx}=7.276 \\ b_{xy}=0.059 \end{pmatrix}$

[a] Correlation coefficient followed by number of plants in parentheses.
[b] Correlation is significant at 5% level.
[c] Correlation is highly significant at 1% level.

Discussion

Contrary to the response of increasing the rate of elongation made by the rhizome to continuous illumination reported by Chen (*4*), the rhizoid maintained a constant rate (8.1 mm/day) of elongation under both the 12L-12D and 24L-0D regimes (Table 1). The rate was higher than the one (5.2 mm/day) reported by Chen and Jacobs (*1*) under similar experimental condition, but same as the one (8.1 mm/day) observed by Mishra and Kefford (*3*) under 400 ft. c. of 12L-12D regime. Under either 12L-12D or 24L-0D, the plants had a tendency to produce the cluster within two specific periods of day, 0400–0900 hr and 1800–2100 hr (Fig. 1), and at certain place on the rhizome, 1.1–1.9 mm (Table 1) from the rhizome tip. Furthermore, the presumptive time and site of initiation persisted irrespective of the differences in the times of light being turned on and off, since both in this experiment, where light was on at 0900 hr and off at 2100 hr, and in the previous work (Fig. 6 of Chen and Jacobs (*1*)), where the on and off were at 0600 hr and 1800 hr, the similar two peaks and the site of initiation were observed.

The plants not only were able to perceive these preferred periods for the cluster initiation, but also inclined to adjust the initiation time of cluster n to be in one of these periods, in case the cluster $n-1$ was initiated outside of the periods (Fig. 3 and 4). Once a cluster was produced in one of the periods, the plants tended to produce the following clusters within the periods (Fig. 4). The mechanism of the adjustment under one regime however was different from under other regime: under 12L-12D regime, increasing plastochron interval to slightly longer than one day, but under 24L-0D regime, slightly shorter than one day.

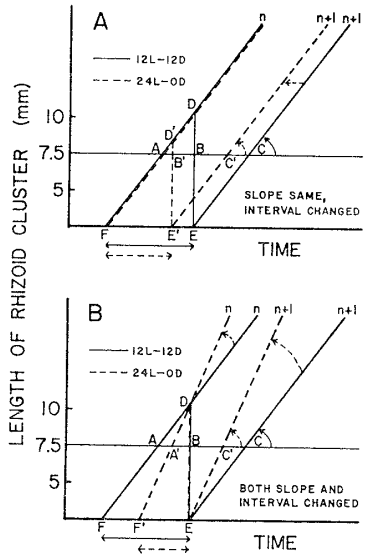

Fig. 5. *Schematic representation of the plot of rhizoid cluster length vs. time.* Solid lines and broken lines represent respectively the data from the 12 L-12 D and 24 L-0 D regimes; and n and $n+1$ represent a given cluster and the next younger one. Graph A is the schematic representation of actually observed situation in *Caulerpa prolifera*. Graph B is a theoretical situation where R.P.I. (rhizoid plastochron index) value at the time of cluster initiation is maintained at a certain constant value, while the rates of cluster elongation (i.e., the slopes of the lines) and plastochron intervals (i.e., the intervals between lines) are allowed to change. Notice that in *C. prolifera* the rates of cluster elongation under 12 L-12 D (solid lines) and 24 L-0 D (broken lines) are the same (broken lines parallel to slid lines); but the plastochron interval of former regime, represented by F E, is longer than that of latter regime, represented by F E′.

This is supported by the facts that in Fig. 3B (under 12L-12D) the peak is at about 1.2 days, while in Fig. 3E (under 24L-0D) the peak is at about 0.7 day; and in Fig. 4 the plastochron intervals (the figure in parentheses) of those clusters (cluster 4 of plant A and 3 of plant B) under 12L-12D are greater than 1.0, but less than 1.0 for those (clusters 11, of plant A and 9, 12, of plant B) under 24L-0D. Both clusters $n-1$ and n being produced outside of the periods probably means that the plant was not able to complete the adjustment in one plastochron interval and was still in the process of the adjustment (Fig. 3C and F). During this process, under 12L-12D the interval was generally still longer than that of Fig. 3B; and under 24L-0D regime, it also was longer than, instead of shorter than, Fig. 3E, suggesting that the plant somehow could not shorten the interval to less than one-half day.

The results of the present experiment can not elucidate how the plant perceives the preferred periods and adjusts the cluster initiation to be within the periods. However, the data at least imply that the plants did not depend on the following factors to detect the initiation time: 1) a certain period of time after the initiation of the youngest cluster; 2) the presumptive site reaching a certain distance from the

youngest cluster; 3) the time for the youngest cluster to reach a certain length; and 4) the time for the youngest cluster to reach a certain R.P.I. (*4*, *6*) value. The followings are supporting evidences: 1) The histograms of Fig. 2 show wide variation of the plastochron intervals, the intervals being shorter under the 24L-0D and longer under the 12L-12D regime. 2) In Table 3 no b_{yx} value is close to zero, i.e., I.C.L. in every case shows wide variation. 3) Chen (*4*) reported that, on the average, under the 12L-12D regime a new cluster was initiated at −0.82 R.P.I. and under 24L-0D, at −0.99 R.P.I. At −0.99 R.P.I., the youngest cluster was only slightly longer than 7.5 mm (the reference length of the plastochron index); but at −0.82 R.P.I. it was distinctively longer than 7.5 mm, i.e., about 10 mm long (Fig. 5A, broken line (24L-0D) vs. solid line (12L-12D) of schematic diagram and the actual plot of Fig. 2 in Chen (*4*)). 4) Similarly, various R.P.I. values were observed at the time of cluster initiation by Chen (*4*, Table 2). Theoretically, the only way to have the event of the cluster initiation occur at a certain constant R.P.I. value under different environments is: a) maintaining the same slope and interval of the plots of cluster length vs. time; or b) changing both the slope and interval of the plots as shown in Fig. 5B (solid line vs. broken line). However, *Caulerpa prolifera* responded to the change in illumination, from the 12L-12D to 24L-0D regimes, by shortening the interval while maintaining the same slope. Naturally, the observed length of the youngest cluster and the R.P.I. value at the time of new cluster initiation under 12L-12D regime were different from those under 24L-0D regime (Fig. 5A, solid line vs. broken line). The above mentioned variations (variations of the plastochron interval, I.C.L., the length of the youngest cluster, and R.P.I.) rather, were probably the result of the plants being able to perceive these preferred periods under both regimes and to adjust the initiation to be in one of these periods. This suggested that under both regimes, the plants possessed both a kind of biological clock which facilitated the perception of the preferred periods, and a free-running cycle which was longer than one day under the 12L-12D and shorter than one day under the 24L-0D regime. The longer or shorter free-running cycle respectively resulted a phase delay or phase advance under either regime. The clock with which the plants judged the preferred periods could be an endogenous one, or could be the one entrained by some subtle environmental factor other than light. Since the plants were able to perceive the preferred periods under both regimes and the ability of the perception persisted irrespective of the differences in times of light being turned on and off under 12L-12D regime. The phenomeon of phase shifting in the free-running could be related to the model of entrainment developed by Pittendrigh and Minis (*8*). According to their model, the phase of a free-running rhythm shifts (delay or advance) respect to a periodic environmental signal until the signal is so positioned that its time of administration compensates for the difference between the period of free-running and the period of the signal.

Although the plants were not returned to the 12L-12D regime after continuous illumination, the reduction of plastochron interval under 24L-0D was not due to an aging effect. Because the plants subjected to the 12L-12D (200 ft. c.) regime for 23 days in the previous work (Fig. 1 of (*1*)) did not reduce their interval and even under both regimes of the present work the S.E. of the intervals were relatively small (Table 2). Dowes and Barilotti (*9*) reported the rhythmic migration of

chloroplasts in *C. prolifera* under 12L-12D regime, and that the rhythm damped out rapidly incontinuous light and gradually in continuous darkness. This means that either the mechanism of perception of time observed in the present study is not linked to the rhythmic migration, or somehow the migration requires alternation of light and dark period.

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