FLOWERING IN LEMNA MINOR

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SUMMARY

A study was made of the photoperiodic induction of flowering in *Lemna minor*, strain 6573. For comparison some experiments were performed with *L. gibba*. Like the latter, *L. minor*, strain 6573, proves to be a typical long-day plant. The critical daylength is about 10.5 hrs in a 24 hrs cycle, the response being, however, very weak. Much better responses are obtained with photoperiods of 13 and 14 hrs (from 25 to 30 per cent flowering). The response to a 13 hrs photoperiod shows a peculiar rhythm in the rate of development of flowers with periods of slow development alternating with periods of rapid development. A possible relation of this phenomenon to the observed rate of multiplication is discussed.

1. INTRODUCTION

Various species of Duckweed (*Lemna* sp.) provide excellent objects for experimental work on the induction of flowering. Amongst the advantages are rapid growth and small size, so that aseptic mass cultures under controlled conditions of nutrition, temperature and photoperiod can easily be maintained.

The *Lemnaceae* comprise long-day (L.D.) plants as well as short-day (S.D.) plants, such as *L. gibba* and *L. perpusilla*, respectively. Most studies have been carried out with various strains of these two species. LANDOLT (1957) mentions that *L. minor*, strain 6573, grown at 30 °C on a medium with sucrose, produces flowers when subjected to a 16 hrs photoperiod. Further data, *e.g.*, the critical daylength, seem to be lacking.

The present study aimed at confirmation of the long-day nature of photoperiodicity in *Lemna minor*, strain 6573, and at establishing the minimum critical daylength required to obtain flowering in this species. Moreover, the percentage of flowering in cultures kept under different inducing light-regimes was studied over extended periods.

2. MATERIAL AND METHODS

2.1. Plant material

Lemna minor L., strain 6573 and *Lemna gibba* L., strain 7007, were obtained from Dr. E. Landolt, Institut für spezielle Botanik der Eidgenössischen Hochschule in Zürich. According to DAUBS (1965) and MCCLURE & ALSTON (1966) *Lemna minor*, strain 6573, has to be regarded as *Lemna gibba*, strain 6573. In this paper we shall use the name *Lemna minor*, strain 6573. *Lemna gibba* L., strain G3, was provided by Dr. C. F. Cleland, Harvard University, Cambridge, Massachusetts.

2.2. Culture media

The following culture media were used:

1. A slight modification of the medium of PIRSON & SEIDEL (1950) which contains: 0.4 g KNO_3 – 0.2 g KH_2PO_4 – 0.3 g $MgSO_4.7H_2O$ – 0.61 g $CaCl_2$ – 0.3 mg $MnCl_2.4H_2O$ – 0.5 mg H_3BO_3 – 5 mg ferric citrate, made up to one liter with deionized water.
2. The E-medium used by CLELAND & BRIGGS (1967), which contains: 0.68 g KH_2PO_4 – 1.515 g KNO_3 – 1.18 g $Ca(NO_3)_2.4H_2O$ – 0.5 g $MgSO_4.7H_2O$ – 2.86 mg H_3BO_3 – 0.22 mg $ZnSO_4.7H_2O$ – 0.12 mg $Na_2MoO_4.2H_2O$ – 0.08 mg $CaSO_4.5H_2O$ – 3.62 mg $MnCl_2.4H_2O$ – 5.40 mg $FeCl_3.6H_2O$ – 3.0 mg tartaric acid – 9.0 mg E.D.T.A., made up to one liter demineralized water.

Sucrose was never added, because in our hands it completely inhibited flowering.

The media were adjusted to pH 4.6 – 4.8 with KOH and were autoclaved for 6 min. at 120°C. The final pH of the autoclaved medium was routinely checked and always turned out to be between 4.7 and 4.9.

2.3. Experimental conditions

The experimental set-up is schematically outlined in *fig. 1.*

Lemna cultures were kept in 300 ml Erlenmeyer flaks at 29°C ± 0.5 in a constant temperature water bath. The water level was adjusted so that it remained constant at 1 cm above the level of the culture medium. Twenty culture flasks could be accomodated in one water bath. Illumination was provided by two incandescent lamps (Osram concentra flood imp. 220–230 V, 150 W, E 27) at 65 cm above the cultures. The average light intensity at plant level was about 4000 lux, but varied between 2000 and 6000 lux. To ensure approximately equal total illumination the positions of the culture flasks were changed twice a day according to a preset scheme.

Preliminary experiments showed that at less than 10 hours of light per day no flowering occurred in any of the *Lemna* strains used. Accordingly, stock cultures were maintained at 7½ hours of light per day. Vegetative growth under these conditions was quite satisfactory.

For a single experiment colonies from one or two 20-day old stock culture flasks were used. On the average the stock cultures used contained about 70 per cent 2-frond colonies, 1- and 3-frond colonies 15 per cent each, and usually a few 4-frond colonies.

Each experimental flask received 15 colonies; no selection was made as to number of fronds per colony. Immediately after inoculation the cultures were subjected to the required light-schedule.

2.4. Counting of flowers and colonies

The flowers were counted directly in the closed culture vessels, scoring only those which were visible with the naked eye. Thus flower primordia were not considered as flowers. The maximum error in flower counts was 5 per cent when 80 or more flowers per culture flask were counted. Per colony never more than

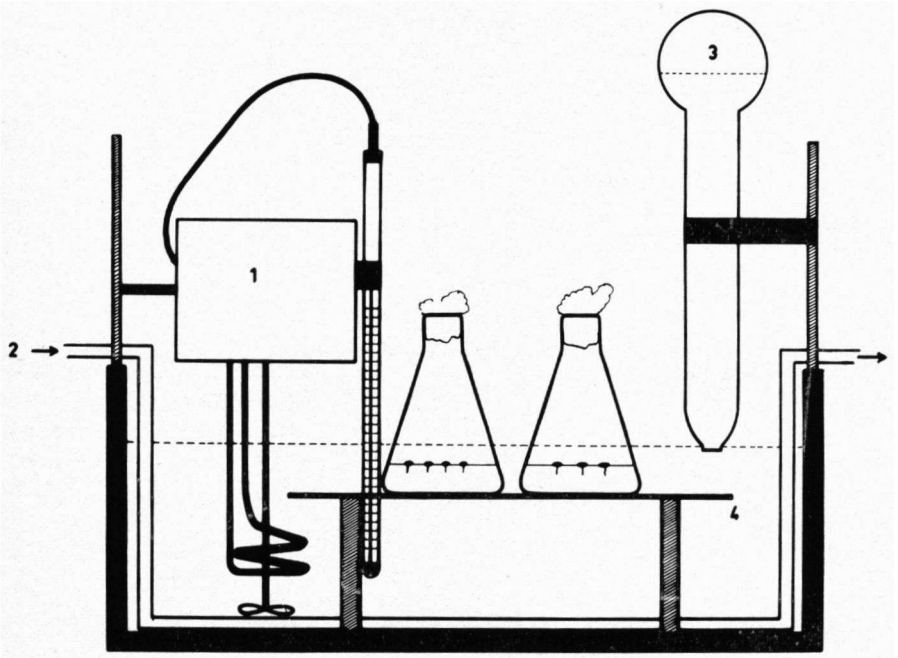

Fig. 1. Experimental set-up for culturing *Lemna*.
1. Thermostat with stirrer; 2. Water-cooling cystem; 3. Water reservoir; 4. Perforated glass support.

one flower was observed. It existed of one pistil or one or two stamens or a combination of these.

To determine the number of colonies photographs were taken through the bottom of the flasks. With a projector the negatives were projected and the colonies were counted. In this way the total number of colonies can be determined exactly. When the number increases to more than 300, an error can be made but this amounts to less than one per cent.

3. RESULTS

Several synthetic media are found in literature such as the medium of Pirson and Seidel and the E-medium, both used for *Lemna gibba*. For our experiments these two media, without sucrose, were used. Vegetative growth was comparable but on the medium of Pirson and Seidel flowering was much better. Therefore the following experiments with *L. minor*, strain 6573, were carried out on the latter medium. Preliminary experiments were performed to roughly establish the minimum critical daylength required for flowering under the present conditions, using *L. minor*, strain 6573, and for the purpose of comparison *L. gibba* strain 7007 and G3. The results are shown in *tables 1* and *2*.

Table 1. Flowering percentages of *L. minor*, strain 6573

hours L : D	days after start of experiment								
	5	10	14	15	17	19	21	23	26
9.5:14.5 10 :14 10.5:13.5	no flowering								
14 :10	–	–	4,5	7	9.5	14.5	22	25.5	33

– means no flowering

Table 2. Flowering percentages of *L. gibba*, strain 7007 and G3

hours L : D	days after start of experiment					
	5	10	13	15	17	20
9.5 : 14.5	*L. gibba*, strain 7007					
10 : 14	–	–	2	6	8	4
11 : 13	–	–	6	17	15	8
12 : 12	–	2	19	35	44	39
13 : 11	–	5	33	59	58	–
14 : 10	–	3	24	42	59	53
	L. gibba, strain G3					
10 : 14	–	–	–	–	–	–
11 : 13	–	–	–	–	–	–
12 : 12	–	–	–	–	2	3
13 : 11	–	–	–	2	9	12
14 : 10	–	–	1	12	22	21

– means no flowering
The flowering percentages are the mean values of three flasks.

The critical daylength for flowering in *L. minor*, strain 6573, turns out to be between 10 1/2 and 14 hours. In *L. gibba*, strain 7007, we find a value of 10 hours; in strain G3 of 12 hours approximately. An attempt was made at establishing the minimum number of inductive cycles required. With up to nine successive cycles of 14 L:10 D still no flowering was obtained. So the chosen light schedules were applied throughout the experiments.

To determine more exactly the critical daylength of *L. minor*, strain 6573, and to follow the course of flowering, groups of 10 flasks were subjected to the following light schedules, respectively: 10 ½ hrs light and 13 ½ hrs dark; 12 hrs light and 12 hrs dark; 13 hrs light and 11 hrs dark; 14 hrs light and 10 hrs dark.

From the 13th day after the start of the experiment daily counts of the numbers of colonies and of flowers were made. (see *figs. 2* and *3*). As a control 3 flasks were subjected to 10 hrs light and 14 hrs dark. No flowering was found in these flasks. In the 10 flasks subjected to 10 ½ L: 13 ½ D only two flowers were found.

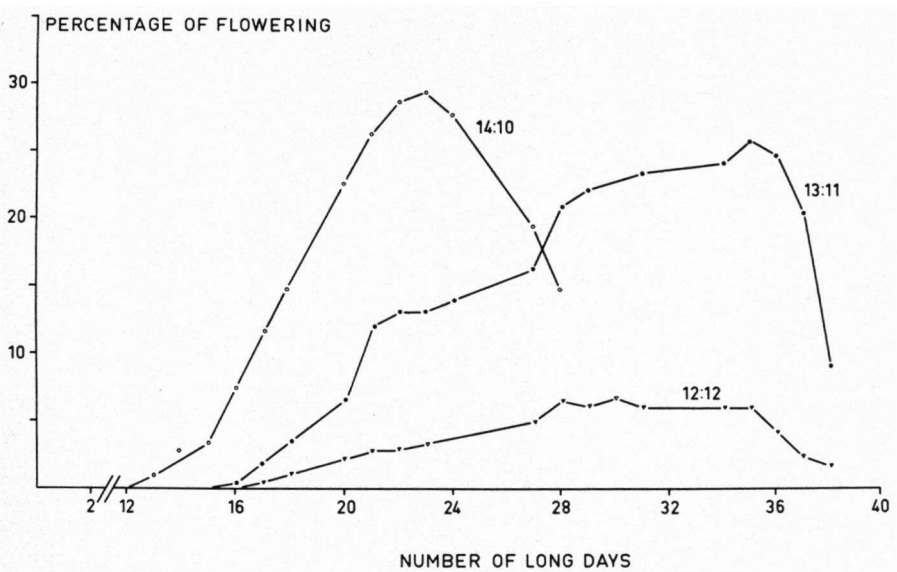

Fig. 2. Flowering in *Lemna minor*, strain 6573. 14:10 etc. means 14 hrs light: 10 hrs dark.

The conclusions from this experiment are the following:

The critical daylength for *L. minor*, strain 6573, is 10½ hours. At this daylength, however, the response is very weak. Much better responses are obtained at 12 hours light or more per day. At 13 L: 11 D a certain periodicity is observed in the development of flowers, periods of slow development alternating with periods of rapid development. At 14 L: 10 D flowering develops rapidly and reaches a maximum after about 23 days.

The various light regimes obviously do not influence the rate of multiplication.

DISCUSSION

Lemna minor, strain 6573, clearly is a typical long-day plant with a critical daylength of 10 ½ hours.

From our experiments (see *table 2*) it is evident that *L. gibba*, strain 7007, is also a long-day plant. This experiment was repeated twice with the same results. *L. gibba*, strain 7007, has a critical daylength of 9 ½–10 hours. This strain is also a very suitable object for experiments on induction of flowering, with a maximum flowering percentage of 60 per cent. From *table 2* it appears that there is little difference in maximum flowering percentage at 12 L: 12 D; 13 L: 11 D and 14 L: 10 D. At the light regimes 11 L: 13 D and 10 L: 14 D maximum flowering is much lower, 17 per cent and 8 per cent, respectively.

Lemna gibba, strain G3, is used in several laboratories. The data about the critical daylength rather diverge. CLELAND & BRIGGS (1967) find a critical day-

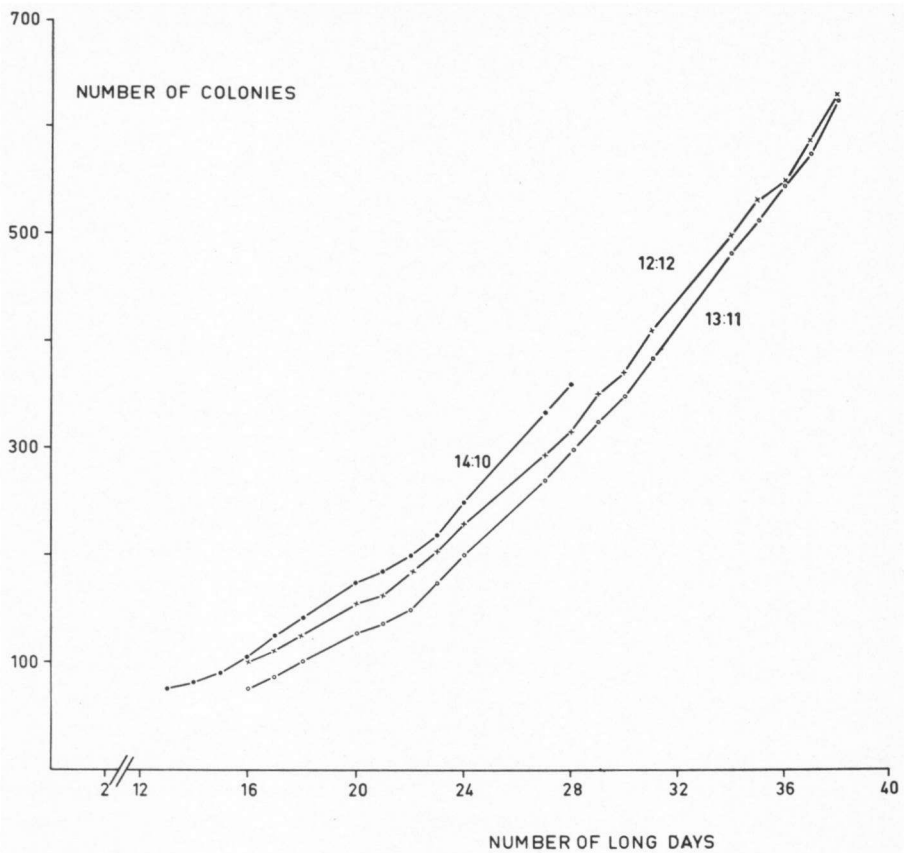

Fig. 3. Increase of colonies in *Lemna minor*, strain 6573, 14:10 etc. means 14 hrs light: 10 hrs dark.

length of 9 ½–10 hours. OOTA (1965) finds a critical daylength of 12–14 hours. The latter found flowering at 12 L: 12 D in the presence of 10^{-5} M GA_3. His experiments were already terminated after eight days, so that perhaps instead of a reduction of the critical daylenth, only an acceleration of flowering was proved.

Our experiments with *L. gibba* G3 show a critical daylength of 11–12 hours. Flowering appears form 13 to 15 days after starting the experiments (see *table 2*). In comparison with *L. gibba*, strain 7007, and *L. minor*, strain 6573, the flowering percentage is rather low and the first flowers at the same light regimes appeared three to five days later. It may be that the influence of the medium plays a role in flowering in these two strains of *L. gibba*.

The differences in the critical daylength in *L. gibba* G3 as it was established by Cleland, Oota and the present authors may be due to the conditions under which the colonies are grown.

From our experiments with *L. minor*, strain 6573, it appears (*fig. 2*) that there is a very strong influence of the light period on the flowering curve. *Fig. 3* shows that there is no influence of the light period on the growth curve. As far as growth is concerned it should be noted that we got the impression that the colonies in the different light schedules have a constant size. However, it would be advisable to examine growth during flowering on the base of dry weight or fresh weight.

The flowering curve at 13 L: 11 D (*fig. 2*) shows a certain rhythm. From this curve it looks as if the cultures are periodically more sensitive to flower induction or that there are periodically more sensitive colonies. Every seven days we found a steep rise in flowering. This rhythm might be connected with the multiplication rate (M.R.) calculated according to HILLMAN (1961) with the formula:

$$k = \frac{M.\,R.}{1000} = \frac{\log \frac{F_d}{F_o}}{d}$$

In this formula k = constant, M. R. = multiplication rate,
F_d = number of fronds on day d,
F_o = number of fronds on day o.

In this formula doubling of the number of colonies each day yields a M. R. = 301.

The values we found are:

Light regime	M.R.	Days required for doubling of number of colonies
14 L : 10 D	43,5	301 : 43,5 = 6,9
13 L : 11 D	48,0	301 : 48,0 = 6,3
12 L : 12 D	41,6	301 : 41,6 = 7,2

So we can say that doubling of the number of fronds takes place every seven days.

Periodicity in flowering at regime 13 L: 11 D is also 7 days. This is an indication that the age of the plants is related to the sensitivity to flower induction, as was also suggested by CLELAND & BRIGGS (1967) and HILLMAN (1962). At 14 L: 10 D no rhythm has been found. The course of the flowering percentage reaches its maximum slope already after seven days, so that an extra rise cannot be seen.

The maxima of flowering at 14 L: 10 D and 13 L: 11 D are almost equal viz. 29 per cent and 27 per cent respectively. However, the moments at which these maxima are reached vary strongly: 9 days and 20 days, respectively, after the first flowers appear. From these experiments we may conclude that it is important when working with *L. minor*, strain 6573, to pay attention to the critical daylength as well as to the fact, that the kind of the experiment also determines

the light-schedule to be used. Examining the factors that have influence on maximum flowering 14 L: 10 D is a suitable light schedule. However, to get information about the rhythm in flowering and the sensitivity of the colonies the 13 L: 11 D regime should be used.

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