MITOSIS IN LEAVES

by

Bernice M. Speese

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SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS

OF

COLLEGE OF WILLIAM AND MARY

for the degree

MASTER OF ARTS

1941

Table of Contents

Acknowledgments

The author expresses her appreciation to Dr. J. T. Baldwin, Jr., for suggesting and criticising this work, to Dr. Donald W. Davis for research equipment and for advice and criticism in the preparation of this manuscript, to Dr. Paul M. Patterson for making available laboratory facilities at Hollins College, to Dr. Orland E. White for the hospitality of the Blandy Experimental Farm, and to Dr. C. V. Morton for identifying the *Smilax* plants investigated.

Mitosis in Leaves

Introduction

Statement of Problem.--This is a preliminary report on nuclear divisions in leaves. Though, because of the peculiar physiology of leaves, pronounced fluctuations in their division curves might be expected, no analysis seems to have been made of the mitotic rhythms in these plant organs. Leaf shape is in part dependent upon the pattern of frequencies of mitosis. Cell division is fundamental to growth and morphogenesis. It would be of some significance to determine for leaves the distribution of mitotic frequencies and to compare in this regard leaves which differ in shape. Any study of mitosis in leaves offers a convenient opportunity for determining the numbers of chromosomes. In the present work the temporal mitotic frequency, the number of nuclear divisions for given areas, and the chromosome numbers of certain species have been studied. Leaves of Smilax Bona-nox L., S. glauca Walt. var. leucophylla Blake., S. hispida Muhl., S. lanceolata L., S. laurifolia L., S. rotundifolia L., and Lathyrus latifolius L. were investigated. Reasons for studying these species of Smilax and Lathyrus were: they smear well; the chromatic mass is large, and dividing nuclei are, therefore, readily observable.

Method.--The method of preparing the leaves for study was essentially that of Baldwin (1939); fixation for at least twelve hours in Carnoy's fluid, treatment for four minutes with a solution of equal parts 95 per cent alcohol and concentrated hydrochloric acid, transference again into Carnoy's, and smearing in iron aceto-carmine. The preparations were sealed with a gum-mastic-paraffin mixture or with Zirkle's (1937) permanent aceto-carmine. For each investigation all the leaf tissues included dorsiventrally in the sections were examined. The stage in prophase when the chromosomes are first easily recognizable and the stage in telophase when the chromosomes are still apparent were used as arbitrary limits for determining the number of nuclear divisions in process in each section.

Review of Literature

Friesner (1920) wrote:

> "the subject of periodicity of growth activities in the plant is by no means a new one, in fact, it is one of the oldest. But a careful review of the available literature shows that there are still certain phases of the work which have not yet been thoroughly investigated."

Indeed the entire field is open for study. As referred to by Friesner (1920), two of the earliest investigators of periodicity, Sachs (1872) and Prantl (1873), concluded that daily periodicity of plant growth depends upon external influences, such as the alternation of light and dark; that the periodicity is lost when plants are grown in darkness. Also, according to Friesner, Baranetzky (1879) made similar observations except that shoots of Brassica rapa maintained a periodicity when grown in darkness. Friesner makes a distinction between this "periodicity" which is lost under constant environmental conditions and "rhythm" which refers to "any oscillation in activity which is definite and regular and not related to any external influences."

Ward (1895) concluded: "growth (i.e. permanent increase in bulk) while in the long run dependent upon cell division, does not synchronize but rather alternates with it."

Lewis (1901), growing Allium Cepa under normal day and night illumination, discovered two waves of cell division, with maxima at midnight and noon and minima at 4:00 a.m. and 4:00 p.m. He found that change in color of light changed the times of maxima and minima, while continuous darkness, for the most part, reversed the time of occurrence of the maxima and minima.

MacMillan (1901) observed the growth of potato tubers, in continuous darkness, to be rhythmic instead of regular. He found the maxima, occurring one to four times during a twenty-four hour period, to be of short duration and to be followed by periods of either slower growth or no growth.

Kellicott (1904) found in roots of Allium, grown under constant conditions, two waves of cell division, with a primary maximum at 11:00 p.m., a secondary maximum at 1:00 p.m., and primary and secondary minima at 7:00 a.m. and 3:00 p.m., respectively. According to Kellicott, Famintzin (1867) and Strasburger (1880) found that, in the majority of algae, cell division was most rapid at night. Friesner (1920) records that de Wildeman (1891) observed no diurnal differences in the rate at which cells of Spirogyra divide, and that Braun (1851) found cell division of this alga to be most rapid at night. Kellicott also records that Kurrsanow (1912) found the maximum period of dividing cells in Zygnema to occur from 9:00 p.m. until midnight. Kellicott agreed with the conclusion of most of the earlier workers that the maximal periods of cell division alternated with the minimal periods of elongation, and that during periods of slow cell division elongation was most rapid. Kellicott further concluded that each plant form seemed to have its own rhythmic variation in rate of growth.

Karsten (1915), working with desmids grown under normal light conditions, discovered, as recorded by Friesner (1920), a daily periodicity of nuclear and cell division; he also found cell-division rhythms, for aerial parts of higher plants, which were independent of changes in illumination and temperature.

Lutman (1911), investigating cell and nuclear divisions in _Closterium_, found divisions occurring from 10:00 p.m. to 5:00 a.m. with the maximum number from about 10:00 p.m. until midnight, the rate of division being conditioned by the weather of the preceding day.

Laughlin (1911), working with the common onion grown under constant conditions, found a daily mitotic and growth rhythm, which he attributed to external influences, and a seasonal rhythm, which he considered was caused by the internal organization of the plant. He also stated that "there is a definite alternation between permanent increase in bulk and mitosis."

Friesner (1920) used for his investigations seedlings of _Cucurbita Pepo_ L., _Lupinus albus_ L., _Pisum sativum_ L., _Vicia faba_ L., _Allium Cepa_ L., and _Zea everta_ Sturt., also roots from germinating bulbs of _Allium Cepa_ L., _A. canadense_ L., and _A. cernum_ L. All the work was done in a dark room; the temperature was kept constant, with few exceptions, to within one degree. Friesner's conclusions were:

1. Under constant uniform conditions elongation in all plants studied proceeds in rhythmic manner, two or more waves occurring during the 24-hour period.

2. Nuclear and cell divisions proceed in a similar rhythmic fashion.

3. The time of occurrence of maxima and minima are dependent upon the time of initiation of metabolic activity and not upon the time of day by the clock.

4. Elongation and cell division, as regards time of maxima and minima, are, in general, reciprocals of each other.

5. This reciprocal relation existing between elongation and cell divisions accounts for a large share, at least of the rhythms found in these plants.

One (1937), investigating nuclear divisions in *Crepis*, found two marked dividing periods and two resting periods, with maxima at 2:30 p.m. and 12:30 a.m. and minima at 5:30 p.m. and 5:30 a.m.

The literature indicates that mitotic divisions occur in waves and are rhythmic.

Observations

Mitotic Rhythms.--Twelve Smilax Bona-nox leaves of approximately the same size were fixed, one each at 2-hour intervals throughout a 24-hour period on August 2-3, 1939, and smears made of one-millimeter-in-diameter samples, punched from each leaf near veins in the positions indicated in figure 1. Since only one young leaf of a given size usually occurred on a single plant of S. Bona-nox, twelve different plants (growing at the edge of the William and Mary campus) were used. The number of nuclear divisions was counted for each of the samples: Sample A, 2:45 p.m., 3734; B, 4:45 p.m., 2003; C, 6:45 p.m., 1248; D, 8:45 p.m., 1756; E, 10:45 p.m., 3615; F, 12:45 a.m., 2559; G, 2:45 a.m., 3978; H, 4:45 a.m., 2064; I, 6:45 a.m., 2521; J, 8:45 a.m., 2149; K, 10:45 a.m., 2143; L, 12:45 p.m., 1846. These data produce a curve (fig. 5 A) with two minima--6:45 p.m. and 12:45 p.m.--and with three maxima--primary maxima, 12 hours apart, at 2:45 p.m. and 2:45 a.m., and a secondary maximum at 10:45 p.m. Since it happened that the leaves fixed at 10:45 p.m. (fig. 1 E) and at 10:45 a.m. (fig 1 K) were smaller than the others, two additional leaves of a more comparable size were fixed at these hours on August 17 and 18 respectively (fig. 2). The number of divisions for the second 10:45 p.m. leaf (fig. 2 A) was considerably lower than for the first (fig. 1 E), 3141 as compared with 3615, yet high enough to constitute a markex maximum in the curve. The number of divisions in the second 10:45 a.m. leaf (fig. 2 B) did not differ significantly from the number in the first (fig. 1 K), 2255 as compared with 2143.

Plants of Lathyrus latifolius were grown in a seed flat. The fourth leaf from the stem base of twelve of these plants was fixed when three millimeters in length and the number of nuclear divisions, for each entire leaf, in process at the time of fixation determined as recorded in table 1.

Table 1. The nuclear divisions in process, at indicated time, in the fourth leaf from the stem base of Lathyrus latifolius, fixed when three millimeters in length.

Date	Time	Mitoses
March 1, 1940	2.45 p.m.	2254
February 21, 1940	4:45 p.m.	1504
February 20, 1940	6:45 p.m.	1435
February 23, 1940	8:45 p.m.	1853
February 23, 1940	10:45 p.m.	2301
February 25, 1940	12:45 a.m.	2244
February 26, 1940	2:45 a.m.	1685
February 26, 1940	4:45 a.m.	871
February 21, 1940	6:45 a.m.	1645
February 24, 1940	8:45 a.m.	49
February 25, 1940	10:45 a.m.	1912
February 25, 1940	12:45 p.m.	1644

Spatial Distribution of Mitoses.--Young leaves of _Smilax rotundifolia_ were used for the determination of the number of nuclear divisions in process for a given area. A leaf of the size shown in figure 3 was fixed at 4:30 p.m. on July 3, 1939; a total of 31,380 mitoses was found in section A of that leaf. There was a general decrease in mitotic frequency from the proximal to the distal part of that area. Comparable results were obtained from random samples designated in figure 4; the leaf was fixed at 3:00 p.m. on July 24, 1939; the number of divisions for each of the samples designated in figure 4 was determined; Sample A, 1952; B, 2012; C, 2166; D, 1787; E, 2089; F, 1469; G, 92.

The first leaf on any stem tip of _Lathyrus latifolius_ is small and scale-like at maturity in comparison with later leaves occurring on the same stem tip. It seemed desirable to compare the number of mitoses in young leaves of like size, but in different positions and having different prospective size at maturity. The first leaf, 7 mm x 3 mm in size, on a potted plant of _L. latifolius_ was fixed at 7:30 p.m. on October 30, 1939 and the nuclear divisions in process in the entire leaf were counted. The third leaf, 8 mm x 3 mm, was fixed at 7:30 p.m. on November 13, 1939 and all the nuclear divisions counted. The results of these two counts are shown in table 2.

Table 2. Comparison of the nuclear divisions in process in the first and third leaves of a stem of <u>Lathyrus latifolius</u>.

Leaf	Leaf size	Mitoses
First leaf	7 mm x 3 mm	37
Third leaf	8 mm x 3 mm	2006

Still another investigation was made using the first, second, third, fourth and fifth leaves from a stem of a potted plant of <u>Lathyrus latifolius</u>; the leaves were fixed at 4:45 p.m., November 14, 1940. Smears were made of samples only, one millimeter in diameter, located beside the midrib and at the midpoint of the length of the leaf. Leaf sizes and the mitoses in process for each sample are shown in table 3.

Table 3. Effect of leaf size and position on the stem upon the number of nuclear divisions in process at a given instance in comparable areas of leaves of <u>Lathyrus latifolius</u>.

Leaf Position	Leaf Length	Mitoses
First Leaf	8 mm	0
Second Leaf	34 mm	0
Third Leaf	34 mm	0
Fourth Leaf	30 mm	2
Fifth Leaf	16 mm	307

A study was made to determine the relation between size of leaf and cessation of nuclear divisions. Material was collected from young shoots of a clone of Smilax rotundifolia (Speese No. 95; specimen deposited in the United States National Herbarium), growing near the Blandy Experimental Farm, Clarke County, Virginia. Since preliminary investigations had given concordant counts only when samples were taken from like areas of leaves of similar size and in corresponding positions on the stem, leaves, fifth in position from the base of the stem, were fixed at 4 p.m. on various days from June 14 to June 24, 1940, and the nuclear divisions determined for samples one millimeter in diameter and located beside the midrib and at the midpoint of the greatest longitudinal axis of the leaf. Leaf size and the number of mitoses determined are given in table 4.

Table 4. Number of mitoses in samples from beside the midrib and at the midpoint of the greatest longitudinal axis of leaves of different sizes, each being the fifth leaf above the base of a shoot from a clone of S. rotundifolia. The leaves were fixed at 4:00 p.m. between the dates June 14 and June 24, 1940.

Leaf size		
Length	Width	Number of Mitoses
12 mm	5 mm	1998
14 mm	7 mm	1703
22 mm	10 mm	1609
35 mm	22 mm	475
46 mm	31 mm	56
55 mm	43 mm	38
68 mm	62 mm	4
82 mm	65 mm	0

A further approach to the problem of distribution of leaf mitoses in space and the possible relation of this distribution to the size and shape of leaves was attempted. Leaves of *Smilax laurifolia* L., *S. glauca* Walt. var. *leucophylla* Blake., *S. lanceolata* L., *S. hispida* Muhl., *S. Bona-nox* L., and *S. rotundifolia* L. (Speese Nos. 133, 134, 119, 110, 135, and 114 respectively; specimens have been deposited in the United States National Herbarium) were fixed at 4:00 p.m. on various days from August 4 to August 24, 1940. Samples, one millimeter in diameter, were taken from along the margins and from beside the midribs at the levels indicated in figure 6. The karyokinetic count for each sample is recorded in table 5.

Table 5. Species of Smilax investigated and the number of mitoses counted in samples from along the margins and from beside the midribs of leaves outlined in figure 6; all the leaves were fixed at 4:00 p.m. between the dates August 4 to August 24, 1940. Leaves of S. laurifolia, S. glauca, S. lanceolata, and S. Bona-nox were fixed from plants growing in the nursery at the Blandy Experimental Farm, Boyce, Clarke County, Virginia: the geographic sources of these plants are shown in table 5.

Collection No.	Species	Geographic Source	Leaf and Level	Mitoses at Margin	Mitoses at Midrib
133	S. laurifolia L.	Wilmington,	A1	84	85
		New Hanover Co.,	A2	164	146
		North Carolina	A3	236	225
134	S. glauca Walt. var	Keysville,	B1	55	18
	leucophylla Blake.	Charlotte Co.,	B2	207	61
		Virginia	B3	264	83
119	S. lanceolata L.	Wrightsville Beach,	C1	108	53
		New Hanover Co.,	C2	284	173
		North Carolina	C3	360	210
110	S. hispida Muhl.	Clarke Co.,	D1	523	607
		Virginia	D2	1135	1198
			D3	1321	1226
135	S. Bona-nox L.	Myrtle Beach,	E1	631	640
		Horry Co.,	E2	912	882
		South Carolina	E3	1532	889
114	S. rotundifolia L.	Clarke Co.,	F1	722	556
		Virginia	F2	977	940
			F3	1289	1064

Chromosome Numbers.--Chromosome determinations for *Smilax rotundifolia* L. (fig. 7) and *S. Bona-nox* L. (fig. 8) were made from smears. Both have 32 chromosomes at leaf metaphase.

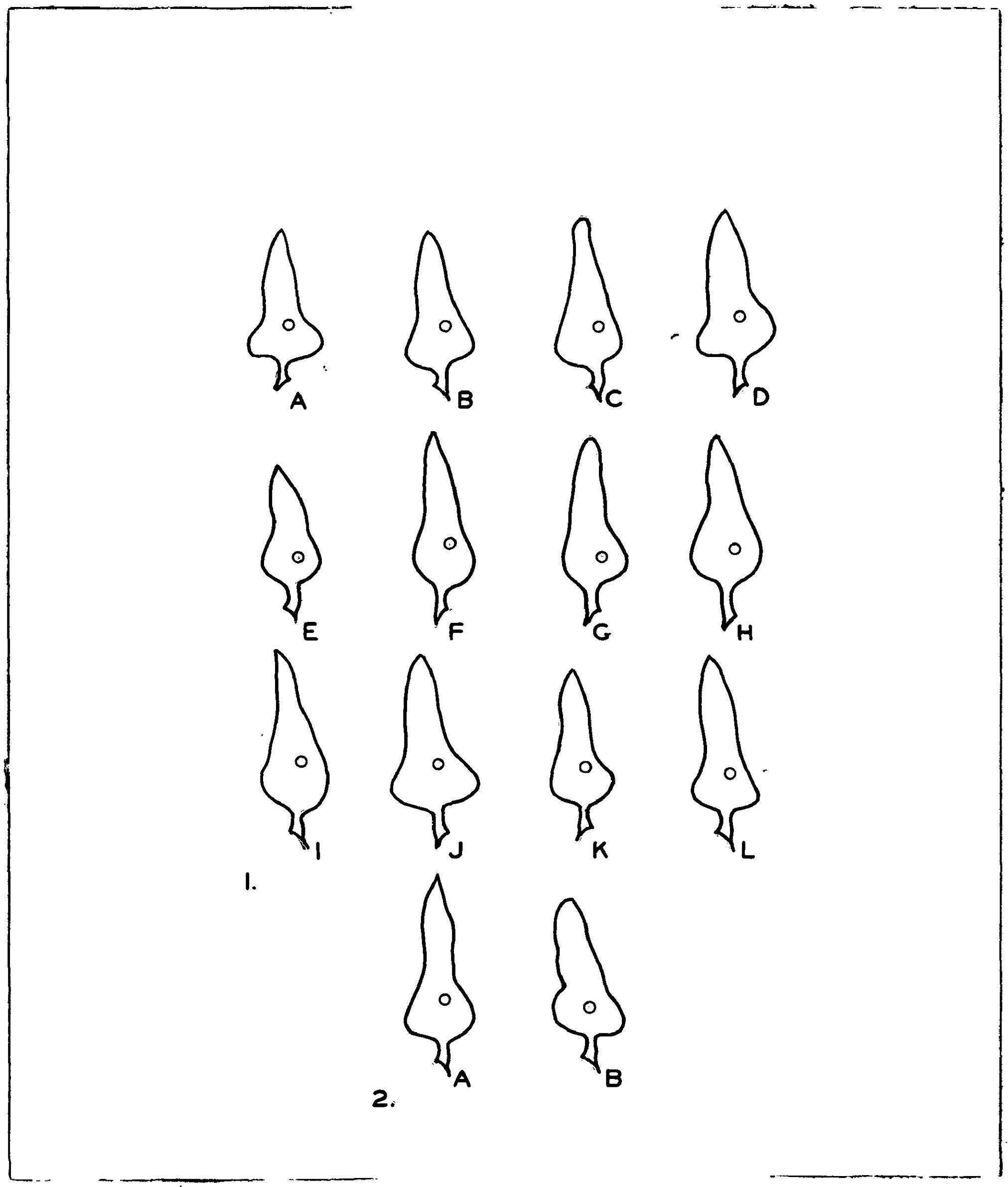


Fig. 1 & 2.--Fig. 1. Outlines of twelve leaves of <u>Smilax-Bona-nox</u> fixed one each at 2-hour intervals throughout a 24-hour period; places from which samples were taken for division counts are indicated. Fig. 2. Outlines of two other leaves of <u>S. Bona-nox</u> studied.

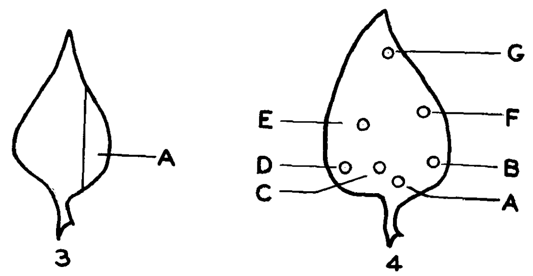


Fig. 3 & 4.--Fig. 3. Natural size outline of Smilax rotundifolia leaf with area in which 31,380 mitoses were counted. --Fig. 4. Natural size outline of S. rotundifolia leaf of which samples A - G were studied cytologically.

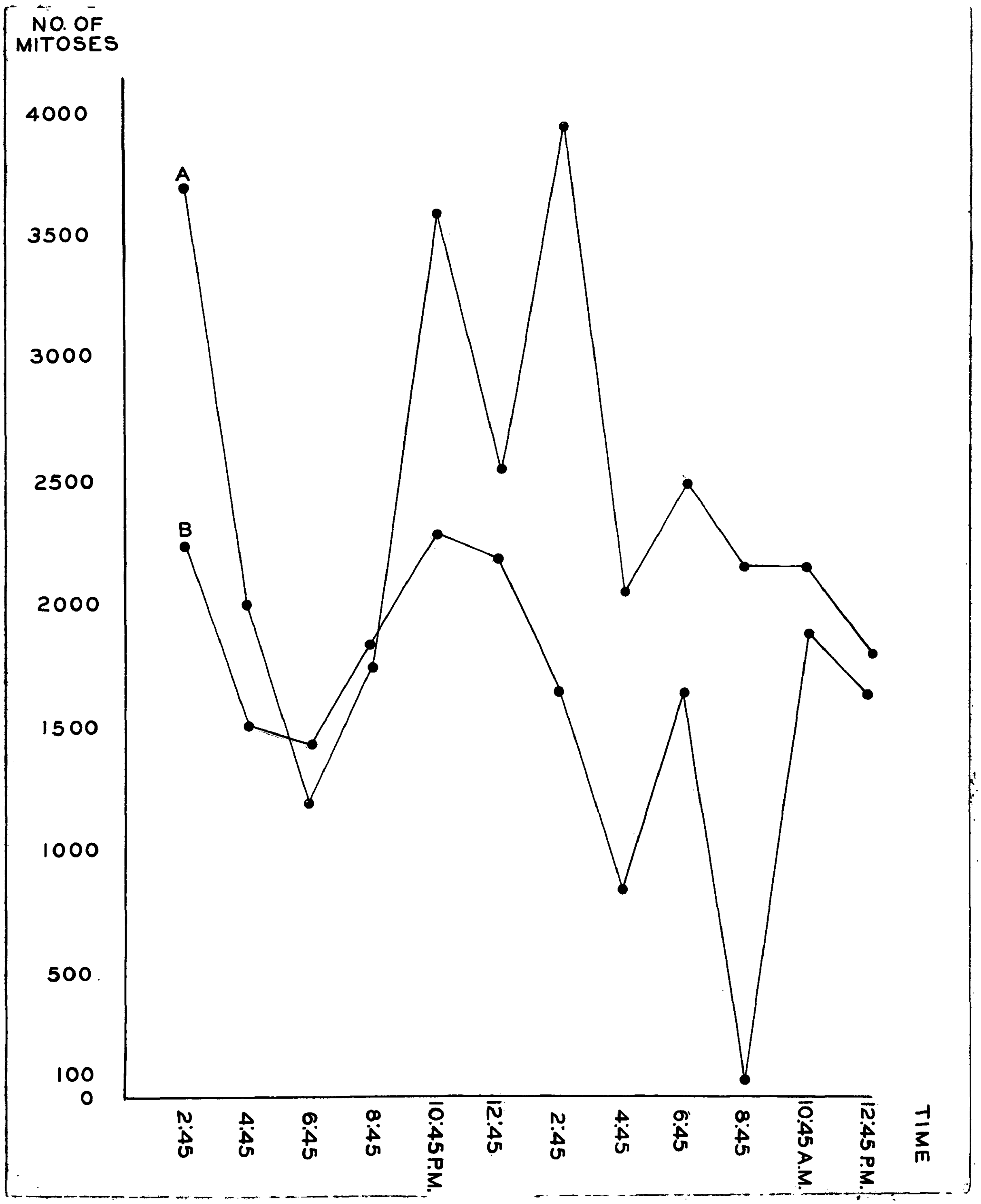


Fig. 5. -- Curve of division frequency in Smilax Bona-nox (line A) and Lathyrus latifolius (line B) leaves, fixed one each at 2-hour intervals throughout a 24-hour period.

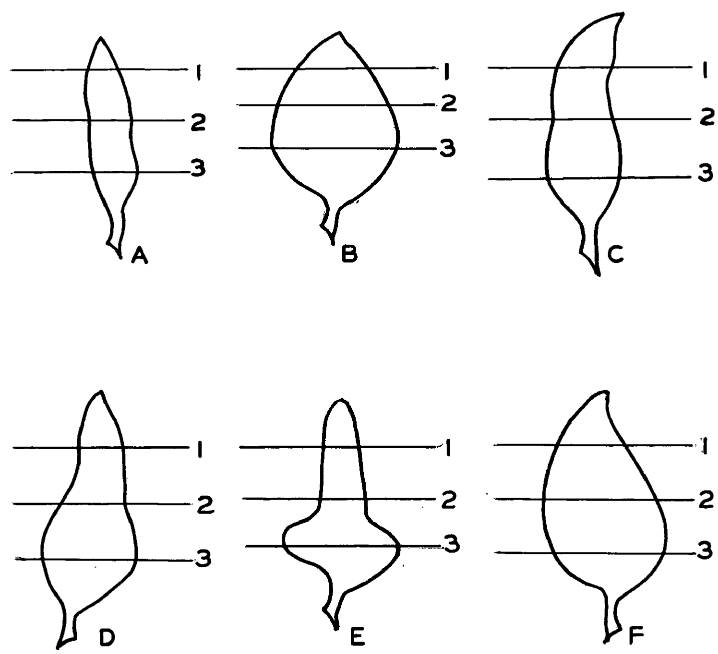
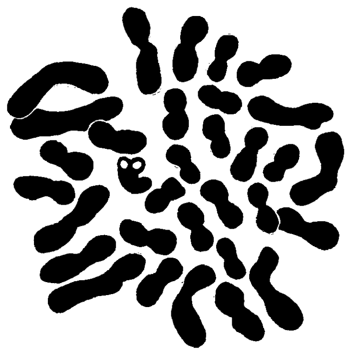
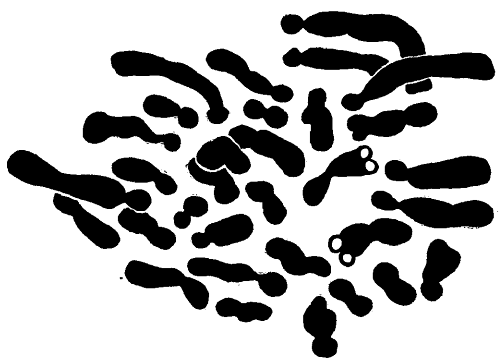


Fig. 6.--Natural size outlines of leaves of *Smilax laurifolia*, *S. glauca*, *S. lanceolata*, *S. hispida*, *S. Bona-nox*, and *S. rotundifolia* with lines indicating levels at which samples one millimeter in diameter were taken from along the margins and from beside the midribs.



7



8

Fig. 7 & 8.--Fig. 7. Leaf metaphase of Smilax rotundifolia L., 2n = 32. Magnification ca. 3800X. -- Fig. 8. Leaf metaphase of S. Bona-nox L., 2n = 32. Magnification ca. 3800X.

Discussion

Mitotic Rhythms. The data on temporal frequency for mitosis in Smilax Bona-nox produce a curve with two minima--6:45 p.m. and 12:45 p.m.--and with three maxima--primary maxima, twelve hours apart, at 2:45 p.m. and 2:45 a.m., and a secondary maximum at 10:45 p.m. Similar data for Lathyrus latifolius produce a curve with three minima--primary minimum at 8:45 a.m. and secondary minima at 6:45 p.m. and 4:45 a.m.--and with three maxima--2:45 p.m., 10:45 a.m., and 10:45 p.m. A comparison of these two curves (figure 5) shows a general trend for mitotic frequency to be high at 2:45 p.m. and low at 6:45 p.m., again high at 10:45 a.m. and low at 4:45 a.m. These observations on mitotic fluctuations in leaves of Smilax and Lathyrus are preliminary, but they support a conclusion reached by Friesner (1920) for roots of several different genera: "the curve of cell division in all plants studied exhibits a number of oscillations in the 24-hour period, in the majority of plants three." Likewise Ono (1937) found in root tips of Crepis "two marked dividing periods".

Spatial Distribution of Mitoses in Space. The data, as recorded in table 2 and table 3, on the frequency of mitoses in leaves of Lathyrus indicate the possibility of a relationship between the size of the leaf at maturity and the frequency of nuclear division. The first leaf, table 2, had only 37 divisions while the third leaf, when of a comparable size, had 2006 dividing nuclei. Similarly, from another plant, the first leaf (8 mm in length) table 3, had no divisions while the fifth leaf (16 mm in length) had 307 mitotic divisions. The first leaves of Lathyrus latifolius are small and scale-like with few divisions while later leaves have a much larger size at maturity and have a correspondingly higher karyokinetic frequency. Mounts (1932) found the size of the leaf at the time cell division ceases to vary considerably in different leaves on the same shoot and to be related to the dimensions of the leaves at maturity. Gregory (1928) maintains that leaf growth depends upon the size of the already existing leaf area. He concluded: "the limited growth of single leaves must be accounted for by the limited growth potentiality of the leaf primordia."

Additional evidence in support of this hypothesis is found in the data recorded in table 5. Smilax glauca, S. laurifolia, and S. lanceolata have, in general, a lower mitotic frequency than have S. Bona-nox, S. rotundifolia, and S. hispida. Mature leaves of the latter three species, as observed by the writer, attain a much larger size than do mature leaves of S. glauca, S. laurifolia, and S. lanceolata.

The data in table 5 show the division frequency at the leaf margin to be, in general, higher than at the midrib. Smith (1934) concludes:

> "the species of the plant under consideration and the stage of development of the leaf determine what portions of the leaf are the most actively meristematic, in some instances the major portion of the meristematic activity is confined to the marginal zone, in others the activity is distributed throughout the leaf."

In the investigation of cessation of mitosis (data recorded in table 4), it was found that concordant counts were obtained only when leaves of comparable size were taken from the same stem positions. Delisle (1938) found typical growth curves in leaves of Aster "with the grand period of growth within each successive leaf appearing only at the time when that of the preceding leaf is falling off". According to Delisle, Kolkunov (1905) found that the size and number of cells per unit area of leaf vary with the leaf position on the stem.

Chromosome Numbers. The 2n-chromosome numbers of S. rotundifolia (fig. 7) and of S. Bona-nox (fig. 8) were found at leaf metaphase to be 32; the sex of the various plants was not known. (Herbarium specimens of the species will be distributed under the numbers: Baldwin 416 and 417.) The chromosomes in both species vary considerably in size and morphology. Jensen (1937) reported an n-number of 16 for S. rotundifolia from the Blue Ridge Mountains of North Carolina. He interpreted certain of his observations to "suggest a record of previous hybridization for the species". It is of consequent interest to note that counts from leaf mitoses (sometimes considered to be probably chromosomally aberrant) of plants in the Virginia coastal plain corroborate the gametic number determined for supposed hybrid plants in the North Carolina mountains. Chromosome numbers reported for Smilax are shown in table 6.

Table 6. Chromosome numbers reported for *Smilax* L.

Species	2n	n	Determined by
S. Bona-nox L.	32		Speese (1939)
S. China L.		30	Nakajima (1937)
S. glauca L.		14	Jensen (1937)
S. hederacea L. var. *nipponica* Maxim.	30		Nakajima (1937)
S. herbacea L.		12-13	Elkins (1914)
		12	Humphrey (1914)
		13	Lindsay (1929; 1930)
S. Oldhami Miq.	30		Nakajima (1937)
S. rotundifolia L.		16	Jensen (1937)
	32		Speese (1939)

Of the above workers only Nakajima (1937) got evidence of heteromorphic chromosomes; he reported sex chromosomes of the X-Y type in three species.

Summary

A temporal frequency established for mitosis in leaves of S. Bona-nox gives a curve with two marked minima--6:45 p.m. and 12:45 p.m.--and with three maxima--primary maxima, twelve hours apart, at 2:45 p.m. and 2:45 a.m., and a secondary maximum at 10:45 p.m. A similar frequency in leaves of L. latifolius gives a curve with three marked minima--primary minimum at 8:45 a.m. and secondary minima at 6:45 p;m. and 4:45 a.m.--and with three maxima--2:45 p.m., 10:45 a.m., and 10:45 p.m.

The number of dividing nuclei was counted for certain selected areas of young leaves of S. rotundifolia. A general reduction in mitotic frequency appears to occur proximo-distally in those leaves.

Mitotic divisions in leaf areas vary with the position of the leaf on the stem, nearness to the stem tip being correlated with high division frequencies.

Increase in size, and accordingly in age, of a given leaf area is regularly correlated with decrease in mitotic frequency, until divisions cease.

As determined for six species of Smilax, there is a basipetal gradient for the mitotic rate in leaves, and divisions cease earlier in the central part of the leaf than at the margin.

S. rotundifolia L. and S. Bona nox L. both have 32 chromosomes at leaf metaphase.

A review of the literature on mitotic rhythms and a table of chromosome counts for Smilax L. are included.

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