THE NUCLEOLAR NUMBERS AND ATTACHMENTS IN *LILIUM*

by

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INTRODUCTION

The cytological literature of the past twenty-five years has reflected an increased interest in the subject of the nucleolus. As shown in Gates' (1942) recent review of all phases of this subject, the majority of the evidence regarding the origin of the nucleolus supports the work of Heitz (1931). He found that nucleoli arise at each of the satellite chromosomes. This has been confirmed many times since, (Resende 1937, Stewart 1942), and now the secondary constrictions are considered of equal rank, for as Gates (1942, p. 372) says, "this appears to mean simply that the nucleolar organizer with its accompanying constriction is situated farther back from the end of the chromosome." Warmke (1941) also has expressed the same thought when he pointed out that there is "a definite and constant relationship between secondary constrictions, including satellite constrictions, and the formation of nucleoli."

Despite all this confirming evidence the inclusion of all secondary constrictions as nucleolar organizers is debatable. Sato (1936, 1937, 1938) reports the presence of non-nucleolar secondary constrictions in seven genera of the Amaryllidaceae. Jacob (1940) in Cassia found non-nucleolar secondary constrictions. Resende (1937 a, b), Fernandes (1936), Bushnell (1936), and Okuno (1937) have also reported non-nucleolar secondary constrictions and satellites.

While observing the number, morphology, and behavior of the chromosomes of some species of the genus Lilium the number and position of the secondary constrictions were determined. Some of these (Stewart and Bamford 1943) in the triploid species, L. tigrinum, were found to be connected with nucleolar formation (fig. 17). This report

was part of a survey of the species of Lilium designed to determine the extent of the variation in chromosome morphology of the genus and its possible contribution to the phylogeny of the group (Stewart unpublished). During these studies two general observations were made; that the species had high nucleolar numbers, and that a great number of secondary constrictions was present. The purpose of this paper is to determine if the number of secondary constrictions is directly related to the maximum number of nucleoli present in any one species and to observe the variations of nucleoli and secondary constrictions between species.

MATERIALS AND METHODS

Root tips were grown in pots in the greenhouse from bulbs obtained from commercial sources and from the wild. Parallel fixations were made in Navashin's fluid and in 3:1 absolute alcohol-glacial acetic acid. Root tips were washed and stored in 85% alcohol. The Navashin's fixed roots were embedded by the butyl alcohol technique, sectioned at 20 u and stained with Haidenhain's haematoxylin for nucleolar counts. For such counts only the regions where mitoses were abundant in the root tip were used.

Root tips from the other fixation were smeared in propionocarmine. The slides were kept as temporary mounts sealed with a gum mastic-paraffin mixture until the nucleoli were differentiated from the cytoplasm. They were made permanent by mounting in Nevillite or Diaphane. The observations were made with the aid of 40x and 90x apochromat oil immersion objectives and 10x and 15x compensating oculars. Camera lucida drawings were made of late prophase cells of each form at a magnification of 2333x. The idiograms plotted directly

from these drawings were reduced 3/4 in reproduction.

RESULTS AND DISCUSSION

The frequencies of the nucleolar numbers found in 500 root tip cells from each form are given in Table I. These data show that the maximum nucleolar number is not frequent, although the same number of organizing regions must be present in each cell. A similar range of nucleolar numbers has been presented by Matsuura (1938). Woods (1937) gives frequency distributions from root tip cells of Tulipa ranging from 1 to 8 and from 3 to 13. The greatest frequencies are at the centers of the curves. Since Van Camp's (1924) study there has been little doubt that reduction in numbers occurs through fusion of several nucleoli into one. Derman (1933) referring to the globules of nucleolar substance present on the telophase chromosomes of root tip cells of Callisia states, "These globules collect, undoubtedly by fusion, into two and finally one body and, as more globules are produced and added together, the nucleolus grows very rapidly and becomes constant in size during further development of the chromosomes."

Because in the resting nuclei fusion had reduced the number of nucleoli in most of the cells the maximum number observed was the best indication of the number of attachment regions present. In prophase more than one of the chromosomes are frequently attached to one nucleolus (Plate VI, fig. 54, Plate VII, figs. 58 and 59, and Plate IX, fig. 60). As many as eight chromosomes have been observed attached to a single nucleolus. The higher the number of organizing regions the greater is the chance that nucleoli will fuse and consequently the lower is the frequency of the occurrence of the maximum number of nucleoli.

TABLE I

NUCLEOLAR NUMBER FREQUENCIES IN *Lilium* SPECIES

	1	2	3	4	5	6	7	8	9	10
SUBGENUS - CARDIOCRINUM										
cordatum		18	96	189	136	57	4			
SUBGENUS - EULIRION										
Section - Leucolirion										
Bolanderi	138	210	135	16	1					
Brownii	18	118	219	124	20	1				
candidum	82	208	174	58	5					
formosanum	108	220	137	35						
Grayi	92	223	150	35	2					
japonicum		18	77	124	182	87	52	10		
longiflorum	19	138	216	115	10	2				
regale	10	135	210	115	28	2				
Washingtonianum purpureum	134	258	102	6						
Section - Archelirion										
auratum	87	216	144	51	2					
Section - Isolirion										
croceum	6	79	211	151	48	5				
concolor		5	24	96	151	133	57	31	5	
dauricum luteum	9	83	185	149	64	10				
dauricum Wallacei	6	101	182	164	45	4				
philadelphicum		6	43	113	169	112	43	14		
tsingtauense		4	42	145	175	94	31	9		
Section - Martagon										
amabile	16	115	197	141	27	4				
canadense	114	238	126	22						

TABLE I - Continued

NUCLEOLAR NUMBER FREQUENCIES IN *Lilium* SPECIES

	1	2	3	4	5	6	7	8	9	10
Section - Martagon - Continued										
carolinianum	42	166	209	76	7					
Davidi	20	104	184	139	46	7				
Duchartrei	58	160	200	80	20	2				
Hansonii	1	18	77	123	132	87	52	10		
Henryi	84	171	190	55						
Humboldtii type	25	276	183	16						
H. bloomerianum	321	179								
H. magnificum	266	234								
Kelloggii	157	245	92	6						
martagon		15	83	157	157	78	9	1		
Maximowiczii	19	148	233	97	3					
pardalinum type	114	250	116	19	1					
pardalinum angustifolium	75	234	162	28	1					
pardalinum giganteum	124	223	132	21						
parvum	122	216	131	30	1					
pumilum		8	35	98	153	140	41	21	3	1
Roezlii	114	228	136	21	1					
speciosum album	45	202	210	43	2					
speciosum album Kraetzeri	72	234	163	31						
speciosum punctatum	54	175	195	71	5					
speciosum rubrum	35	149	188	103	23	2				
superbum	8	105	225	119	38	5				
Willmottiae	12	81	177	158	64	10				

TABLE I - Continued

NUCLEOLAR NUMBER FREQUENCIES IN *Lilium* SPECIES

	1	2	3	4	5	6	7	8	9	10
HYBRIDS										
Davmottiae	4	59	203	177	51	6				
Phildauricum		22	86	187	156	67	1	1		
Scottiae	5	72	226	156	37	4				
HORTICULTURAL VARIETIES										
Elegans Mahogany	17	148	215	100	17	3				
tigrinum flore pleno		35	157	181	106	41				
tigrinum fortunei	13	77	162	144	87	17				
tigrinum malmo	9	50	136	180	96	22	5			

Nucleolar sizes further indicate this fusion of nucleoli for within a single species nucleoli are larger where they are fewer in number. This has been shown in other genera by de Mol (1928), Woods (1937), and Gates (1942). Gates and his students have used telophase nuclei in their studies where the nucleoli were not yet fused and consequently have presented their observations as a single number of nucleoli for each form studied. Their method is not suited to Lilium where the nucleolar attachments are not mainly terminal and the chromosomes lose their individuality early in the telophase stage.

Nucleolar attachments are presented diagrammatically as idiograms in Plates I-V, figs. 1-53. The primary constrictions are represented as breaks and are arranged in a horizontal line. The secondary constrictions are represented as breaks above or below that line. All of the chromosomes represented in one figure are from a single cell and include all of the nucleolar attachments of that cell but none of the non-nucleolar chromosomes. Comparisons are limited by the fact that at this prophase stage the chromosomes are rapidly contracting and differences between cells may be due merely to differences in the stage of division. Variation within any one pair may be the result of the distortion that is unavoidable when the chromosomes are spread. Wide variation in the positions of the nucleolar attachments is evident in spite of these limitations.

The length of the achromatic region is variable; the distal end is frequently separated from the rest of the chromosome even before the chromosomes are spread by pressure in the preparation of the slide. Nucleoli have generally disappeared before the chromosomes approach the metaphase plate but do persist longer than in many genera (Stewart and Bamford 1943).

Since the long achromatic thread does not persist into metaphase as it does in what are considered typical satellites, the attachment regions of Lilium may all be classed as secondary constrictions. However, in some forms the constriction may be so near the end that the distal portion is shorter than the average width of the chromosome, (Plate VI, figs. 54 and 55). In Lilium as in Narcissus (Sikka 1940) and unlike Crocus (Pathak 1940) and tomato (Lesley 1939) there is no definite relation between the size of the nucleolar fragment and the activity of the nucleolar organizer, (Plate IX, figs. 60 and 61).

The maximum nucleolar numbers and the number of attachments represented in the idiograms exactly correspond in 25 of the 49 forms studied. Since the work of Heitz (1931) many workers have reported nucleolar numbers in agreement with the number of attachment regions. Gates' (1942) review of this subject cites 21 reports which confirmed this point. In many of these observations on several groups are presented, and they furnish abundant evidence that this is the most usual behavior.

In nine of the forms studied the maximum number of nucleoli is one greater than the number of attachments. The greatest frequency of any of these maxima is seven in L. carolinianum. Comparable cases have been observed by Heitz (1931), Dermen (1933), Resende (1937), and Matsuura (1938). The latter made counts of 100 resting nuclei of Trillium kamtschaticum where six attachments are known. One cell having eight and one cell having nine nucleoli were observed. He has interpreted his results in terms of nucleolar valency, stating, "However, under a special condition this competition might be disturbed, especially when the difference in valency is small. This would be

taken to be the exceptional cases of supernumeral nucleoli in somatic cells." The low frequency in the cases observed here seems to support his conclusion that these numbers are the result of some exceptional behavior. McClintock (1934) in Zea and Matsuura (1938) in Trillium have demonstrated that each of the non-nucleolar chromosomes is capable of forming one or more nucleoli when separated from the nucleolus forming regions. Most observers of meiotic behavior in interspecific hybrids are well acquainted with the fact that lagging chromosomes, regardless of the type, will form nucleoli when isolated in micronuclei.

In the single case of L. longiflorum a maximum nucleolar number of six is recorded but only four attachments are presented in the idiogram (Plate I, fig. 8). Small chromosome fragments are visible on the nucleolus at early prophase. Later prophases where the individual chromosomes can be distinguished in their entirety show no nucleolar fragments of this size. In anaphase a pair of short I chromosomes shows minor secondary constrictions in the short arm, as described by Stewart and Bamford (1945) in L. tigrinum. These nucleolar organizers are very difficult to locate because they show no pronounced achromatic area in the later stages.

An excess of attachment points over the maximum number of nucleoli is present in each of the remaining fourteen forms studied. Further search failed to show the presence of any nucleolar numbers greater than the maxima of the first 500 cells studied. Although no separate nucleoli are evident for some of these secondary constrictions the extra constrictions are indistinguishable on the nucleolus in the prophase stage (Plate VII, fig. 56 and Plate VIII, figs. 58 and 59). The cases of non-nucleolar secondary constrictions previously cited (Sato 1938,

1937, 1938, Jacob 1940, Resende 1937 a, b, Fernandes 1936, Bushnell 1936, and Okuno 1937) may be comparable to these. Differences in the activity of individual organizing regions have been recorded by Lesley (1939), Pathak (1940), and Matsuura (1938). If the difference in activity were expressed in the telophase stage as a delay in the formation of a nucleolus and prophases were not studied, such nucleolar organizers would pass as non-nucleolar constrictions.

de Mol (1928, 1937) observed in Hyacinthus that polyploidy was reflected in a higher maximum number of nucleoli in the resting nuclei. Diploids showed maxima of two, triploids three, and a tetraploid form had four. Gates (1942) has extended this concept to other genera, stating, "Poincinia regia with 2n=28 chromosomes appears to be heptaploid as it has seven nucleoli." He uses similar data in several other cases as evidence of polyploidy. In the diploid species of Lilium polyploidy has not been a factor in phylogeny. The chromosome numbers are constant at 24 except in some forms where an extra chromosome or fragment may be observed (Stewart 1943). However, nucleolar numbers range from two to ten. Therefore some process besides polyploidy has been responsible for the duplication of nucleolar organizing regions in this genus.

The path of phylogeny within the genus Lilium can only be explained on the basis of structural changes which have altered the morphology of the nucleolar chromosomes and in some way added nucleolar organizing regions or secondary constrictions. The positions of the secondary constrictions range from the distal half of the long arm (Plate I, fig. 1), to either side of the primary constriction (Plate II, figs. 18 and 28), to the distal half of the short arm (Plate II, fig. 15). The reports of other workers on the meiotic pairing of interspecific

hybrids also show that structural differences exist in Lilium, (Richardson 1936, Ribbands 1937, and Stewart and Bamford 1943).

Variability, both in position and in number of nucleolar attachment regions, is evident within either the taxonomic sections or the natural geographic groups outlined by Slate (1939). The extremes of high and low number fall within the Martagon section; and within the single species, L. Humboldtii, the two varieties Bloomerianum and Magnificum show maxima of two nucleoli and two attachment regions while the type has four. The Western American species show two, four, and six nucleolar attachment regions. Those native in Eastern North America range from four to eight, European and Western Asiatic species show from six to eight, and the Eastern Asiatic species have four, six, eight, and ten attachment regions.

Similarities in the nucleolar numbers and attachments within a genus may reflect close phylogenetic relationship. Babcock (1942) states, "It is certain that morphologically similar species have similar chromosomes and, conversely, close similarity of the chromosomes has often proved to be a helpful clue to a closer degree of relationship than was at first surmised from superficial gross morphology of the plants. Thus it has been possible to work out with greater accuracy the classification of species into groups and to ascertain phylogenetic relations. Certain groups of closely related species have been shown to possess almost identical karyotypes without any notable structural differences in their chromosomes. This shows that close similarity in the chromosomes is usually correlated with close taxonomic relationship in Crepis." Gates (1942) includes nucleoli and their attachments in his statement on this subject, "Comparative studies of the nucleoli and sats. in related species and genera, when

combined with other lines of cytological evidence, such as secondary meiotic pairing and chromosome morphology, throw much light on nuclear phylogeny."

Similarity is evident in the L. Humboldtii varieties, Magnificum (fig. 27) and Bloomerianum (fig. 26), which have only two attachments and show no significant difference. L. Humboldtii type (fig. 25) shows the same attachments as its varieties but in addition has an extra pair on the short arms of a pair of V chromosomes. This pair is unique in position among the Western American lilies studied. The difference within the species, L. Humboldtii, represents a case of addition or loss of a pair of secondary constrictions.

Remarkable similarity is evident between the Western American species regardless of taxonomic section. L. pardalinum type (fig. 31) and variety Angustifolium (fig. 32) are alike, having four attachments, each on the short arm of an I chromosome very near the primary constriction. L. pardalinum variety Giganteum (fig. 33) differs from the type in the addition of two more secondary constrictions on another pair of I chromosomes. Maximum nucleolar numbers are only four in each of these types. L. Roezlii (fig. 36) is like L. pardalinum variety Giganteum except that there is less separation between the primary and secondary constrictions on one pair of chromosomes. L. Kelloggii (fig. 28) differs from L. pardalinum in having one of the secondary constrictions on the other side of the primary constriction, suggesting the possibility of an inverted region including both constrictions. L. parvum (fig. 34) shows the same relation to L. Kelloggii that L. pardalinum Giganteum does to L. pardalinum type, having an additional pair of attachments on the short arms of I chromosomes.

L. Washingtonianum (fig. 10) of the Leucolirion section is like

L. Kelloggii (fig. 28) of the Martagons and differs from all other species of Leucolirion studied. Similarly L. Bolanderi (fig. 2) is like L. Roezlii (fig. 36) and is also distinct from other species of Leucolirion.

A monophyletic origin is indicated for the Western American lilies by the number and the positions of the nucleolar attachments. With one exception all of the attachment regions are on the I chromosomes and adjacent to the primary constrictions. The differences between species are comparatively small when considered with the rest of the genus. The distinctions between species of the Leucolirion and species of the Martagon sections are no greater than those within the sections. In each case the differences are such as might be caused by a single structural change.

The Eastern American lilies show similarities to both Western American and European species. L. canadense (fig. 19) and L. carolinianum (fig. 20), Martagons, are alike. L. Grayii (fig. 6), Leucolirion, is similar having one of the two pairs of attachments slightly further removed from the primary constriction. All of these constrictions are on the short arms of I chromosomes and similar to the Western American species. L. superbum (fig. 41), Martagon, is distinct, having one pair of attachments on the short arms of a pair of V chromosomes and two other pairs on the long arms of I chromosomes. L. philadelphicum (fig. 16), section Isolirion, shows eight secondary constrictions; four in the long arms of I chromosomes, two in the short arms of I chromosomes, and two in the long arms of V chromosomes. In these attachment regions it resembles the other Isolirions native in Europe and Asia.

The Eastern American lilies show greater differences in the attachments of the nucleoli. Three of the species are similar to the Western American species. The remaining two are so distinct that several structural changes as well as reduplication of nucleolar organizers would have to be assumed to explain this differentiation. Slate (1939) has described L. carolinianum as similar to L. superbum but this evidence clearly shows that the form studied was more closely allied with L. canadense.

L. bulbiferum subsp. croceum (fig. 12), a European Isolirion, shows eight attachments like the American Isolirion. One pair is unusual in position, being close to the primary constriction on the long arm of a V chromosome. One pair is in the short arms of the other pair of V chromosomes and the remaining two pairs are in the long and short arms of two pairs of I chromosomes. The Asiatic Isolirion, L. dauricum var. luteum (fig. 14), is like L. croceum except that the secondary constriction adjacent to the primary constriction on a large V chromosome is on the other side of it, again suggesting a difference of an inverted region including the two constrictions, between the two species. L. dauricum variety Wallacei (fig. 15) and the horticultural form, Elegans Mahogany, differ from luteum in lacking the fourth pair of constrictions. The nucleolar attachments found in L. concolor (fig. 16) are most nearly like those of L. dauricum variety luteum but differ in the position of one secondary constriction which is on the other side of a primary constriction and considerably further away from it. L. tsingtauense (fig. 17) shows four pairs of I chromosomes attached to the nucleolus at secondary constrictions in the long arms.

The similarities between widely separated species of this section suggest that it may be a good phylogenetic unit. L. philadelphicum (fig. 16) is more like the Isolirions of Europe and Asia than the other species of North America where it is native. The European and the Asiatic Isolirions show similar attachments in some cases. L. tsingtauense (fig. 17), described by Slate (1959) as very similar to L. concolor, is the most distinct cytologically of the group.

Members of the Leucolirion section show the least similarity in their attachment regions. Of those native in the Eastern Asiatic region L. longiflorum (fig. 8) shows four attachments, one pair being in the long arm of a pair of I chromosomes and one in the short arm of another pair. A minor secondary constriction is present in the short arm of an I chromosome. Nucleolar maxima indicate that this constriction is functional. L. Brownii (fig. 3) differs in having its third pair of constrictions located in the long arm of a shorter I chromosome. L. formosanum (fig. 5) has three pairs of attachments, like L. longiflorum. L. regale (fig. 9) shows two pairs of constrictions near the primary constrictions in the short arms of the two pairs of V chromosomes. Two other pairs of constrictions are located in the short and long arms of two pairs of I chromosomes. L. japonicum (fig. 7) shows one pair of constrictions located in the short arms of a pair of V chromosomes, two pairs in the short arms of I chromosomes, and one pair near the primary constriction in the long arms of a pair of I chromosomes. The three American Leucolirions previously described are distinct from these, showing greater resemblance to the other species of the same region. L. regale and L. japonicum are distinct from the other species of the group.

A great deal of variability is present within the large group of Asiatic Martagons. Each of five shows three pairs of attachments. L. Davidi (fig. 21) and L. Willmottiae (fig. 42) both show a pair of attachments near the primary constriction on the short arm of a V chromosome and another pair in the long arm of an I chromosome. They differ in the position of the third pair of attachments. In L. Davidi it is near the primary constriction on the short arm of an I chromosome. In L. Willmottiae it is near the primary constriction on the long arm of an I chromosome. L. amabile (fig. 18) is similar to L. Davidi in nucleolar attachments. It is distinct morphologically and geographically. L. Duchartrei (fig. 22) has two pairs of attachments in the short arms of I chromosomes, and one pair in the distal halves of the short arms of a pair of V chromosomes.

L. speciosum in its varieties Album Kraetzeri (fig. 38), Rubrum (fig. 39), and Punctatum (fig. 40), is uniform. Each variety shows two pairs of attachments in the I chromosomes, one in the short arm of one pair and one in the long arm of another pair. One pair of V chromosomes shows the remaining attachments in the short arm approximately one third of the distance from the primary constriction to the end of the arm. L. speciosum variety Album (fig. 37) shows evidence of structural hybridity in two of its nucleolar chromosomes, only one V chromosome showing a secondary constriction and one pair of nucleolar I chromosomes differing in the length of the long arms. It does have three chromosomes representing each of the three pairs in the other varieties.

L. pardalum (fig. 35) with a maximum of ten has the highest number of nucleoli of any of the species studied. It also shows ten nucleolar attachments, four being on the short arms of the two pairs

of V chromosomes, four on the long arms of two pairs of I chromosomes and two on the short arms of a pair of I chromosomes. L. Hansonii (fig. 24) shows the same number of attachments but the expected maximum number of nucleoli was not observed. One pair of attachments is to the short arms of a pair of V chromosomes, three pairs to the long arms of I chromosomes, and one pair to the short arms of a pair of longer I chromosomes.

L. auratum (fig. 11) is the only species of the Archelirion section. It shows two pairs of attachments, both very near the primary constriction in the short arms of I chromosomes.

L. cordatum (fig. 1) is one of the three species of the subgenus Cardiocrinum distinguished by their heart-shaped, netted veined leaves. It shows eight attachments; one pair in the short arms of a pair of V chromosomes, and three pairs in the long arms of I chromosomes. In this, which is considered a primitive species, all of the secondary constrictions are located in the distal halves of the chromosome arms.

Of the nucleolar attachments of the varieties of L. tigrinum (figs. 51, 52, and 53) little need be said except that they do not differ significantly from the secondary constrictions described by Stewart and Bamford (1943).

The hybrid lily, Davmottiae, (fig. 43) shows six attachments as does each of its parents (figs. 42 and 44). The chromosomes of each parent are distinguishable in the hybrid.

Phildauricum (fig. 46) is a hybrid between L. philadelphicum (fig. 45) and L. dauricum produced by F. L. Skinner in 1921. The nucleolar chromosomes of L. dauricum (fig. 47) are fully distinguishable from those of the other parent. The nucleolar V chromosome from

L. philadelphicum does not show any secondary constriction. It is recognizable and there can be no doubt that the secondary constriction, present in L. philadelphicum, is absent in this hybrid. M. Navashin (1935) has demonstrated the loss of secondary constrictions in Crepis hybrids and named the phenomenon amphiplasty. He has attributed the loss to competition between attachment points for the nucleolar material.

The third hybrid, Scottiae (fig. 48), has six attachment regions as does each of its parents, L. Willmottiae (fig. 50) and the horticultural variety Elegans Mahogany (fig. 49). The latter is supposed to have arisen as a hybrid between L. concolor and L. dauricum although the production of the original hybrid is not recorded. Slate (1930) has stated that seedlings of Elegans Mahogany are easily raised but do not come true to the parent type. Scottiae shows all of the nucleolar attachments of both its parents without visible alteration.

CONCLUSIONS

In *Lilium* the number of nucleolar attachment regions varies from two to ten.

Fusion of nucleoli results in an approximately normal distribution of nucleolar number frequencies in resting cell nuclei.

Maximum nucleolar numbers equal the number of attachment regions in 25 of the forms studied.

In nine cases the maximum number of nucleoli exceeds the number of attachments by one. This is concluded to be a result of irregular behavior.

Reduplication of nucleolar organizing regions is shown to be independent of polyploidy for most of these species of *Lilium*.

Similarity of the attachment regions in *Lilium* coincides neither with the taxonomic divisions of the genus nor with the natural geographic regions.

Each of the secondary constrictions previously reported in *L. tigrinum* is found to be a nucleolus organizing region.

SUMMARY

In this study an attempt has been made to correlate the numbers of nucleoli found in resting cells with the secondary constrictions of the chromosomes in 49 forms of *Lilium*. Evidence is presented to show that the secondary constrictions are the points of origin of the nucleoli. All except one of the secondary constrictions have been observed connected with the nucleoli in prophase although in fourteen cases there are excesses of attachment points over the maximum number of nucleoli observed.

In *Lilium* duplication of nucleolar organizing regions takes place without duplication of the whole chromosome complement. Variation in number and position of nucleolar attachments is evident within each of the taxonomic sections and natural geographic groups. Certain similarities of nucleolar attachments are discussed as a possible contribution to the evidence on the phylogenetic relations within the genus.

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PLATE I

Subgenus Cardiocrinum

Figure 1.	L. cordatum	Japan

Subgenus Eulirion

Section Leucolirion

Figure 2.	L. Bolanderi	Western North America
Figure 3.	L. Brownii	South China
Figure 4.	L. candidum	Eastern Mediterranean
Figure 5.	L. formosanum	Formosa
Figure 6.	L. Grayi	Eastern North America
Figure 7.	L. japonicum	Japan
Figure 8.	L. longiflorum var. Creole	Formosa
Figure 9.	L. regale	Western China
Figure 10.	L. Washingtonianum	Western North America

Section Archelirion

Figure 11.	L. auratum	Japan

Section Isolirion

Figure 12.	L. bulbiferum subsp. crocum	Eastern Europe
Figure 13.	L. concolor	Central China
Figure 14.	L. dauricum var. luteum	Northeastern Asia

PLATE I

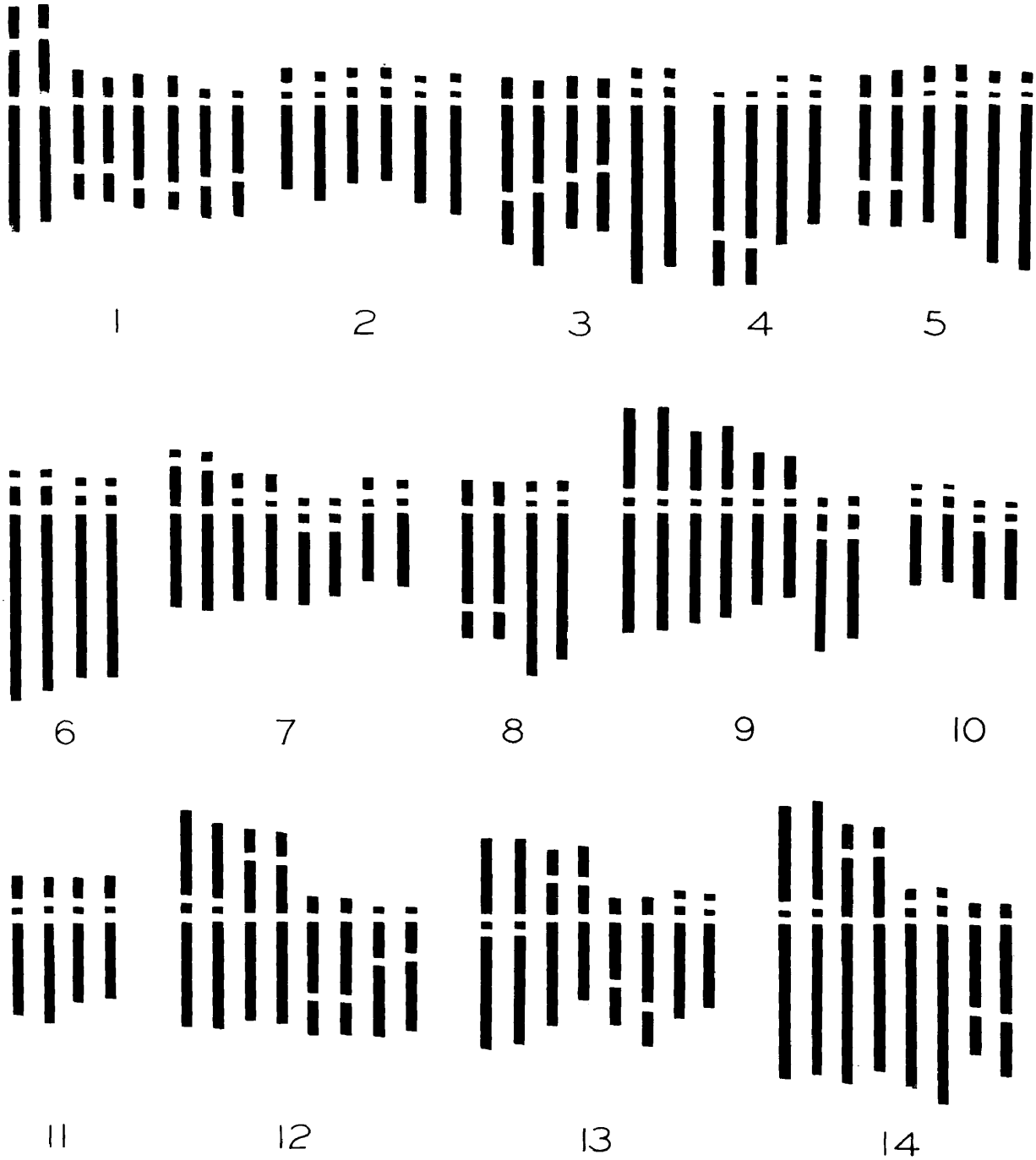

PLATE II

Section Isolirion - Continued

Figure 15.	L. dauricum var. Wallacei	Northeastern Asia
Figure 16.	L. philadelphicum	Eastern North America
Figure 17.	L. tsingtauense	Korea

Section Martagon

Figure 18.	L. amabile	Korea
Figure 19.	L. canadense	Eastern North America
Figure 20.	L. caroliniarum	Eastern North America
Figure 21.	L. Davidi	Western China
Figure 22.	L. Duchartrei	Western China
Figure 23.	L. Henryi	Central China
Figure 24.	L. Hansonii	Korea
Figure 25.	L. Humboldtii	Western North America
Figure 26.	L. Humboldtii var. Bloomerianum	Western North America
Figure 27.	L. Humboldtii var. Magnificum	Western North America
Figure 28.	L. Kelloggii	Western North America

PLATE II

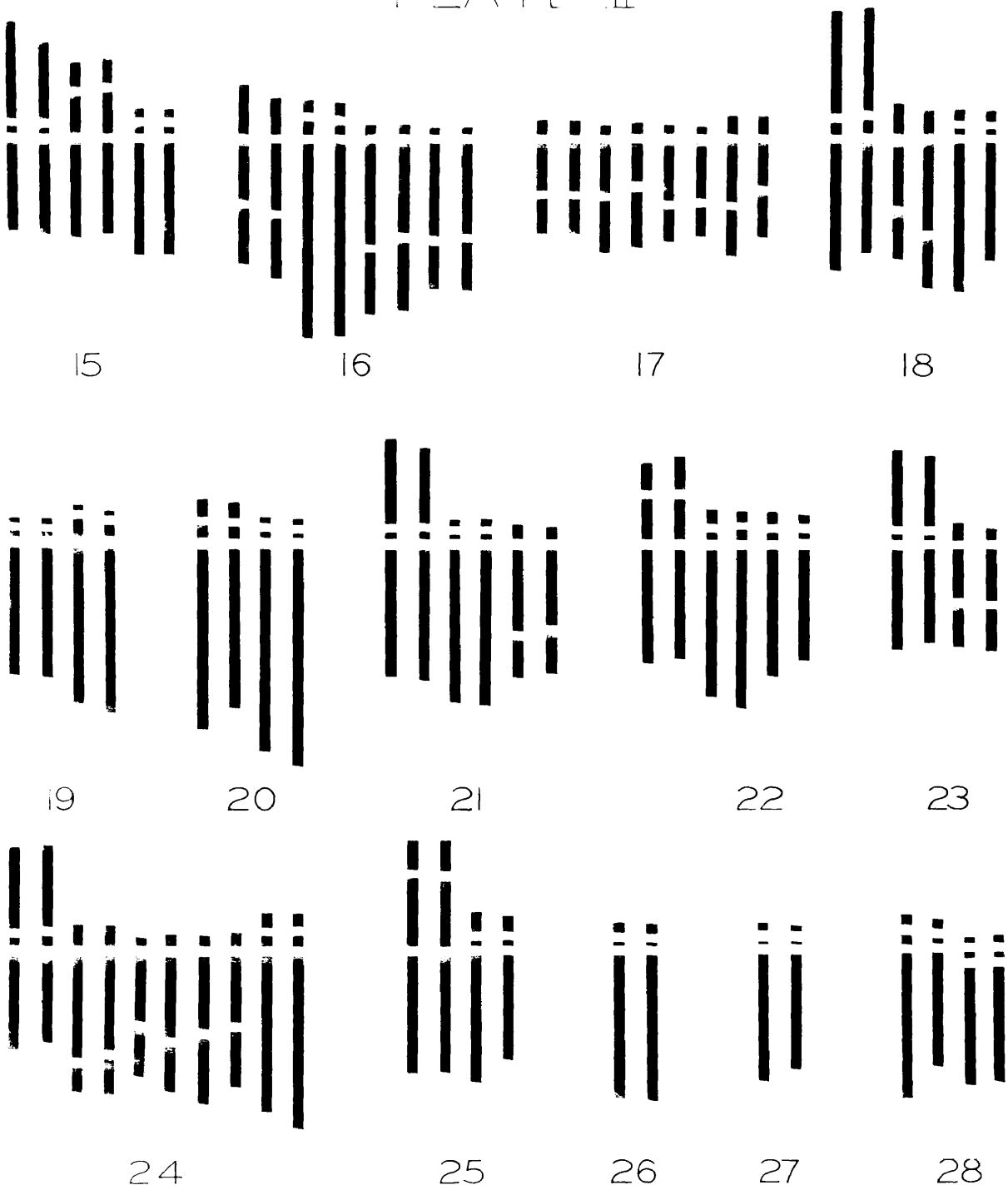

PLATE III

Section Martagon - Continued

Figure 29.	L. martagon var. Album	Europe
Figure 30.	L. Leichtlinii var. Maximowiczii	Japan
Figure 31.	L. pardalinum	Western North America
Figure 32.	L. pardalinum var. Angustifolium	Western North America
Figure 33.	L. pardalinum var. Giganteum	Western North America
Figure 34.	L. parvum	Western North America
Figure 35.	L. pumilum	North China and Korea
Figure 36.	L. Roezlii	Western North America
Figure 37.	L. speciosum var. Album	Japan
Figure 38.	L. speciosum var. Album Kraetzeri	Japan
Figure 39.	L. speciosum var. Punctatum	Japan
Figure 40.	L. speciosum var. Rubrum	Japan
Figure 41.	L. superbum	Eastern North America

PLATE III

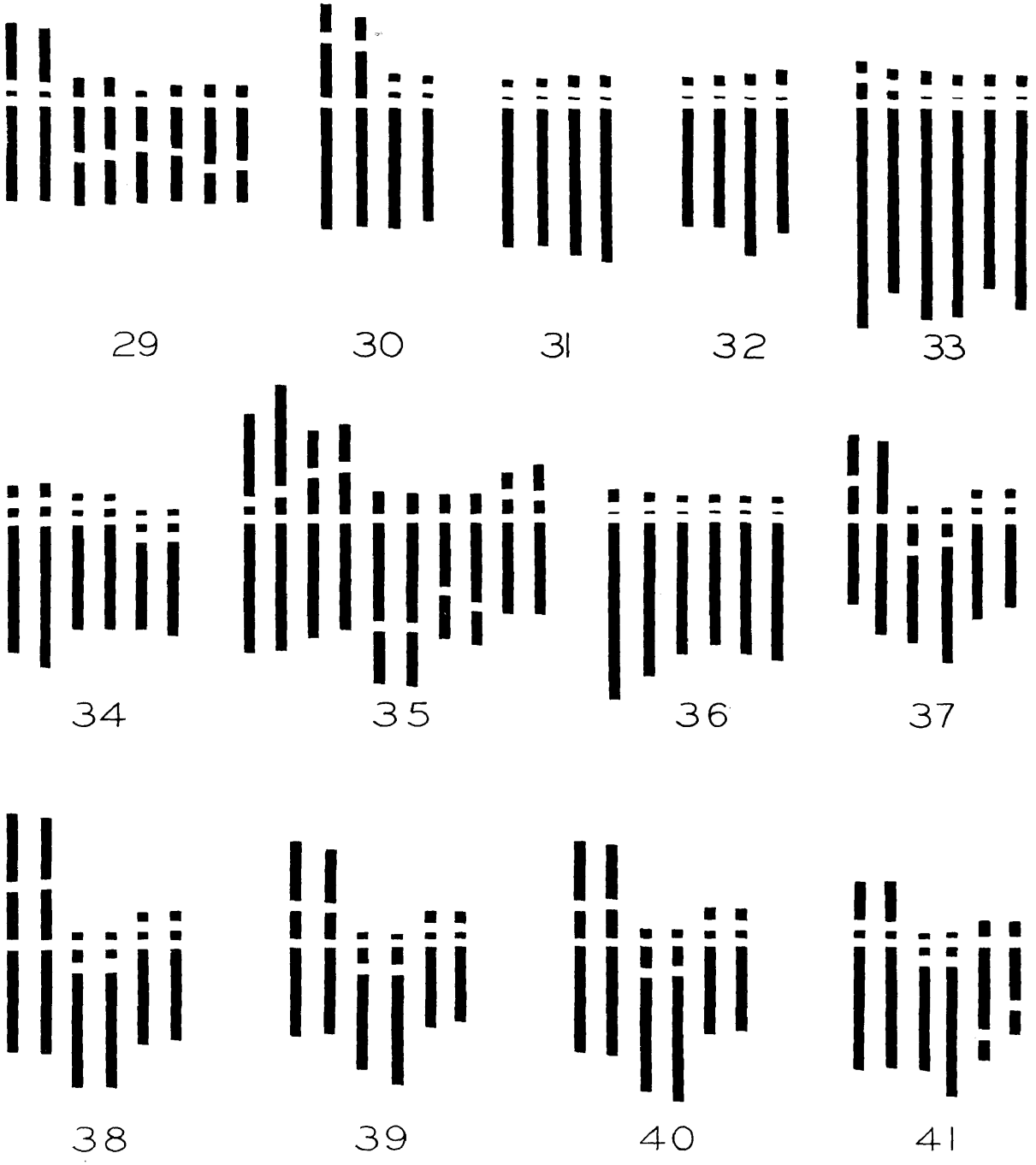

PLATE IV

Section Martagon - Continued

Figure 42.	L. Willmottiae	Central China
Figure 43.	L. Davmottiae	Hybrid
Figure 44.	L. Davidi	
Figure 45.	L. philadelphicum	
Figure 46.	L. Phildauricum	Hybrid
Figure 47.	L. dauricum var. Wallacei.	
Figure 48.	L. var. Elegans Mahogany	
Figure 49.	L. Scottiae	Hybrid
Figure 50.	L. Willmottiae	

PLATE IV

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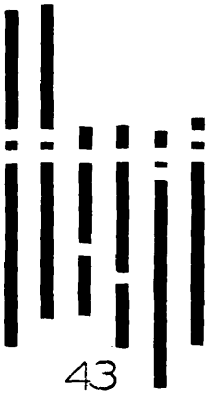

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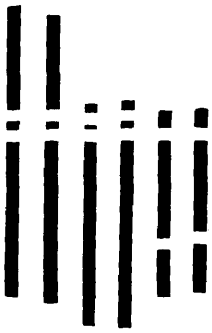

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PLATE V

Section Martagon - Continued

Figure 51. L. tigrinum var. Flore Pleno

Figure 52. L. tigrinum var. Fortunei

Figure 53. L. tigrinum var. Malmo

PLATE V

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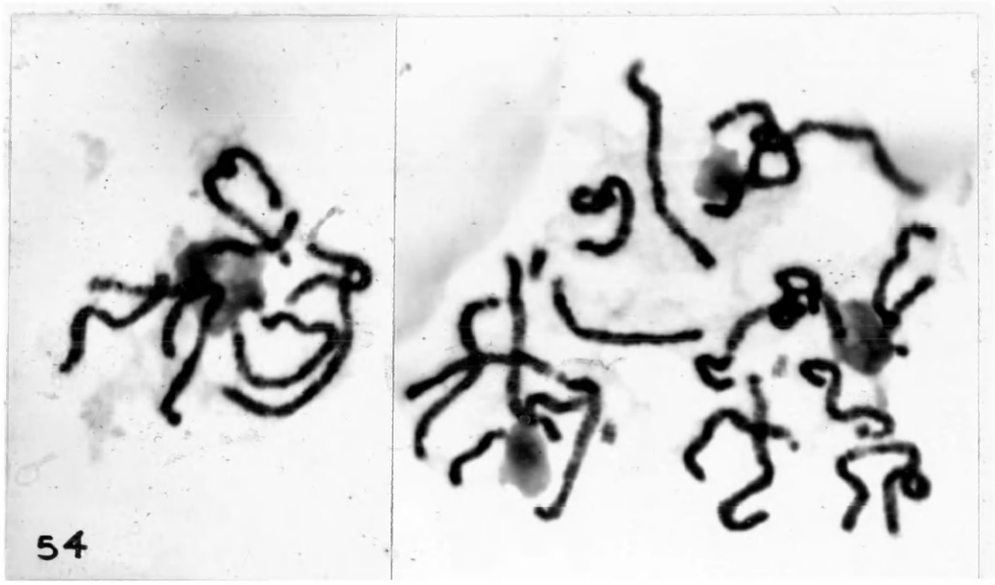
PLATE VI

Figure 54. L. Brownii. Prophase showing four nucleoli and six chromosomes attached. X 1000.

Figure 55. L. Brownii. Early anaphase showing the difference between the primary and the secondary constrictions. X 1000.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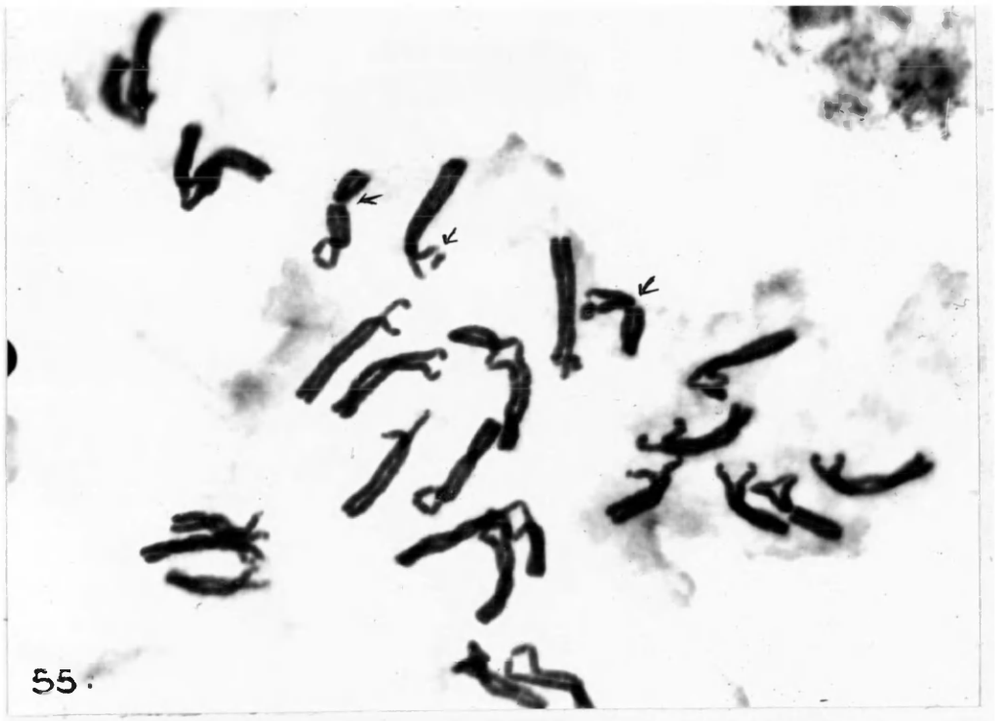
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PLATE VII

Figure 56. L. Bolanderi. Late prophase showing four nucleoli and six chromosomes attached. X 600.

Figure 57. L. carolinianum. Prophase showing four nucleoli and two of the chromosomal attachments. X 1000.

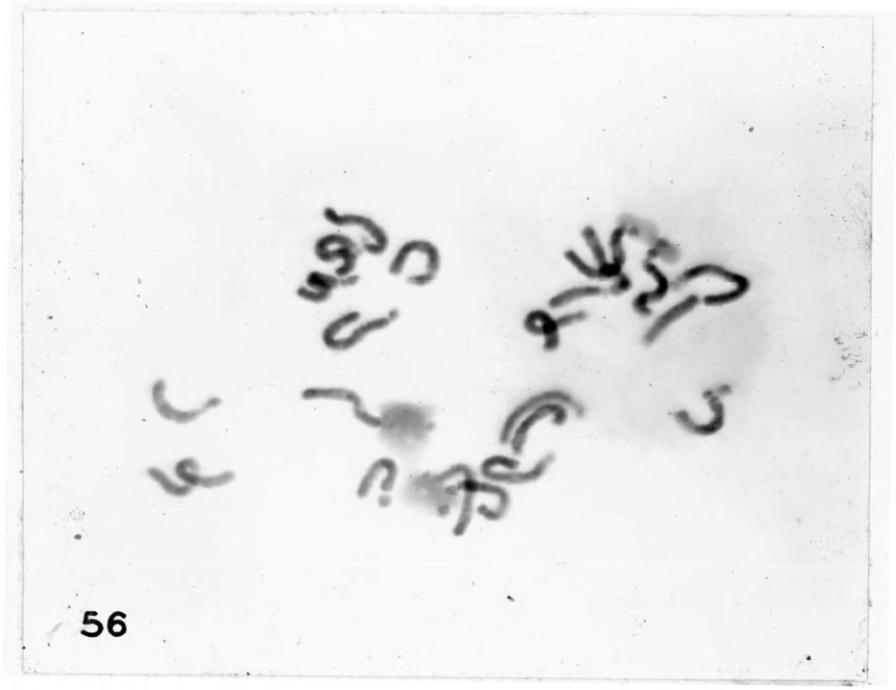
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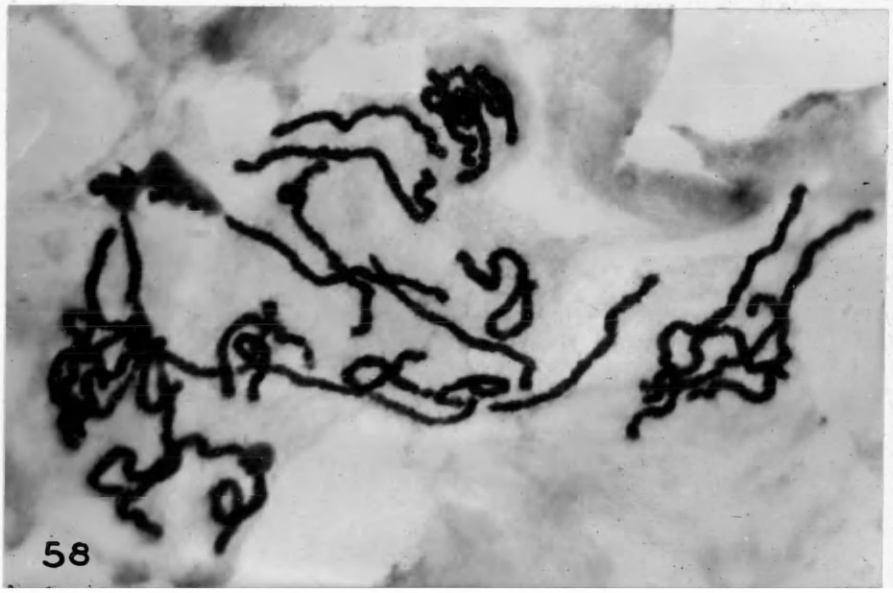
PLATE VIII

Figure 58. L. pardalinum var. Giganteum. Prophase showing two nucleoli and six nucleolar attachments. X 1000.

Figure 59. L. Roezlii. Prophase showing six nucleolar fragments on two nucleoli. Two chromosomes have been pulled away from the nucleoli. X 1000.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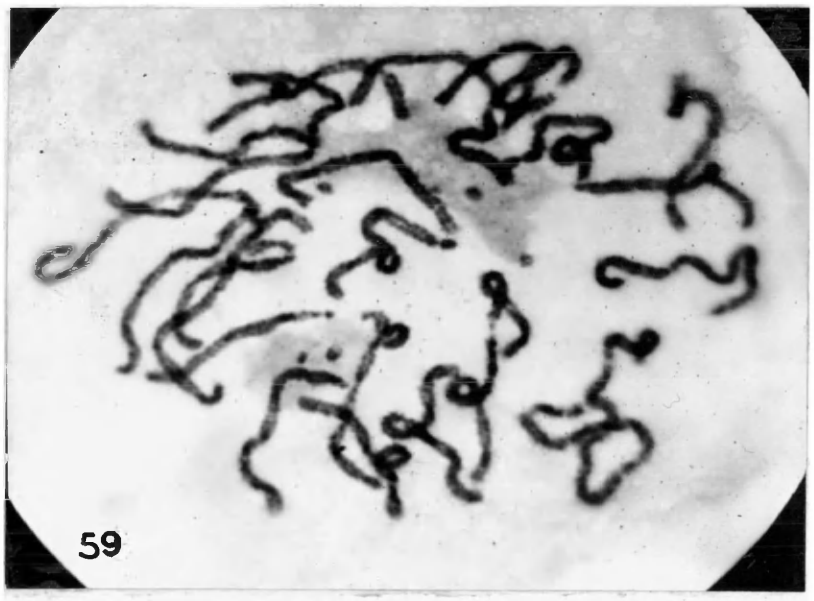
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PLATE IX

Figure 60. L. pumilum. Late prophase showing nine nucleoli. Some of the chromosomes have been pulled away from the nucleoli. X ca. 1000.

Figure 61. L. pumilum. Camera lucida drawing of cell shown in fig. 60. X ca. 1000.

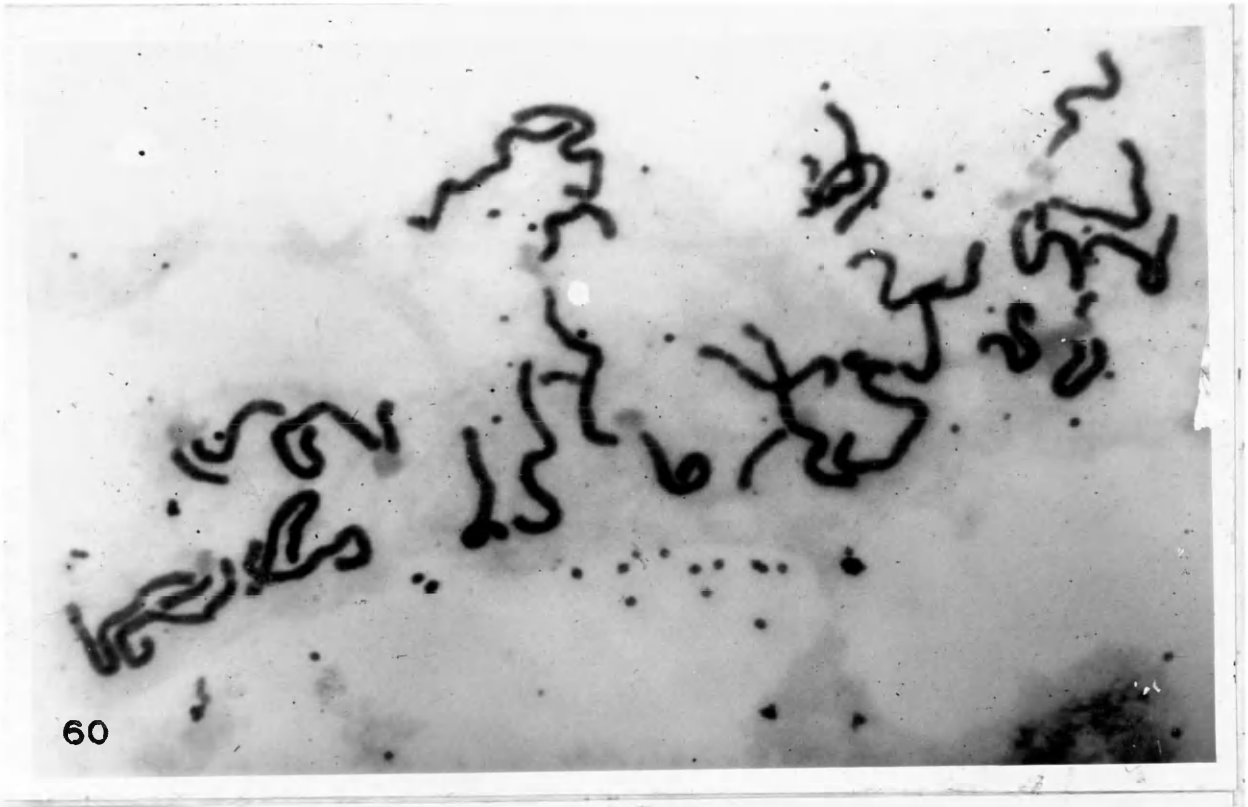

FIGURE 61