THE MORPHOLOGY OF THE SOMATIC CHROMOSOMES IN LILIUM

By

Robert N. Stewart

Thesis submitted to the Faculty of the Graduate School of
the University of Maryland in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy

UMI Number: DP71129

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

UMI DP71129

Published by ProQuest LLC (2015). Copyright in the Dissertation held by the Author.

Microform Edition © ProQuest LLC.
All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGMENT

The writer expresses his deep appreciation of the constant and valuable advice and criticism of Dr. Ronald Bamford throughout the course of the work and the preparation of this thesis.

TABLE OF CONTENTS

Page

INTRODUCTION 1

MATERIALS AND METHODS 2

RESULTS 5

DISCUSSION 17

SUMMARY 55

LITERATURE CITED 57

INTRODUCTION

The cytogenetic literature is full of instances where species relationships have been clarified by cytological evidence. In many plant groups, chromosome number has been shown to be the barrier separating species and information on chromosome number alone has solved these problems for the plant breeder and taxonomist. However, in other cases chromosome numbers have been found to be so widely variable that such knowledge alone merely serves to add confusion or the numbers are all the same and they furnish no new evidence. The genus Lilium has been investigated by numerous workers and all species are reported as having a somatic number of twenty four (Sato, 1932; Sansome and LaCour, 1934; Mather, 1935; Beal, 1942 and Stewart, 1943) with the exceptions of the triploid species L. tigrinum (Takenaka and Nagamatsu, 1930) and several species in which aneuploids are found (Sansome and LaCour, 1934; Mather, 1935; Beal, 1942 and Stewart, 1943). A similar situation obtains in many other genera and in these cases useful information can be obtained from a comparative study of the chromosome morphology of the members of the group. Other cytogenetic methods available have been utilized in the work recently reviewed on Crepis (Babcock et al, 1942; Babcock, and Jenkins, 1943; Babcock, 1944), Nicotiana (Goodspeed, 1945), and in the great mass of material on Zea and Drosophilla. It is recognized that the new systematics utilizes taxonomy, morphology, cytology, genetics, physiology, ecology, paleobotany and all other divisions of plant science.

The earlier reports of chromosome number in Lilium were based on observations of sectioned material. The limitations of this method are obvious when dealing with chromosomes which are as much as twenty eight microns in length at their most contracted stage, above the optimum thickness of sections

to obtain satisfactory staining and enable thorough examination. Cooper (1935-1936) utilized pollen grain divisions in L. Henryi and L. regale, but his illustrations show neither primary nor secondary constrictions. In recent investigations utilization of the propriono or aceto carmine smear technique and excised pretreatment with colchicine (Stewart and Bamford, 1945; Emsweller and Stewart, 1944) has given clear flat figures of the very long chromosomes of Lilium and allow a critical study of their morphology. This is a report of such a study to serve as a base for more extensive investigations of the phylogeny of the genus and allow a more efficient as well as a more fruitful breeding program.

MATERIALS AND METHODS

Root tips were used exclusively for this study and these were taken from bulbs which were obtained from reliable commercial dealers in the United States. Those species native to this country were obtained from dealers specializing in native plants on both the east and west coasts. A few collections were made of native species and garden escapes in the vicinity of College Park. Few of the plants used in previous studies (Stewart and Bamford, 1943; Stewart, 1945) survived the interval and plants reported here represent additional data on the occurrence of aneuploids in Lilium (Stewart, 1943). This collection represents a large majority of the species, varieties and horticultural forms now available. Identification has been checked with the following sources: Elwes (1880), Wilson (1925), Woodcock and Coutts (1935), Slate (1939), and various articles in the Royal Horticultural Society Lily Year Books.

Root tips were fixed in 3:1 absolute alcohol - glacial acetic acid for

twenty four hours, then rinsed and stored in 80 percent alcohol until used. Smears were made by the propriono-carmine technique. The omission of acid hydrolysis and the use of glass tools throughout to eliminate any trace of iron in the stain resulted in the nucleoli staining a bright red color from twenty four to forty eight hours after the preparation of the slide. The roots prepared in this way showed cells at the critical stages of mitosis for the classification of constrictions as shown in Plate 7.

The morphology of the chromosomes at somatic metaphase was determined from divisions in root tips given excised pretreatment for thirty minutes in a .2 percent aqueous solution of colchicine followed by washing in water for ninety minutes. This is a slight modification of previous methods (Burrell, 1939; O'Marra, 1939; Stewart and Bamford, 1943; Stewart, 1943; Emsweller and Stewart, 1944). Buds and mature pollen have been collected and preserved for future work.

Observations were made with the aid of 43x and 90x apochromatic oil immersion objectives and 15x compensating oculars. Camera lucida drawings at table level (approximately 2000x) were made of all the metaphase chromosomes separately in from one to three cells of each plant of a species or variety. These were cut out and for each cell arranged in pairs on the basis of total length, position of primary constriction, number and position of nucleolar secondary constrictions. The idiograms (Plates 8-13) are these drawings of a haploid set from a typical cell which was the least distorted in preparation and traced so that the primary constrictions appear on a horizontal line. The order left to right in each case is of increasing arm length ratio (long, short) rising from approximate unity. Letters are assigned to the chromosomes according to their order in this scheme. Both chromosomes of pairs which are heteromorphic in the position of the secondary constrictions are shown and designated by a letter and its prime. The centric

fragments and extra chromosomes which are not duplications of one of the normal complement are placed on the right regardless of the length of its short arm and are designated by the letter M. In those cases where the heteromorphic pairs differ by a measurable amount of chromatin, they are also placed at the far right but given the letter of the position they would occupy in the idiogram assuming loss of material from the smaller member of the pair.

In addition to the camera lucida idiograms a series of photoidiograms of representatives of the divisions of the genus Lilium; subgenus Cardiocrinum (L. giganteum) and the four sections of subgenus Eulirion; Leucolirion (L. Brownii), Archelirion (L. auratum), Isolirion (L. concolor), and Martagon (L. monadelphum). These photoidiograms are constructed in exactly the same way as the other idiograms except that photomicrographs are used in place of camera lucida drawings. Plates 2 and 3 are photomicrographs of somatic metaphases in roots of L. concolor and L. Brownii respectively from which enlargements were made to construct the photoidiograms.

Table 1 presents the percentage of the total length of all the chromosomes in the idiograms represented by each of the chromosomes. Pairs heteromorphic for secondary constrictions shown twice in the idiograms are figured only once in the table. For pairs heteromorphic for a measurable amount of chromatin, the average is used. Plants having extra chromosomes are figured twice, with and without the extra chromosomes.

The idiograms thus constructed present an exact picture of the haploid set of chromosomes from a typical cell of the species. However, from an examination of these idiograms alone, no conclusions as to the variation in size of karyotypes can be drawn. There is no indication that there is more chromatin in cells of any one species of Lilium than in any other. There is as great difference in cell and chromosome mass between adjacent cells in the

same root as between cells from different species. The idiograms, being camera lucida drawings all at the same magnification, reflect only the latter variation. None of the gradual curves or spirals in the chromosomes shown are characteristic of the morphology of the chromosome. They represent only the chance result of all the forces of coiling, movement, and smearing pressure. However, the indentations or "incomplete" constrictions such as that in the short arm of the C and D chromosomes of auratum, in the short arm of the G chromosome of giganteum, and in many others are constant morphological details as characteristic and definite as any other feature.

RESULTS

The results are presented almost completely by the idiograms (Plates 8 - 13). There are no large variations in chromosome morphology in the genus, all species having two long pairs of chromosomes with submedian centromeres and ten pairs with subterminal centromeres. The 2n number of all species reported here is 24 and, although individuals were found in L. auratum, L. tsingtauense, L. Sargentiae, and L. pumilum with 2n = 25 and one plant in L. Henryi with 2n = 26, the additional chromosomes are, with two exceptions, centric fragments and in all cases are unlike any of the normal complement. However, the variations in the length of the chromosomes and the variation in position and function of the constrictions differentiate the species into two groups.

The following is a detailed classification of the constrictions under each of the species and varieties observed. (Plates 8 - 13).

L. concolor: Seven plants were examined and the karyotype of five of these (type 1 in Table 1) is represented by the idiogram in figure 1. Both chromosomes of pairs A, B, F, I, and K were associated with nucleoli at their secondary constrictions in prophase. A maximum of ten nucleoli were observed in resting cells. The constrictions in the short arms of pairs C and D were non-nucleolar. In the sixth plant the chromosomes were the same except that the secondary constriction in the I pair of chromosomes was absent. The I pair was not associated with nucleoli at prophase and a maximum of eight nucleoli were observed in resting cells. The seventh plant (type 2 in table 1) differed from the first five in that the secondary constriction in one of the B pair of chromosomes was nearer the end of the short arm (fig. 2 and photoidiogram plate 1.)

L. Brownii: Three plants were examined and their karyotype is represented by the idiogram in figure 3 and the photoidiogram in Plate 1. The secondary constrictions in the chromosomes D, F, and G were nucleolar, having been observed attached to nucleoli in prophase. A maximum of six nucleoli were observed in resting cells. The constrictions in the short arms of chromosome pairs C and E were non-nucleolar.

L. candidum: Three plants were examined and their karyotype is represented by the idiogram in figure 4. The I pair was heteromorphic for the secondary constriction in the short arm. The satellite was so small that it was impossible to determine whether or not it was present but it seemed probable that it was fused with the short arm of the I chromosome. Another irregularity was the presence of three K chromosomes and only one J chromosome. This is one of very few cases where a pair is

heteromorphic for a measurable amount of chromatin. The secondary constrictions in the D and F pairs, and the I chromosome are nucleolar. A maximum of five nucleoli were observed in resting cells. The secondary constrictions in C and E were non-nucleolar.

L. callosum: (fig. 5) All five plants were alike having six pairs with nucleolar secondary constrictions; A, C, F, G, and I and also the distal constriction in short arm of the B chromosomes. The proximal secondary constriction in the short arm of the B pair and the secondary constriction in the short arm of D were non-nucleolar.

L. Davidii: (fig. 6) Three plants had four pairs of nucleolar secondary constrictions in chromosome pairs A, D, F, and G. The constriction in the short arm of C was non-nucleolar.

L. speciosum: The karyotypes of two plants of var. album, two of var. rubrum and four of var. magnificum proved to be identical and are represented by the idiogram in figure 7. The distal constriction in the short arm of A and those in C, E, and K were nucleolar. The proximal constriction in the short arm of A is non-nucleolar. Figure 8 represents the karyotype found in two plants of var. punctatum. It differs from the other three varieties only in the A pair of chromosomes where one secondary constriction in each was in the long arm and was nucleolar. The maximum nucleolar count in all varieties was eight.

L. monadelphum: (fig. 9 and Plate 1) The three plants all had eleven nucleolar secondary constrictions; distal in the short arm of both of the C pair, in both of the D, E, and G pairs, one in the long arm of F' and two in the long arm of the F chromosome. The F chromosome was the

only one observed in Lilium with two nucleolar constrictions. In almost every prophase observed, both constrictions were associated with nucleoli. The proximal constriction in the short arm of the A and C pairs were non-nucleolar. The length of the short arm of the C pair was much greater than that of any but the A and B pairs in any of the other species.

L. auratum: In seven plants of this species that were examined, three karyotypes were found (figs. 10, 11, 12). All were identical in the first ten pairs, A through J. The A, B, and D pairs had nucleolar secondary constrictions in their long arms and non-nucleolar secondary constrictions in their short arms. The C pair had nucleolar secondary constrictions proximal and non-nucleolar secondary constrictions distal in their short arms. The E pair had non-nucleolar secondary constrictions in their short arms. Four plants (type 1 in table 1) are represented in figure 10 and Plate 1. The L pair had a nucleolar secondary constriction in the long arm and there was a twenty fifth chromosome, or centric fragment, designated M. One plant (type 2 in table 1) is similar except that it did not have the centric fragment (fig. 11). Two plants (type 3 in table 1) were like type 2 except that there was a nucleolar secondary constriction in the long arm of both of the K pair in the same position as in the L pair, from which they could not be distinguished (fig. 12). It is possible that the first two types are heteromorphic for secondary constrictions in the K and L pairs as no evidence of genetic or pairing homology has been obtained and, except for the secondary constrictions, the K and L pairs are indistinguishable.

L. giganteum: (fig. 13 and Plate 1). All three plants examined were alike having B, C, and D pairs with nucleolar and G with non-nucleolar secondary constrictions.

L. tsingtauense: The two plants (type 1 in table 1) represented in figure 14 and the one plant (type 2 in table 1) represented in figure 15 were identical except for the chromosome M which was an extra chromosome present only in the single plant. Both types had nucleolar secondary constrictions in the long arms of chromosome pairs C, D, F, and J and non-nucleolar secondary constrictions in the short arm of pairs C and D.

L. Grayi: (fig. 16) The two plants examined were identical and had nucleolar secondary constrictions in the long arm of pairs C and K and non-nucleolar secondary constrictions in the short arm of C and D.

L. japonicum: (fig. 17) The two plants examined were alike with nucleolar secondary constrictions in pairs B, proximal in the short arm of D, and in L. There were non-nucleolar secondary constrictions in A, C, distal in the short arm of D, and in F.

L. leichtlinii var. Maximowiczii: (fig. 18) Three plants examined were alike, having nucleolar secondary constrictions in pairs A, B, and G and a non-nucleolar secondary constriction in C.

L. Henryi: Three plants were examined. Two had twenty four chromosomes (fig. 19 and type 1 in table 1) and one had twenty four plus two centric fragments (fig. 20 and type 2 in table 1). Except for the fragments they were alike. There were nucleolar secondary constrictions in the A and F pairs and non-nucleolar secondary constrictions in the C pair.

L. martagon var. album: (fig. 21) Three plants were all alike, having nucleolar secondary constrictions in A, B, long arm of C, F, and K.

There were non-nucleolar secondary constrictions in the short arms of the C and D pairs.

L. longiflorum Creole, Estate, and Slocums Ace: (fig. 22) Five plants of Creole, two of Estate, and three of Slocums Ace were alike, having nucleolar secondary constrictions in D, G, and the long arm of C. Non-nucleolar constrictions were present in the short arm of C and E.

L. formosanum: (fig. 22) Three plants proved to be exactly like L. longiflorum.

L. regale: (fig. 24) Seven plants examined were alike, having nucleolar secondary constrictions in the short arms of pairs A and C, and in the long arms of B, D, and E. There were non-nucleolar secondary constrictions in the short arms of B, D, and E.

L. myriophyllum: (fig. 25) Two plants examined were exactly like L. regale.

L. Sargentiae: (fig. 26 and type 1 in table 1) Three plants examined were exactly like L. regale.

L. Sargentiae Horsford: (fig. 27 and type 2 in table 1) One plant available was exactly like the type and like L. regale but with one additional centric fragment labelled M in the idiogram.

L. leucanthum var. chloraster: (fig. 28) Three plants examined were exactly like L. regale.

L. dauricum: (fig. 29) Four plants of L. dauricum were alike. There were nucleolar secondary constrictions in the A, B, and G pairs and non-

nucleolar secondary constrictions in the short arm of the C pair. The secondary constrictions in the long arms of both the C and F pairs behaved in the same way. At metaphase the constriction always appeared in one and ocassionally in both chromosomes of a pair. In a smaller number of observations of prophase cells, one chromosome of each was usually associated with a nucleolus, but never both chromosomes of either pair. It was impossible to determine if it was always the same member of the pair. The secondary constriction in the member of each pair not associated with the nucleolus was usually visible in these prophase cells. A maximum of eight nucleoli were observed in resting cells. Three plants of L. dauricum var. Wilsoni were examined and were identical with the type except that the secondary constrictions in the long arms of the C and F pairs appeared very rarely and none were ever seen associated with nucleoli in prophase. The maximum nucleolar count in the resting cells of the variety was six.

L. Duchartrei: (fig. 30) Two plants examined were alike, having nucleolar secondary constrictions distal in the short arm of the B pair, and in the C and G pairs. There were non-nucleolar secondary constrictions proximal in the short arm of the B pair.

L. Wardii: (fig. 31) Only one plant was available. There were nucleolar secondary constrictions in the A and D pairs.

L. amabile: (fig. 32) Three plants of amabile and two of var. luteum were all alike. There were nucleolar secondary constrictions in the A, F, and G pairs and non-nucleolar secondary constrictions in C and D.

L. pumilum: (figs. 33, 34, and 35) Ten plants of L. pumilum (fig. 35 and type 1 in table 1) and six plants of L. pumilum Golden Gleam (fig. 34 and type 2 in table 1) were alike. A seventh plant of L. pumilum Golden Gleam (fig. 35 and type 3 in table 1) had the same twelve pairs plus one extra chromosome labelled M (fig. 35). All seventeen plants had nucleolar secondary constrictions in the A, B, D, and F pairs and in the long arm of C. There were non-nucleolar secondary constrictions in the short arms of C and E.

L. superbum: Two karyotypes were found in collections of L. superbum from a very small area in a swamp near College Park. Two plants are shown in figure 36 (type 1 in table 1) and five more from the collection and three plants from a commercial dealer are shown in figure 37 (type 2 in table 1). Type 1 had nucleolar secondary constrictions in pairs C, J, and K and non-nucleolar secondary constrictions in the short arm of D. Type 2 had nucleolar secondary constrictions in the long arms of C, D, and K and non-nucleolar secondary constrictions in the short arms of the D pair. The two types were very similar in the distribution of chromatin (table 1) and the only difference is the position of one of the three pairs of secondary constrictions in each.

L. philadelphicum: (fig. 38) Three plants examined were alike, having nucleolar secondary constrictions in pairs D, F, K, and L and non-nucleolar secondary constrictions in C.

L. Catesbaei: (fig. 39) Only one plant was available. Metaphases were abundant and it was possible to determine the position of the constrictions and from anaphase figures it was possible to classify them as

primary or secondary. However, there were many nucleoli and many chromosomes attached to them in prophase and the nucleolar activity of some of the constrictions could not be determined. Both secondary constrictions in B, C, K, L, and the distal ones in the long arm of the F pair were all identified in prophase as associated with nucleoli. In those prophases where the distal constriction in the long arm of an F chromosome was seen associated with a nucleolus it was determined that the proximal constriction was not and it is probable that it is non-nucleolar. At least two and probably all four of the secondary constrictions in the D and E pairs were nucleolar, but no clear cut case of association of all four was found.

L. carolinianum: (fig. 40) Four plants examined were alike, having nucleolar secondary constrictions in the F and L pairs and non-nucleolar secondary constrictions in the C, E, and G pairs.

L. michiganense: (fig. 41) Five plants were alike, having nucleolar secondary constrictions in chromosome pairs E and G and non-nucleolar secondary constrictions in C and D.

L. canadense: (fig. 42) Five plants of L. canadense, two of L. canadense var. rubrum and two of L. canadense var. flavum all possessed identical karyotypes and all were indistinguishable from L. michiganense.

L. pardalinum var. giganteum: (fig. 43) Three plants examined had identical karyotypes. There were nucleolar secondary constrictions in the long arms of pairs H, I, and K and non-nucleolar secondary constrictions in the short arms of C and D and in the long arms of C and L. The F pair is hetermorphic for a measurable amount of chromatin. They are

lettered F, and loss of material from F' presumed, because they differ only in that way from the species type and the other Western North American species to which this variety is obvicusly closely related.

L. Roezlii: (fig. 44) Two plants were examined and were alike, having nucleolar secondary constrictions in the long arms of pairs H, I, and K and non-nucleolar secondary constrictions in the short arms of C and D and in the long arms of C and L. The karyotype appears identical to that of L. pardalinum var. giganteum except for the F' chromosome.

L. pardalinum: (fig. 45) Three plants were examined and were found to have karyotypes identical with that of L. Roezlii.

L. Parryi: (fig. 46) Three plants were found to have identical karyotypes and all were similar to that of L. Roezlii except that the long arm of the G pair of chromosomes is measurably shorter.

L. occidentale: (fig. 47) Two plants were found to have identical karyotypes. The B pair was heterozygous for a nucleolar secondary constriction in the short arm and there were also nucleolar secondary constrictions in the long arms of pairs H, I, K, and L. There were non-nucleolar secondary constrictions in the short arms of C and D and in the long arm of C.

L. columbianum: (fig. 48) Three plants were found to have identical karyotypes similar to that of L. occidentale except that the B pair of chromosomes was homozygous for the nucleolar secondary constriction in the short arm.

TABLE I

Percentage of chromosome length to total length in idiograms

SPECIES	A	B	C	D	E	F	G	H	I	J	K	L	M
concolor (1)	12.1	11.0	7.8	7.6	7.6	7.5	8.7	8.2	8.7	6.6	6.9	7.6	
concolor (2)	12.1	11.2	7.8	7.6	7.6	7.3	8.7	8.2	8.7	6.6	6.6	7.6	
Brownii	12.8	10.9	8.6	7.4	7.2	6.9	8.6	8.1	7.9	6.9	6.7	7.9	
candidum	11.9	10.5	8.4	8.1	7.0	6.6	8.6	8.8	8.9	7.0	6.8	7.3	
callosum	11.9	10.8	7.6	8.0	7.1	8.0	8.9	8.5	8.5	6.9	6.6	7.3	
Davidii	12.6	10.6	7.8	7.2	7.8	7.2	9.1	8.9	8.5	6.7	6.7	6.9	
speciosum vars. album, rubrum, magnificum	12.8	12.1	7.3	5.5	6.6	7.3	9.6	8.9	8.4	5.7	7.8	8.0	
speciosum var. punctatum	11.9	11.1	7.9	6.2	6.7	7.2	9.6	9.1	8.9	5.7	7.9	7.9	
monadelphum	11.8	10.8	9.2	8.4	7.0	6.3	8.9	8.4	8.7	6.5	7.2	6.7	
auratum (1)	12.7	12.0	6.5	7.7	8.0	6.0	8.9	8.0	8.2	5.8	7.2	7.5	1.4
" "	13.0	12.2	6.6	7.8	8.1	6.1	9.0	8.1	8.3	5.9	7.3	7.6	
auratum (2)	12.0	11.7	6.2	7.0	8.5	6.2	9.5	8.2	8.7	5.7	8.0	8.2	
auratum (3)	12.3	12.2	6.4	7.2	7.9	6.1	9.5	8.4	8.7	5.6	7.9	7.9	
giganteum	13.7	12.2	8.2	7.6	7.6	7.1	8.9	8.7	8.5	5.3	6.2	6.5	
tsingtauense (1)	12.6	11.9	8.1	7.2	7.6	7.9	8.8	8.3	8.8	5.8	6.3	6.7	
tsingtauense (2)	12.6	11.8	8.2	7.3	7.8	8.0	8.6	8.2	8.6	6.0	6.2	6.7	
" "	12.0	11.2	7.8	6.9	7.4	7.6	8.2	7.8	8.2	5.7	5.9	6.3	5.1
Grayi	11.6	10.4	8.2	6.9	7.3	8.0	9.2	8.8	8.6	6.3	6.9	7.8	
japonicum	12.1	10.8	7.9	6.7	8.3	6.1	9.7	8.8	8.1	6.3	7.4	7.9	
Leichtlinii var. Maximowiczii	12.9	11.5	8.3	7.1	8.1	6.9	9.2	9.0	8.1	5.6	6.0	7.3	
Henryi (1)	12.9	11.5	8.4	6.7	7.9	6.7	8.6	8.4	8.4	6.2	7.2	7.4	
Henryi (2)	12.7	11.6	8.3	6.4	7.5	6.6	8.5	8.3	8.3	6.1	6.8	7.3	1.4
" "	12.9	11.7	8.4	6.5	7.7	6.9	8.6	8.4	8.4	6.2	6.9	7.4	
martagon album	12.2	11.2	8.4	7.6	7.6	6.6	8.6	8.8	8.4	6.2	6.8	7.8	
longiflorum Hfs. Creole, Estate, Slocum's Ace	12.4	11.2	8.0	7.2	9.1	7.4	7.2	9.1	8.8	6.3	6.3	7.2	
formosanum	12.8	11.3	7.9	7.1	7.3	7.3	9.0	9.0	8.6	6.2	6.4	7.1	
regale	12.9	12.2	7.3	7.7	8.2	7.4	9.2	8.0	8.0	5.6	6.6	7.3	
myriophyllum	11.9	11.1	7.3	7.8	8.4	7.1	9.0	8.2	8.6	6.1	6.7	7.8	
Sargentiae Horsford	12.2	11.1	6.6	7.2	7.8	6.8	8.9	8.5	8.7	6.0	6.6	7.4	2.3
" "	12.4	11.4	6.8	7.4	8.0	7.0	9.1	8.6	8.9	6.1	6.8	7.6	
Sargentiae	12.5	11.1	6.9	7.9	8.6	7.6	8.8	8.1	8.6	6.0	6.5	7.4	

leucanthum	12.3	11.0	7.2	7.7	8.3	7.2	9.0	8.3	8.6	5.7	6.8	7.9	
dauricum and dauricum Wilsonii	12.7	10.9	8.4	8.2	7.0	7.3	8.8	8.4	8.6	6.1	6.6	7.0	
Duchartrei	13.4	12.2	8.0	7.3	6.1	7.0	9.9	9.4	8.7	5.4	5.9	6.8	
Wardii	12.7	11.1	8.2	7.2	6.6	8.2	9.0	8.8	8.2	7.4	6.4	6.1	
amabile	11.9	10.8	8.4	7.8	7.3	7.3	9.1	8.6	8.4	7.5	6.3	6.7	
pumilum	13.8	12.1	8.1	7.7	7.0	6.8	8.1	7.4	8.5	8.1	6.2	6.2	
pumilum Golden Gleam (1)	12.4	11.2	8.2	8.0	7.3	6.4	8.9	7.6	8.7	8.2	6.2	6.9	
pumilum Golden Gleam (2)	11.6	10.2	7.2	7.0	6.6	6.2	8.2	7.6	8.4	7.8	5.8	6.0	7.6
" " " "	12.5	11.0	7.8	7.6	7.1	6.7	8.9	8.2	9.1	8.4	6.3	6.5	
superbum (1)	11.9	10.2	8.1	6.4	7.2	9.8	8.7	7.2	8.5	6.6	7.9	7.4	
superbum (2)	12.0	11.0	8.1	6.5	7.3	9.4	9.0	7.5	8.6	6.1	7.3	7.1	
philadelphicum	11.1	11.1	8.5	8.7	8.0	9.5	8.2	8.0	8.0	6.4	6.2	6.4	
Catesbaei	11.5	10.6	8.7	7.1	8.7	8.7	6.9	8.7	8.3	7.4	6.4	6.9	
carolinianum	11.4	9.7	8.3	8.8	6.8	9.0	9.2	6.6	8.5	7.5	6.3	7.8	
michiganense	11.6	10.8	8.0	8.0	9.0	9.2	8.0	8.6	7.3	6.8	6.6	6.2	
canadense	12.0	10.6	7.8	7.8	9.2	9.4	7.8	8.5	7.6	6.7	6.5	6.0	
pardalinum var. giganteum	11.8	11.1	6.8	8.1	9.2	8.5	8.5	7.5	7.7	7.1	6.4	7.3	
Roezlii	12.3	11.2	6.6	7.8	9.1	8.7	8.2	7.3	7.8	6.8	6.6	7.5	
pardalinum	11.8	10.5	6.8	7.4	9.4	9.0	8.1	7.4	8.1	7.0	7.0	7.6	
parryii	11.6	10.8	7.3	8.1	9.4	8.7	7.1	7.3	8.1	7.1	6.9	7.7	
occidentale	12.1	10.8	6.9	8.2	9.2	8.6	8.0	7.1	8.2	7.1	6.9	6.9	
columbianum	12.0	10.4	6.4	7.8	9.4	8.8	8.4	7.0	7.8	7.0	7.6	7.2	

DISCUSSION

An accurate determination of the karyotype of a species must include a thorough analysis of the activity of the constrictions observed at metaphase. Failure to do this will give false pictures of chromatin distribution if any comparison other than total length of the chromosomes is used. The chromosome pairs F and L in L. carolinianum (fig. 40) illustrate this. The two chromosomes appear to be very similar at metaphase, but classification of the constrictions separates them widely in the arrangement used and the effect on arm length ratio is obvious. If one defines that portion of a chromosome distal from the nucleolar organizing region in respect to the centromere as a satellite, then the satellite in the F chromosome is a very small part of the total mass of the chromosome, while in the L pair the satellite makes up approximately ninety percent of the total mass of the chromosome. An even more extreme case of this type is the I chromosome of the West Coast species (figs. 45-48).

There are critical stages of mitosis which must be examined to determine the activity of constrictions (plate 7). It is necessary, for example in L. Sargentiae, which has five nucleolar chromosomes with more than one constriction, to examine four different cells, one at prophase, one at metaphase, one at late metaphase or early anaphase, and one at later anaphase. The activity of the constrictions in the metaphase chromosomes have been labelled (P = primary constriction: S_N = nucleolus forming secondary constriction: and S = non-nucleolar secondary constriction) as determined in the other three phases of division. The prophase cell shows nucleoli associated with constrictions which could therefore be classified as nucleolar secondary constrictions. In chromo-

somes with only two constrictions, the other can be classed as a primary constriction or centromere. The E chromosome at prophase shows that the two constrictions which are unassociated cannot be differentiated and additional evidence is necessary. In one particular cell, none of the three constrictions in either of the D pair of chromosomes was associated with a nucleolus. However, in approximately eighty percent of the prophases examined, both chromosomes of the pair, and, in approximately eighteen percent of the cells, only one of the chromosomes of the pair were associated with nucleoli at the constriction classified as nucleolar. This failure of constrictions which are usually nucleolar to be associated with nucleoli (variation in nucleolar activity between members of a pair) is typical of all the nucleolar constrictions of which a large number of observations were made. In this species the frequency of failure to associate with nucleoli in prophase was very low in the nucleolar secondary constrictions of the A, B, and C pairs and significantly higher in the D and E pairs. The variation in nucleolar activity (between pairs) is common throughout the genus. The difference in the frequency of nucleolar association of the secondary constrictions in the C and F pairs between L. dauricum and L. dauricum var. Wilsoni was the only difference in their karyotypes. This indicates the need of examining large numbers of prophases. In this report, those secondary constrictions which were definitely associated with nucleoli in more than one of the prophases examined, were classified as nucleolar. Those never associated with nucleoli were classified as non-nucleolar. From 15 to 20 prophases were analyzed in every species and as many as 30 in several.

The primary constriction or centromere can be identified in late metaphase (plate 7). The chromosomes appear double and at the very

beginning of anaphase movement, the centromeres can be seen separating while the remainder of each chromosome, including the secondary constriction, has not separated.

At anaphase the centromere can be distinguished by its orientation towards the pole. The attenuation of the secondary constrictions as seen in chromosomes A, B, and D is related to the mass of chromosome distal to the secondary constriction. Secondary constrictions in the short arms of the ten pairs of chromosomes with subterminal centromeres are never attenuated at anaphase while those in the long arms near the centromere are almost always attenuated (H, I, and K chromosomes of the west coast species, fig. 43-48).

It is of interest to note, while examining the anaphase chromosomes, that it is not necessary to set an arbitrary arm length ratio to separate the I type and J type chromosomes in the karyotype. In chromosomes with very short arms the short arm does not bend back during anaphase movement (the K chromosome alongside the A chromosome and the L chromosome next to E in the anaphase in plate 7). The other chromosomes, G through J, which are not illustrated, also appear as I type chromosomes at anaphase. The short arms of chromosomes A through F bend back during anaphase movement (anaphase in plate 7) and they appear as J type chromosomes.

All the chromosomes of a species can be identified in prophase and their nucleolar attachments determined. In the prophases of root cells which had not received colchicine pretreatment (plates 4 and 5 are a camera lucida drawing and photomicrograph respectively of an untreated prophase cell in L. callosum whose idiogram is presented in fig. 5), it is evident that the large numbers of secondary constrictions found in

Lilium are not artifacts resulting from the colchicine treatment used to obtain large numbers of metaphases. Confirmation of the constrictions observed in colchicine treated metaphases was obtained in untreated metaphases in several species and in untreated prophases in all species. In L. callosum (plates 4 and 5), both chromosomes of pairs A, G, and K and one of pair F are almost invariably attached to nucleoli. However, in some prophases both of the F pair, one or both of the C pair, and one or both of the B pair at the distal constriction in the short arm are also attached to nucleoli. The proximal constriction in the short arm of the B pair is typical of the three cases of constrictions which always appear as full constrictions at metaphase but are never attached to nucleoli. The indentation or "incomplete" secondary constriction in the short arm of the D pair is typical of those of that type which are, with the exception of the C pair in L. japonicum never associated with nucleoli but are constant morphological features of the chromosome. L. callosum shows marked variation in frequency in the association of nucleolar secondary constrictions with nucleoli at prophase. The F chromosomes display differences within a pair. Difference in frequency between pairs distinguishes the secondary constrictions of the A, F, G, and K pairs, which are associated with nucleoli in over ninety-five percent of the prophases from the secondary constriction in the C pair and the distal secondary constriction in the B pair which are associated in approximately twenty-five percent of the prophases. These, in turn, are distinguished from the secondary constrictions proximal in the B pair and in the D pair which are never associated and are classified as non-nucleolar. It is probable that the frequent observation in resting cells of fewer nucleoli than nucleolar secondary constrictions is due not only to fusion of nucleoli, but also

to failure of nucleolar secondary constrictions to form nucleoli.

Variation in nucleolar activity between a pair of nucleolar secondary constrictions is also found in L. giganteum (plate 6). The secondary constriction of one B chromosome illustrated is not associated with nucleoli in this particular cell. However, it is associated in a large percentage of the prophases observed and is therefore classified as nucleolar.

This data confirms the association of secondary constrictions and nucleoli reported by Heitz (1931), Resende (1937), Stewart and Bamford (1942), and the innumerable cases mentioned in Gates' review (1942). Previous reports of non-nucleolar secondary constrictions (Fernandes, 1936; Resende, 1937; Sato, 1938; Jacob, 1940; Stewart and Bamford, 1943, etc.) are supplemented. However, the failure of normally nucleolar secondary constrictions to be associated with nucleoli and the measurable variability of this feature has not been previously reported. Enough reports of non-nucleolar secondary constrictions have found their way into the literature to make it necessary to differentiate between secondary constrictions or satellite constrictions and the nucleolar attachment regions. Statements as to the correspondence of attachment regions and nucleolar numbers are meaningless (Gates 1942). The maximum number of nucleoli in resting cells was determined for all the species of Lilium reported, and was in all cases equal to the maximum number of chromosomes observed associated with nucleoli at secondary constrictions in prophases. In all but one species of Lilium of over forty examined there were additional non-nucleolar secondary constrictions.

Van Camp (1924), Dermen (1933), Woods (1937), and Matsurra (1938) give reduction of number by fusion as the cause of the high frequency of

less than the maximum number of nucleoli observed in resting cells. Failure of nucleolar constrictions to form nucleoli must be recognized as an additional factor.

Polyploidy as a source of variation in nucleolar number within a genus (Gates 1942) is ruled out in the homoploid genus Lilium where, nevertheless, maximum nucleolar numbers of species varies from four to fourteen with several instances of odd numbers resulting from chromosome pairs heteromorphic for secondary constrictions.

The order of the idiograms (plates 8 - 13) is an attempt to arrange the species on a basis of similarity of distribution of chromatin within the karyotype. Since the chromosomes in each idiogram are arranged from A to L on a basis of the decreasing length of the short arms, the curve formed by connecting the ends of the successive long arms will indicate relative distribution of chromatin.

All species had two long pairs of chromosomes with submedian centromeres, the A and B pairs. The small variations in length of these has been ignored in the arrangement of the species. In all the other chromosomes of the regular complement of all species the centromeres were subterminal. The variation of distribution of chromatin among these chromosomes is discontinuous on several levels and first allows separation into two groups. In Groupe 1, the C, D, E, and F pairs are relatively short with low arm length ratios, the G, H, and I pairs are long with high arm length ratios, and the J, K, and L pairs short but still with high arm length ratios. This relative distribution is very definite in the species represented in figures 1 through 30. The species represented in figures 31 through 35 differ only in that the increasing length of the J chromosome

moves it from the JKL class to the GHI class. Group 2 consists of the rest of the species examined (figs. 36 - 48). In L. superbum (figs. 36 and 37) the CDEF class is reduced to three pairs and the GHI class is increased to four. In L. philadelphicum the classes are only slightly different but the species represented in figures 39 and 40 are markedly distinct from these and from each other. The karyotype of L. canadense (fig. 42) is identical with that of L. michiganense (fig. 41). The variation is again stabilized in the remaining species (figs. 43 - 48) and appears identical except for the relative shortness of the G pair in L. Parryi (fig. 46).

Further subdivision based on variation of chromatin distribution alone depends on smaller differences and obviously becomes less accurate. Group 2, however, can easily be divided into two sections. First, the species represented in figures 36 through 42 where there is relatively large variation in karyotype, and second, the rest of the species in Group 2 which have almost identical karyotypes. Within Group 1, the arrangement was first made on the basis of similar variation in the length of the JKL class of chromosomes; J and K relatively short and L long (figs. 1 - 6), J short and K and L long (figs. 7 - 13), increasing length from J to L (figs. 14 - 30), decreasing length from J to L (fig. 31), and J relatively long and K and L short (figs. 32 - 35). Within these groups the order in the series was determined on the basis of variations in length in the GHI class of chromosomes and then, within these, in the variations in the CDEF class. Group 1 does not readily fall into distinct sections as does Group 2. The karyotypes of L. regale, L. myriophyllum, L. Sargentiae, and L. leucanthum var. chloraster (figs. 24 - 28) do form

one clear group, but among the other species the variation is on about the same level, and while the scheme used for arrangement seems to give the most distinct division that is the only indication from evidence of chromosome morphology that the arrangement is natural.

With the exception of one species, L. Grayi (fig. 16), the position and distribution of secondary constrictions supports the separation of the genus into two groups. Although there are numerous exceptions, the secondary constrictions are found in the chromosomes with very short arms, nearer the centromere, and in the long arms, more often in Group 2 than in Group 1. Secondary constrictions are present in the A chromosome pair of all the species of Group 1 except L. Grayi, L. Duchartrei, L. longiflorum, L. formosanum, L. giganteum, L. tsingtauense, L. candidum, and L. Brownii, and in the A chromosome of none of Group 2. Secondary constrictions are present in the B chromosomes of approximately two thirds of Group 1 and in less than one fifth of the B chromosomes in Group 2. One type of chromosome is peculiar to Group 2 and L. Grayi (fig. 16). These chromosomes have very small short arms and the occurrence of secondary constrictions in the long arms, very close to the centromeres, make eighty five percent or more of each chromosome a satellite.

That these divisions represent natural groups could be determined by cytological methods only if the structural changes in the chromosomes, few of which result in changes in chromosome morphology, are not so complex as to preclude analysis. The groups must be closely enough related that hybrids can be obtained for analysis. From the cytological analysis of two hybrid forms as reported by Richardson (1935) and Stewart and Bamford (1943) it can be inferred that the structural differences between

species are numerous and complex. Thus any thorough analysis is probably impossible. Information from other sources commonly used to evaluate natural relationships consists of geographical distribution, interfertility or sterility, morphological structure, and physiological and growth characteristics. Information on these features gathered from the literature is considered in relation to the groups arrived at on the basis of chromosome morphology.

Lilies are indigenous to the Northern hemisphere. Elwes (1880) gives maps showing their distributions in three general areas. In North America, 8 species are found in the Eastern United States and Canada, two or three of them extending to the Central States. Thirteen or fourteen species are found along the Pacific Coast. L. philadelphicum has the widest range of the eastern lilies probably extending to the range of those on the Pacific Coast. In Europe and Western Asia, eight or nine species seem to be native. The range of L. Martagon extends across Siberia and probably to the areas occupied by the East Asiatic group. L. candidum has been cultivated for so many centuries that its origin is in doubt but it is probably from far east of its present concentration in South-Eastern Europe. The third area of distribution is Eastern Asia where by far the largest number of species are found, at least forty-five being recognized at the present time.

It is to be noted that the species of Group 2, second section (figs. 43–48) are all natives of the Pacific Coast of North America. The species of Group 2 first section (figs. 36 to 42) are all natives of Eastern North America. Only three species of the European-West Asian group are reported here. As previously noted, the origin of L. candidum is uncertain and the distribution of L. martagon reaches to the edge of the East Asiatic group.

Two features of the karyotype of L. monadelphum (fig. 9 and plate 1) distinguish it from all other species. First the short arm of the C chromosome pair is much longer than that of any chromosome in all the other species except for the A and B pairs. Secondly, the F chromosome is the only one found with two nucleolar secondary constrictions. However, lacking more complete representation of the species native to this region, the three are placed with the East Asiatic group which they resemble in all characteristics much more than they do the North American group. Group 1 (figs. 1 - 34) includes these three, twenty-five species native to Eastern Asia, and L. Grayi (fig. 16) found only in a small area in Southeastern United States. Thus, all the species of Group 1 except L. Grayi are indigenous to the Old World and all the species of Group 2 are from the New World. Variation in distribution of chromatin is correlated with geographical distribution.

The situation in L. Grayi is of considerable interest because it emphasizes how small the variations in chromatin distribution are. If the F chromosome suffered a structural rearrangement which resulted in decreasing the length of its short arm enough to make it intermediate between the H and I chromosomes a karyotype would result almost identical with that of the L. superbum type represented in figure 57. The idiogram would then have been placed in with those of Group 2, section 1. That the reverse of such a structural rearrangement has occurred is probable because in every other feature, L. Grayi is common with the species of Group 2. L. Grayi has no secondary constrictions in the A and B pairs of chromosomes which is the usual situation in Group 2. Chromosomes like its K pair are found elsewhere only in Group 2. Its characteristics and behavior in all the phases of the following discussion are those of a

member of Group 2 and it is hereafter considered one of that group.

Of approximately sixty named species hybrids listed by Slate (1939) and Woodcock and Coutts (1935), fifty-eight were crosses within Group 1 or Group 2 and only two were between the two groups. Emsweller (1937) lists the interspecific hybrids reported to that date. There were 161 within one of the two groups and only seven were between groups. Two of the seven were the same ones reported by Slate and Woodcock and Coutts. Simmonds (1939) gives a list of species hybrids of which one of 100 was between groups. Preston (1933) lists both successful and unsuccessful crosses. Of fifty-eight attempted inter-group crosses, only one "succeeded." She recorded as successful an attempt which produced "apparently good seed." It was not recorded whether the seeds were hybrids or apomictic as so many seeds produced in Lilium interspecific crosses are (Stout 1933).

Slate (1939) lists groups of species he recommends as promising for the production of new hybrids because a survey of the literature and his experience has shown relatively high fertility in interspecific crosses within these groups. The first group includes L. regale, L. sargentiae, L. myriophyllum, and L. leucanthum. These species are all natives of Eastern Asia and have identical karyotypes falling in Group 1, adjacent in the arrangement within the group. Slate's second group includes L. candidum, L. chalcedonicum, and L. testaceum. L. testaceum is a hybrid between the two species (Emsweller and Stewart 1944) which are both representatives of the European-West Asian group. Only L. candidum was available for the present study but while the chromosomes of these species were not presented by Emsweller and Stewart (1944) in a form directly comparable to the idiograms in this report, it is evident that the L. candidum

of this study has the same karyotype as their Type III which included all the bulbs they obtained from commercial sources. Their L. chalcedonicum evidently has the distribution of secondary constrictions and of chromatin characteristic of Group 1. Slate's third group includes L. martagon and L. Hansoni. Only L. martagon was examined. It is probable that the range of L. martagon extends from Europe to East Asia where L. Hansoni is indigenous. The fourth group includes L. tigrinum, L. Leichtlinii var. Maximowiczii, L. willmottiae, L. suchtuense, L. dauricum, L. croceum, and L. batemanniae. All are natives of East Asia except L. croceum which is probably European although this species is another, cultivated for food since ancient times, whose origin is doubtful. L. Leichtlinii var. Maximowiczii and L. dauricum, the only two represented in this report, both fall into the third section of Group 1 although they are rather widely separated within that section (figs. 18 and 20). L. tigrinum has previously been reported (Stewart and Bamford, 1943). Rearrangement of the idiograms to the present order of decreasing length of the short arms from left to right shows that they fit in the fourth section of Group 1. Slate's fourth group includes L. pardalinum, L. parryi, L. humboldtii, L. washingtonianum, L. maritimum, L. columbianum, L. Roezlii, and L. parvuum. All these are natives of the Pacific Coast of North America and the idiograms of those reported here fall into the second section of Group 2. Slate's fifth group includes L. speciosum and L. auratum. These are both Asiatic and their idiograms were placed in the second section of Group 1, separated only by one species, L. monadelphum (fig. 9). Slate's sixth group adds L. Henryi to his first group. This species is also a native

of East Asia and its idiogram fell in the same section of Group 1 as the L. regale group. These data show interspecific sterility to be correlated with the variation in distribution of chromatin.

Stout (1928) classifies the bulbs and bulb habits of Lilium. The European and Asiatic species (Group 1), with few exceptions, have concentric bulbs. The bulbs of the North American species (Group 1) are all rhizomatous. The bulbs of the Pacific Coast species (section 2) differ from those of the Eastern species (section 1) in that the rhizome between the mother and daughter bulbs is covered with scales. Thus variation in bulb structure is correlated with variation in the distribution of chromatin.

Lilium has not been the subject of scientific researches designed to elucidate the physiological systems of the species. Slate (1939) makes the following general statements based on his experience growing many species and from a survey of the literature. On page 48, he states: "The Asiatic lilies are rich in color and diversity of form. They hybridize well among themselves, but poorly with the other lilies.

"Many of this group are fairly easy in gardens and usually flower well the first year. As a group, they grow rapidly from seed, although a few do not come up until the second year. They are mostly stem rooters and a few have wandering stem bases." On page 49, relative to the species native to Europe and Western Asia, he states: "Few are stem-rooting and as a group they tend to sulk for a year or two after removal. They grow rather slowly from seed ——." On page 49, relative to the species native to Eastern America and the Central States: "These are slow from seeds, which come up the second year, mostly base-rooting or with weak stem roots, and have stoloniferous bulbs and pendulous flowers except L. philadelphicum and L. Catesbaei. Except for L. philadelphicum,

they do not hybridize with other lilies." On page 50, concerning the species native to Western America: "The Pacific Coast lilies, except for L. pardalinum and L. Humboldtii, are usually more or less difficult garden subjects, slow from seed, and have rhizomatous or sub-rhizomatous bulbs. The bulbs of some do not handle well and are not easily established. Some have jointed scales. They are a clannish lot and hybridize only among themselves!"

It is probable that physiological and growth reactions to cultural conditions are correlated with geographical distribution and thus with distribution of chromatin.

The genus Lilium is at present divided into two subgenera. Cardocrinum includes the species with netted veined, heart-shaped leaves with long petioles. The flowers are long, narrow, funnel-shaped, and horizontal in position. The only three species included are Asiatic; namely, L. cathayanum, L. cordatum, and L. giganteum. The subgenus, Eulirion, contains all the other lilies and is divided into four sections on the basis of shape and position of the flower. Leucolirion has funnel-shaped flowers usually horizontal in position and representatives of this section are found in North America, Europe, and Eastern Asia. Archelirion has horizontal bowl-shaped flowers and the one repsentative of this section, L. auratum, is Asiatic. Isolirion has erect, bowl-shaped flowers and representatives are found in all three geographical areas of distribution. Martagon has nodding flowers with strongly recurved perianth segments. Representatives of this section are found in all the areas of distribution.

Endlicher (1836) proposed the present classification and it has been accepted with small modifications by Baker (1874), Wilson (1925), and

others. However, evidence of new intermediate species and breeding behavior has indicated need for revision of the genus into more natural groups. Elwes (1880) was probably the first to suggest this. More recently, Woodcock and Coutts (1935) have stated on page 71: "It is obvious that too much attention has been paid to a single feature - the curving and poise of the perianth-segments (i.e., the sepals and petals). Probably this rather artificial and by no means satisfactory system will be considerably revised in the future, and we may expect to see the elaboration of a new and more natural classification based on a greater range of characters, including the form of the bulb and its mode of development and increase; the American species now referred to Martagon and Leucolirion will be recognized as having no close affinity with the Old World representatives of these groups."

Plate 1 consists of photoidiograms of one species from each of the sections in Eulirion and one species from Cardocrinum. It is evident that all five species have very similar idiograms. The distribution of chromatin in all is characteristic of Group 1. Shape and position of flower, the basis for the present classification, is negatively correlated with chromosome morphology, interspecific sterilities, geographical distribution, bulb structure and growth, and physiological and growth characteristics expressed in reaction to cultural conditions. The latter are positively correlated and are suggested as a basis for revision of the genus.

There is no direct evidence to be obtained from a study of somatic chromosome morphology as to the method of origin of the variation between karyotypes. Indirect evidence was secured from a consideration of the lower levels of the variation evident within a species type and between

a species and its varieties. There was variation of two kinds. First, there was the presence of extra chromosomes in L. auratum (fig. 10), L. tsingtauense (fig. 15), L. Henryi (fig. 20), L. Sargentiae Horsford (fig. 27). and L. pumilum Golden Gleam (fig. 35). These centric fragments and chromosomes appear very much like those reported by Stewart (1943). Examination of meiosis at that time revealed evidence of non-homology with any and all of the normal complement and no indication of their origin. The second type of variation is in the number, position, and activity of secondary constrictions with no change in chromatin distribution. This was found in L. concolor (figs. 1 and 2), L. speciosum varieties (figs. 7 and 8), L. auratum (figs. 10, 11 and 12), L. dauricum and L. dauricum var. Wilsoni (fig. 29), and L. superbum (figs. 36 and 37).

Two cases seem particularly significant. Examination of the karyotypes of two groups of individuals in L. superbum, represented by the idiograms in figures 36 and 37 and of the idiograms of the West Coast species in figures 43 - 48, reveals the fact that, if the change were from the idiogram presented in figure 37 to the idiogram in figure 36, it would be toward the karyotypes found in the West Coast species. This change could have been accomplished by a change in the nucleolar organization of the karyotype only, which has been shown to be the most variable feature of the karyotype in Lilium.

Examination of the metaphase and anaphase figures in L. dauricum and L. dauricum var. Wilsoni (fig. 29) showed identical chromatin distribution as well as the same number and distribution of secondary constrictions. The two karyotypes were found to differ only in the nucleolar activity of the secondary constrictions in the long arms of the C and F pairs. In the type, one chromosome of each pair was always associated

with a nucleolus at prophase or always showed the secondary constriction at metaphase. The other members of the pairs rarely showed the constriction at metaphase and were not seen associated with nucleoli in approximately thirty prophases examined. In the variety, the constrictions showed very rarely at metaphase and were not observed associated with the nucleoli at prophase. It is probable that examination of large numbers of prophases would have revealed the rare associations indicated by the equally rare appearance of the constrictions at metaphase.

A possible case of difference within a species in a measurable amount of chromatin is indicated by the examination of the F pair of chromosomes of L. pardalinum (fig. 45) and of the F and F' pair of L. pardalinum var. giganteum (fig. 43). The origin of L. pardalinum var. giganteum is doubtful and many consider it a hybrid of the L. pardalinum type with some other Pacific Coast species. None of the Pacific Coast species reported here has a chromosome or chromosome pair corresponding to the F' chromosome of L. pardalinum var. giganteum, and no critical evidence has appeared.

Emsweller and Stewart (1944) found three types of variation within species. In L. candidum the plants were either heterozygous or homozygous for the secondary constriction in their C chromosome pair. In one clone, the distribution of chromatin was markedly altered by the translocation of a large part of the long arm of a K chromosome to an A chromosome. Their type III, similar to the three plants here reported, and type IV differed from the rest in that one of the I pair of chromosomes had measurably less chromatin in its short arm giving one I chromosome and three J chromosomes in contrast to two of each for types I and II. Five

L. chalcedonicum plants showed high frequencies of several different sets of hetermorphic pairs but a comparison of length showed the difference to be only in the presence or absence of secondary constrictions. Haga (1943) reports a plant of L. Hansonii heterozygous for a reciprocal translocation. His figure 10, pp. 22 indicates the plant is also heterozygous for two inversions, one in a chromosome with a submedian centromere and one in a chromosome with a subterminal centromere.

The chromosomes of Lilium species hybrids are known to maintain the arm length ratios and constrictions of the chromosomes of the parent species (Haney 1943; Emsweller and Stewart 1944). This knowledge of the karyotypes of the parent species would allow selection of sexual hybrids from the predominantly apomictic progenies usually resulting from interspecific crosses in Lilium. This should be especially valuable in plant groups which require several years to flowering and selection on the usual morphological basis.

The limitations of somatic chromosome morphology as a tool in tracing phylogenetic relationships has been emphasized by many geneticists who point out that "the similarity or dissimilarity of the chromosomes as seen at the metaphase plate stage is not at all necessarily proportional to the similarity of their gene arrangements" (Dobzhansky, pp. 133, 1941). Several types of structural changes, such as inversions and reciprocal translocations where exchange is equal, may occur within chromosomes and not affect their external morphology. These changes can be detected only at meiosis and a cytological study of hybrids is indicated as necessary to determine species relationships and differentiation. Very few gene mutations have been shown to affect chromosome morphology. However, the

accumulation of mutations has been accepted as a more important factor in speciation than structural rearrangement of chromosomes. Thus, genetic studies are the most critical means of evaluating relationships.

The data on chromosome morphology of Lilium has shown that it is not critical evidence at a species level. In three instances, groups of plants recognized as containing several distinct species have proved to possess identical karyotypes. On the other hand, closely related species and even different individuals of a single species have shown variation in karyotype. It is at this level that cytology and genetics of hybrids will give the best evidence of phylogeny. The similar karyotypes will probably reveal hidden inversions, translocations, and gene differences. The variable karyotypes of single species or closely related species will probably be shown to differ by relatively simple chromosome rearrangements and small changes in genomes. It is at the higher levels of differentiation that chromosome morphology must replace those tools. When the sterility barriers between groups become complete chromosome morphology and numbers are the most critical evidence that can be obtained. At these higher levels chromosome morphology has indicated natural relationships in Lilium.

SUMMARY

The karyotypes of forty-eight species and varieties of Lilium have been determined. The idiograms presented represent the haploid complement of a somatic metaphase arranged with centromeres along a horizontal line and with the chromosomes in order of decreasing length of short arms. The activity of all constrictions was determined. The nucleolar activity of secondary constrictions classed as nucleolar was found to be variable and in one case this variation was the only difference between the karyotypes

of a species and its variety. Failure of normally nucleolar secondary constrictions to form nucleoli was found to be a cause, along with fusion, of the frequent reduction from the maximum in the number of nucleoli in resting cells. The maximum number of nucleoli in Lilium species was found to vary from four to fourteen. The maximum number of nucleoli in resting cells was determined for all the species of Lilium reported. It was in all cases equal to the maximum number of chromosomes observed associated with nucleoli at secondary constrictions in prophases. In all but one species of over forty examined, there were additional secondary constrictions which were non-nucleolar. Variation in position of secondary constrictions was found to be correlated with differences in geographical distribution.

The variation of distribution of chromatin within species was the basis for arranging them into related groups. These groups are considered natural because the same groups are reached on the basis of geographical distribution, interspecific fertility and sterility, bulb structure and physiological and growth responses to cultural conditions. The present classification provides entirely different groupings and revision is suggested. While chromosome morphology has indicated natural groups within the genus Lilium, its usefulness in differentiation of species is limited by the independent occurrence of karyotype variation and gene mutation. The accumulation of gene differences is recognized as the most important factor in speciation.

LITERATURE CITED

1. Babcock, E. B. 1944. New light on evolution from research on the genus Crepis. Am. Nat. 78:385-409.

2. _____ and J. A. Jenkins. 1943. Chromosomes and phylogeny in Crepis. III. The relationships of one hundred and thirteen species. Univ. Calif. Publ. in Bot. 18:241-291.

3. _____, G. L. Stebbins, Jr., and J. A. Jenkins. 1942. Genetic evolutionary processes in Crepis. Am. Nat. 76:337-363.

4. Baker, J. G. 1874. Revision of the genera and species of Tulipeae. Jour. of the Linnean Soc. (Botany) 14:211-310.

5. Beal, J. M. 1942. Chromosome fragments in Lilium willmottiae and hybrids between it and L. davidii. Bot. Gaz. 103:617-619.

6. Cooper, D. C. 1935. Macrosporogensis and development of the embryo sac of Lilium Henryi. Bot. Gaz. 97:346-355.

7. _____. 1936. Development of gametes of Lilium. Bot. Gaz. 98:169-177.

8. Dermen, H. 1933. Origin and behavior of the nucleolus in plants. Jour. Arn. Arb. 14:282-323.

9. Dobzhansky, T. 1941. Genetics and the origin of species. Columbia Univ. Press, New York.

10. Elwes, J. H. 1880. A monograph of the genus Lilium. Taylor and Francis, London.

11. Emsweller, S. L., P. Brierley, D. V. Lumsden, and F. L. Mulford. 1937. Improvement of flowers by breeding. U.S.D.A. Yearbook Separate No. 1591. pp. 890-998.

12. _____, and Maydelle B. Stewart. 1944. The origin of Lilium testaceum. Jour. of Heridity. 35:301-308.

13. Endlichers, S. 1836. Genera plantarum. Vindobonae, Vienna.

14. Fernandes, A. 1936. Les satellites chez les Narcissis. II. Les satellites pendant la mitose. Bot. Soc. Broteriana 11:87-146.

15. Gates, R. R. 1942. Nucleoli and related nucleolar structures. Bot. Rev. 8:337-409.

16. Goodspeed, T. H. 1945. Cytotaxonomy of Nicotiana. Bot. Rev. 11: 555:592.

17. Haga, T. 1943. A reciprocal translocation in Lilium Hansonii Leicht. Cytologia 13:19-25.

18. Haney, W. J. 1943. The nucleolar numbers and attachments in Lilium. Ph. D. Thesis, Univ. of Maryland.

19. Heitz, E. 1931. Die ursache der gesetzmassigen zahl, lage, form und grosse pflanzlicher nucleolen. Planta 12:774-844.

20. Jacob, K. T. 1940. Chromosome numbers and the relationship between satellites and nucleoli in Cassia and certain other Leguminosae. Ann. Bot. n.s. 4:201-226.

21. Mather, K. 1934. The chromosomes of Lilium. II. R.H.S. Lily Yearbook 3:38-40.

22. _____ 1938. Meiosis in Lilium. Cytologia 6:354-380.

23. Matsuura, H. 1938. Chromosome studies in Trillium kamtschaticum Pall. VI. On the nucleolus chromosome relationship. Cytologia 9:55-77.

24. Preston, I. 1933. Hybridization of lilies. R.H.S. Lily Yearbook 3:173-195.

25. Resende, F. 1937. Uber die ubiquitat der SAT-chromosomen bei den Blutenpflanzen. Planta 26:757-607.

26. Richardson, Margaret. 1936. Hybridity in Lilium martagon album X L. Hansonii. Jour. Gen. 32:411-451.

27. Sansome, E. R., and L. LaCour. 1934. The chromosomes of Lilium. III. R.H.S. Lily Yearbook 4:40-45.

28. Sato, D. 1932. Chromosome studies in Lilium. Bot. Mag. (Tokyo) 46:68-88.

29. _____. 1938. Karyotype alteration and phylogeny. IV. Karyotypes in Amaryllidaceae with special reference to the SAT-chromosome. Cytologia 9:203-242.

30. Simmonds, A. 1939. List of names of hybrid lilies. R.H.S. Lily Yearbook 9:40-51.

31. Slate, G. L. 1939. Lilies for American Gardens. Scribners, New York.

32. Stewart, R. N. 1943. The occurrence of aneuploids in Lilium. Bot. Gaz. 104:620-626.

33. _____, and R. Bamford. 1942. The chromosomes and nucleoli of Medeola virginiana Amer. Jour. Bot. 29:301-303.

34. _____, _____. 1945. The nature of polyploidy in Lilium tigrinum. Amer. Jour. Bot. 30:1-7.

35. Stout, A. B. 1928. The bulbs and bulb habits of lilies. Jour. N. Y. Bot. Garden 29:25-42.

36. _____. 1933. Sterilities in lilies. R.H.S. Lily Yearbook. 3:142-167.

37. Takenaka, Y., and T. Nagamatsu. 1930. On the chromosomes of Lilium tigrinum Ker-Gawl. Bot. Mag. (Tokyo) 44:386-391.

38. Wilson, E. H. 1925. The lilies of Eastern Asia. Stratford, Boston.

39. Woodcock, H. D., and J. Coutts. 1935. Lilies. Country Life, London.

40. Woods, M. W. 1937. The nucleolus in Tulipa. Amer. Jour. Bot. 24: 528-536.

41. Van Camp, G. M. 1924. Le role du nucleole dans la caryocinese somatique. La. Cellule 34:1-50.

LIST OF SPECIES NAMES

L. concolor Salisbury

L. Brownii F. E. Brown

L. candidum Linnaeus

L. callosum Siebold and Zuccarini

L. Davidii Duchartre

L. speciosum var. album Masters

L. speciosum var. rubrum Masters

L. speciosum var. magnificum Masters

L. speciosum var. punctatum Courtois

L. monadelphum Bieberstein

L. auratum Lindley

L. giganteum Wallich

L. tsingtauense Gilg

L. Gravi S. Watson

L. japonicum Thunberg

L. Leichtlinii var. Maximowiczii Baker

L. Henryi Baker

L. martagon Linnaeus

L. longiflorum Thunberg, Horticultural forms Creole, Estate and Slocum's Ace.

L. formosanum Wallace

L. regale Wilson

L. myriophyllum Franchet

L. Sargentiae Wilson

L. Sargentiae Wilson, Hort. form Horsford

L. leucanthum var. chloraster Wilson

L. dauricum Ker-Gawler

L. dauricum subsp. Thunbergianum f. Alice Wilson Wilson

L. Duchartrei Franchet

L. Wardii Stapf

L. amabile Palibin

L. pumilum De Candole

L. pumilum De Candole Hort. var. Golden Gleam

L. superbum Linnaeus

L. philadelphicum Linnaeus

L. Catesbaei Walter

L. carolinianum Michaux

L. michiganense Farwell

L. canadense Linnaeus

L. canadense var. flavum Pursh

L. canadense var. rubrum Britton

L. pardalinum var. giganteum Kellog

L. Roezlii Regel

L. pardalinum Kellog

L. Parryi S. Watson

L. occidentale Purdy

L. columbianum Hanson

PLATE I

Photomicrographs of representatives of subgenera and sections of genus Lilium.

Subgenus Eulirion, Section Isolirion. . . L. concolor

Subgenus Eulirion, Section Leucolirion. . L. Brownii

Subgenus Cardiocrinum. . . . . .L. giganteum

Subgenus Eulirion, Section Martagon. . . .L. monadelphum

Subgenus Eulirion, Section Archelirion . .L. auratum

All X1700

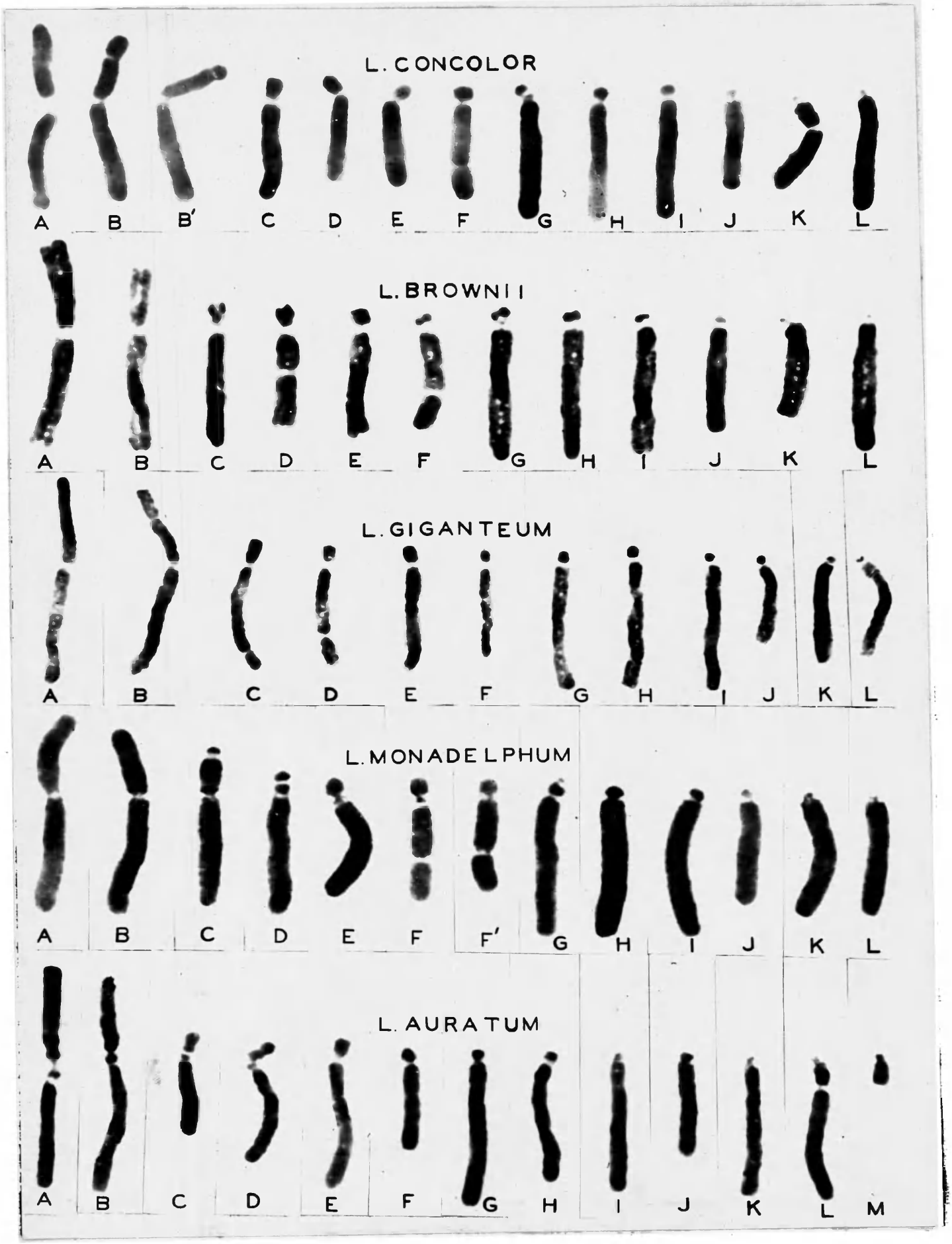

L. CONCOLOR
A B B' C D E F G H I J K L
L. BROWNII
A B C D E F G H I J K L
L. GIGANTEUM
A B C D E F G H I J K L
L. MONADELPHUM
A B C D E F F' G H I J K L
L. AURATUM
A B C D E F G H I J K L M

PLATE I

PLATE 2

Photomicrograph of somatic metaphase plate from root tip of *L. concolor* (type 2) pre-treated with colchicine. Photographs of the chromosomes from this cell were used in the photoidiogram in Plate 1. X2300.

PLATE 2

PLATE 3

Photomicrograph of somatic metaphase plate from root tip of L. Brownii pre-treated with colchicine. Photographs of the chromosomes from this cell were used in the photoidiogram in Plate 1. X2000.

PLATE 3

PLATE 4

Camera lucida drawing of prophase cell from root tip of *L. gallosum* showing attachment of the chromosomes to nucleoli at secondary constrictions. Both chromosomes of pairs A, G, and K and one chromosome of pair F are shown attached to nucleoli. X2500.

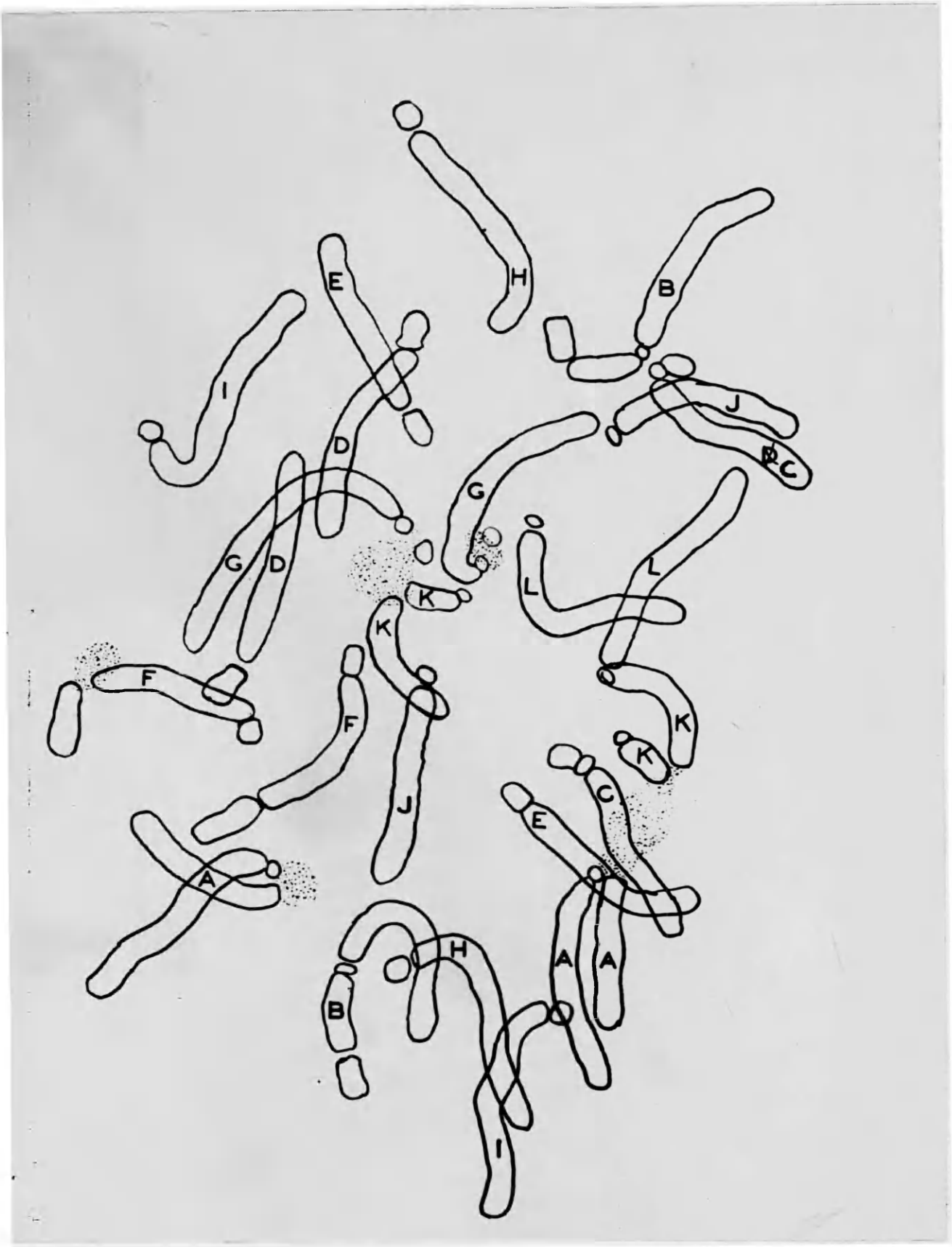


PLATE 4

PLATE 5

Photomicrograph of prophase cell of *L. callosum* **drawn in Plate 4. X2500.**

PLATE 5

PLATE 6

Photomicrograph of prophase cell from root tip of *L. nigrumbrum* showing nucleolar attachment of both chromosomes of the C and D pairs and one chromosome of the B pair. X1500

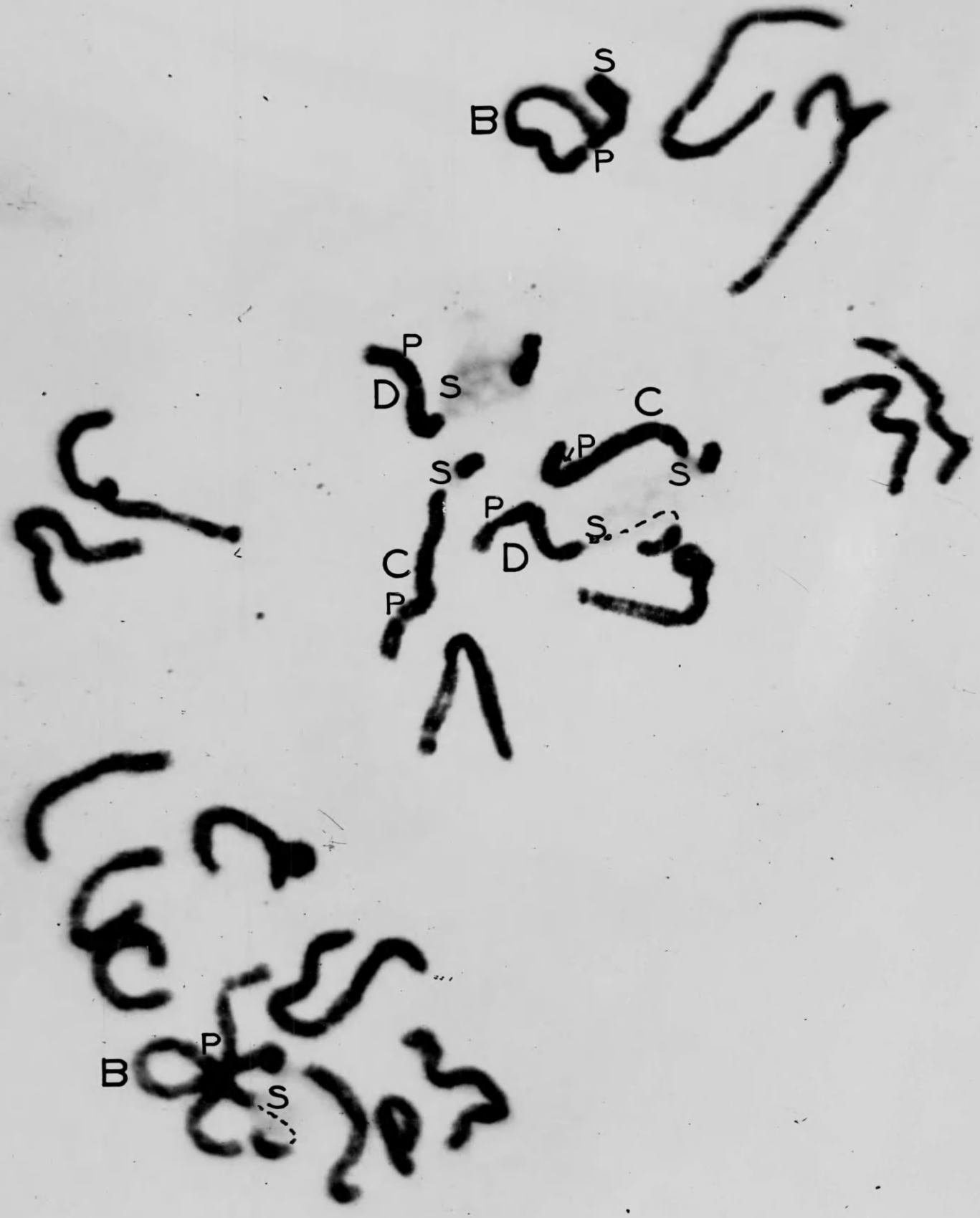
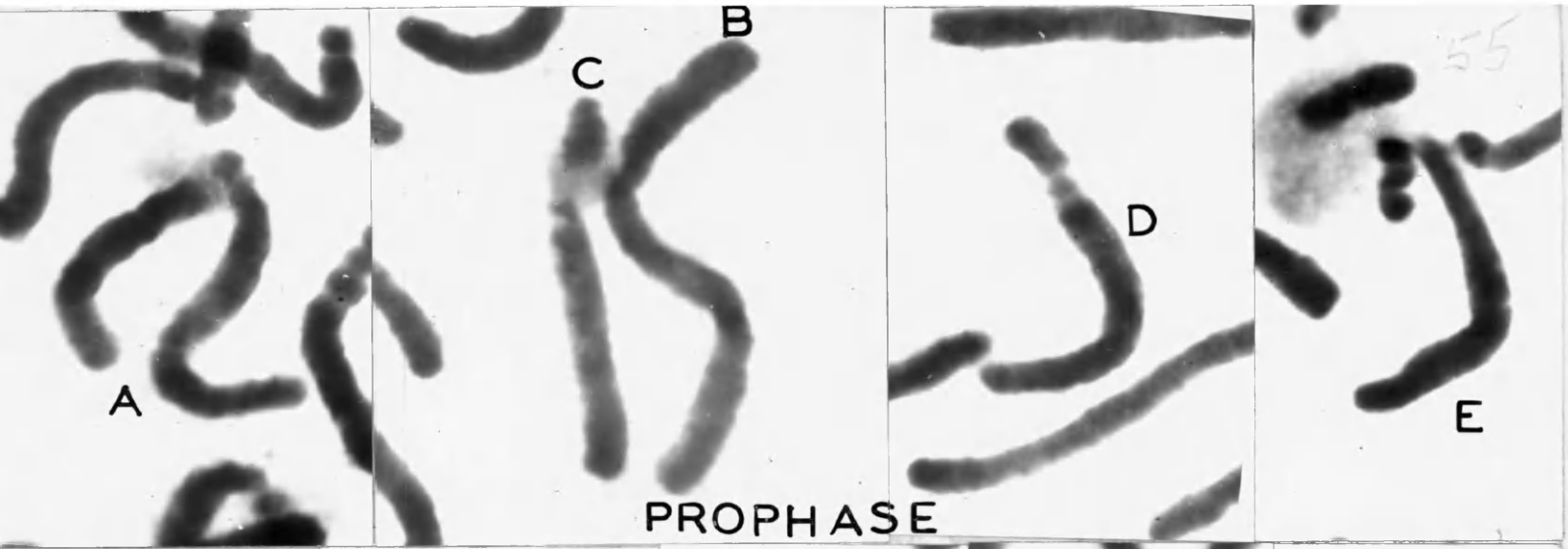


PLATE 6

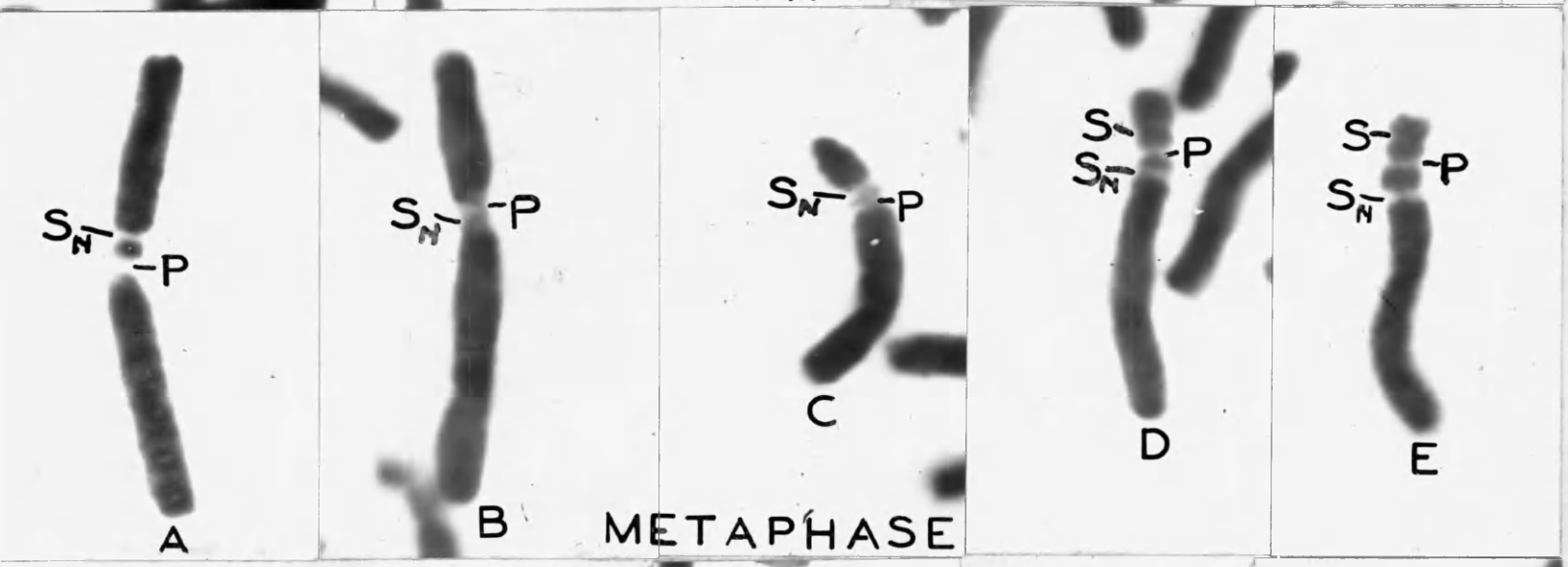
PLATE 7

Photomicrographs and camera lucida drawings of the nucleolar chromosomes of L. Sargentiae f. Horsford. The constrictions of the five chromosomes pictured at metaphase are classified utilizing information from the other mitotic phases shown. P = primary constriction or centromere. S_N = secondary constriction, nucleolus forming. S = secondary constriction, non-nucleolus forming. Prophase shows nucleoli at the nucleolar secondary constrictions except in the D pair, neither of which were attached to nucleoli in this particular cell.

The non-nucleolar constrictions are never found attached to nucleoli. The late metaphase chromosomes show the beginning of separation at the primary constrictions while secondary constrictions still lie together. Anaphase chromosomes show the positions of the primary constrictions oriented toward the poles and secondary constrictions usually attenuated if they cut off a large mass of the chromosome. X2600.



PROPHASE



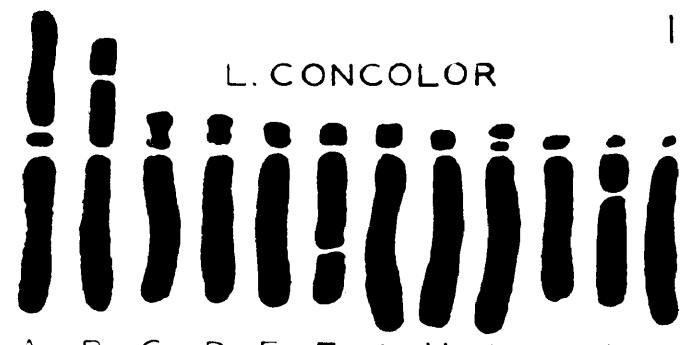
METAPHASE

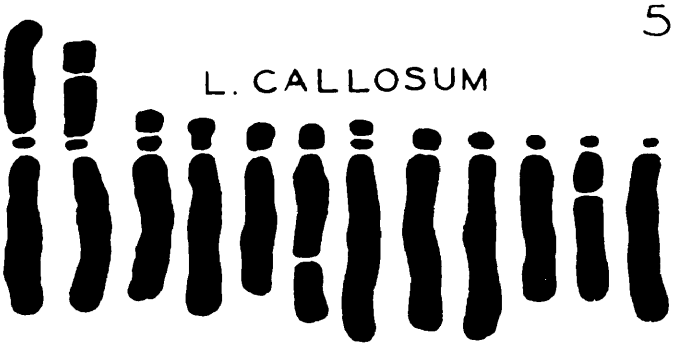
LATE METAPHASE

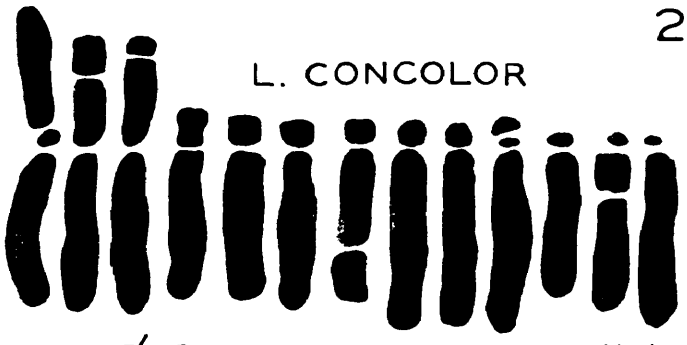
ANAPHASE

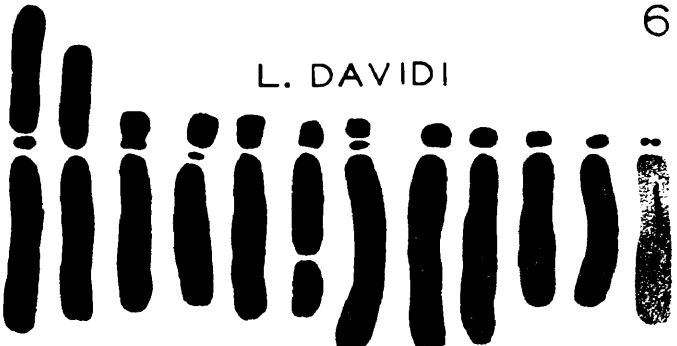
PLATES 8, 9, 10, 11, 12, 13.

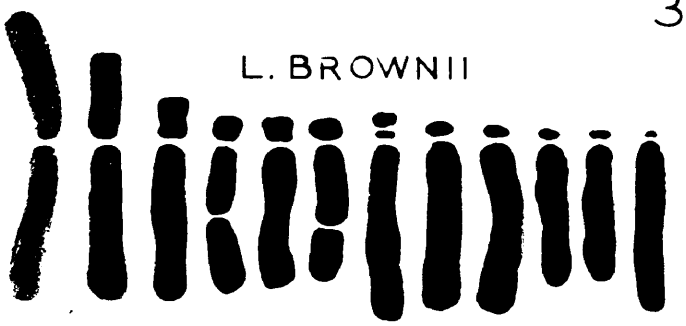
Idiograms of species and varieties of Lilium. Variations within species are shown separately. X2000.

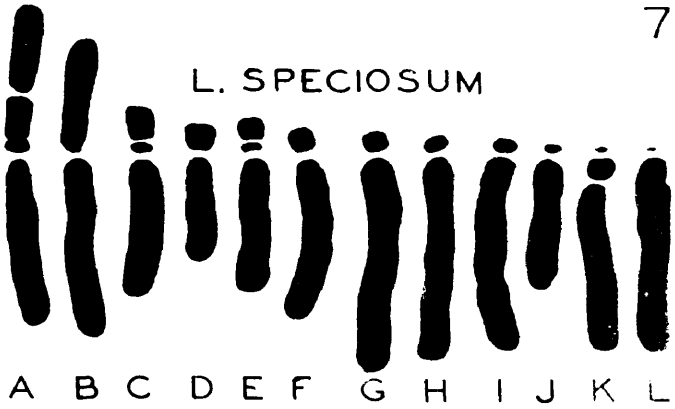

1
L. CONCOLOR
A B C D E F G H I J K L

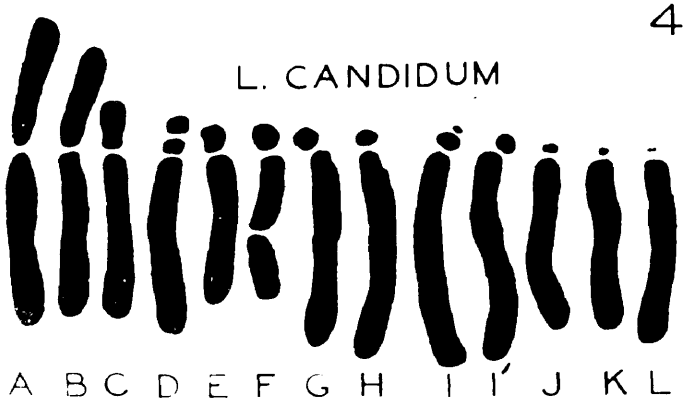

5
L. CALLOSUM
A B C D E F G H I J K L


2
L. CONCOLOR
A B B′ C D E F G H I J K L


6
L. DAVIDI
A B C D E F G H I J K L


3
L. BROWNII
A B C D E F G H I J K L


7
L. SPECIOSUM
A B C D E F G H I J K L


4
L. CANDIDUM
A B C D E F G H I I′ J K L

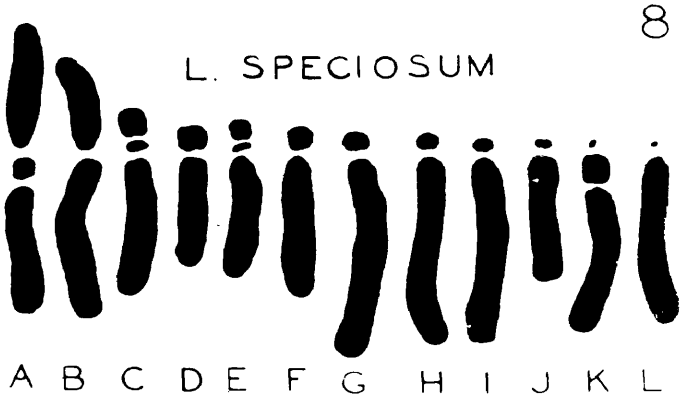

8
L. SPECIOSUM
A B C D E F G H I J K L

PLATE 8

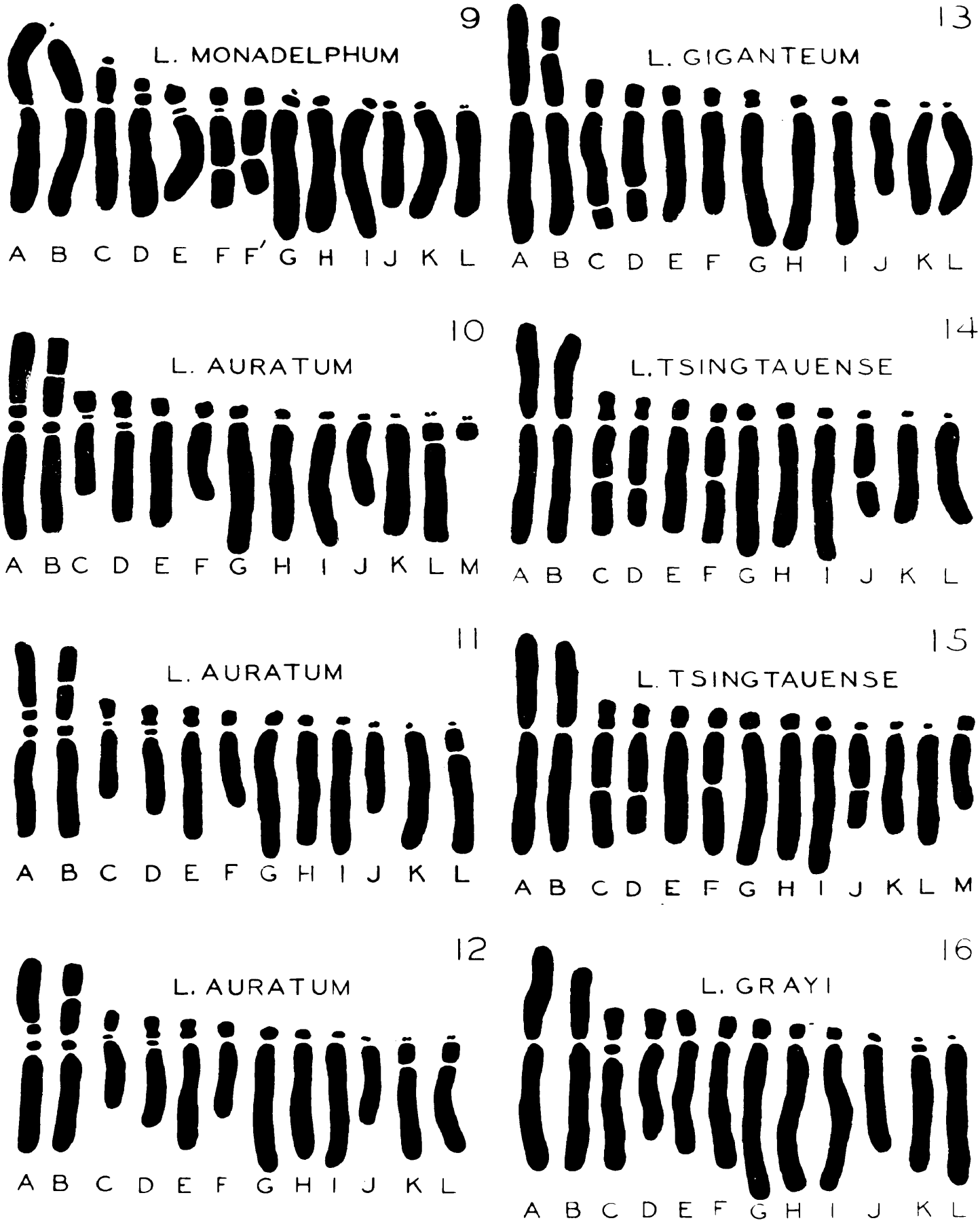
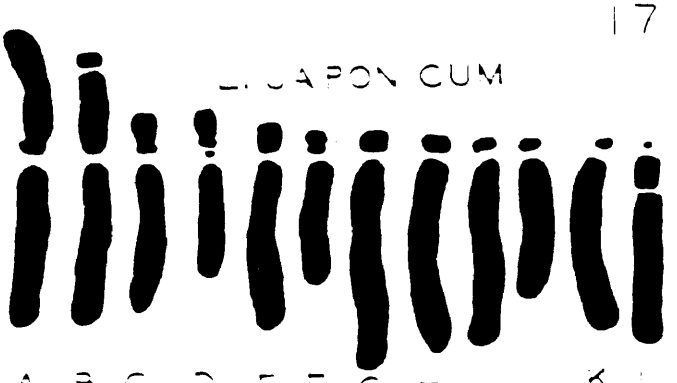

9
L. MONADELPHUM
A B C D E F F' G H I J K L
13
L. GIGANTEUM
A B C D E F G H I J K L
10
L. AURATUM
A B C D E F G H I J K L M
14
L. TSINGTAUENSE
A B C D E F G H I J K L
11
L. AURATUM
A B C D E F G H I J K L
15
L. TSINGTAUENSE
A B C D E F G H I J K L M
12
L. AURATUM
A B C D E F G H I J K L
16
L. GRAYI
A B C D E F G H I J K L

PLATE 9

17

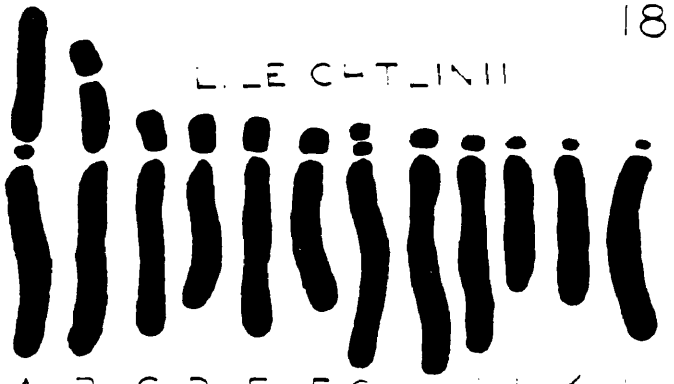
L. JAPONICUM



A B C D E F G H I J K L

18

L. LEICHTLINII



A B C D E F G H I J K L

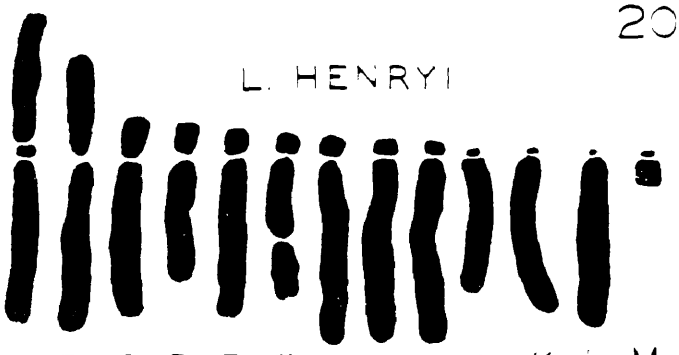
19

L. HENRYI

A B C D E F G H I J K L

20

L. HENRYI



A B C D E F G H I J K L M

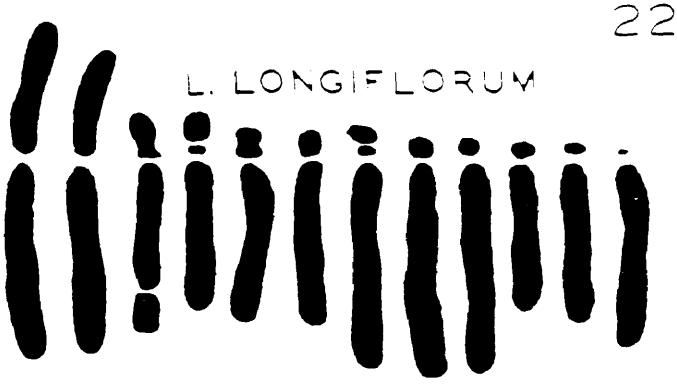
21

L. MARTAGON

A B C D E F G H I J K L

22

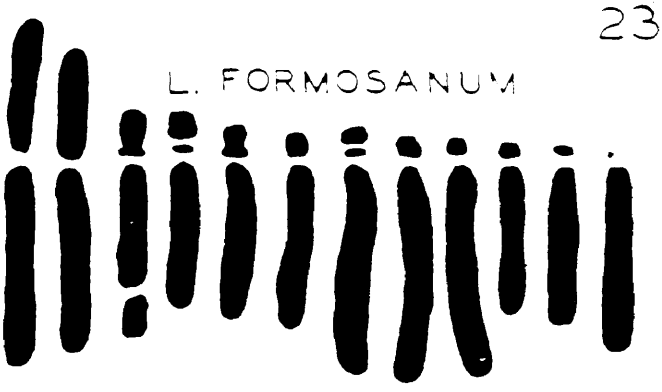
L. LONGIFLORUM



A B C D E F G H I J K L

23

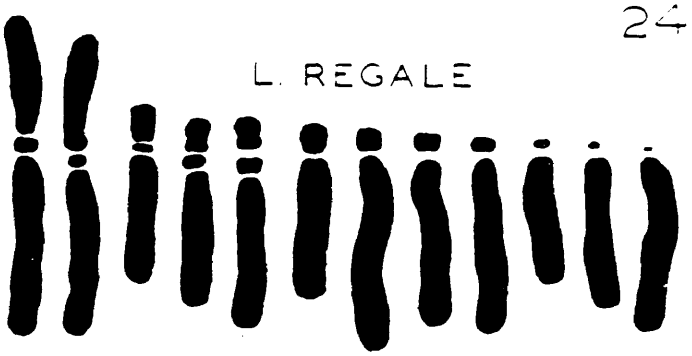
L. FORMOSANUM



A B C D E F G H I J K L

24

L. REGALE



A B C D E F G H I J K L

PLATE 10

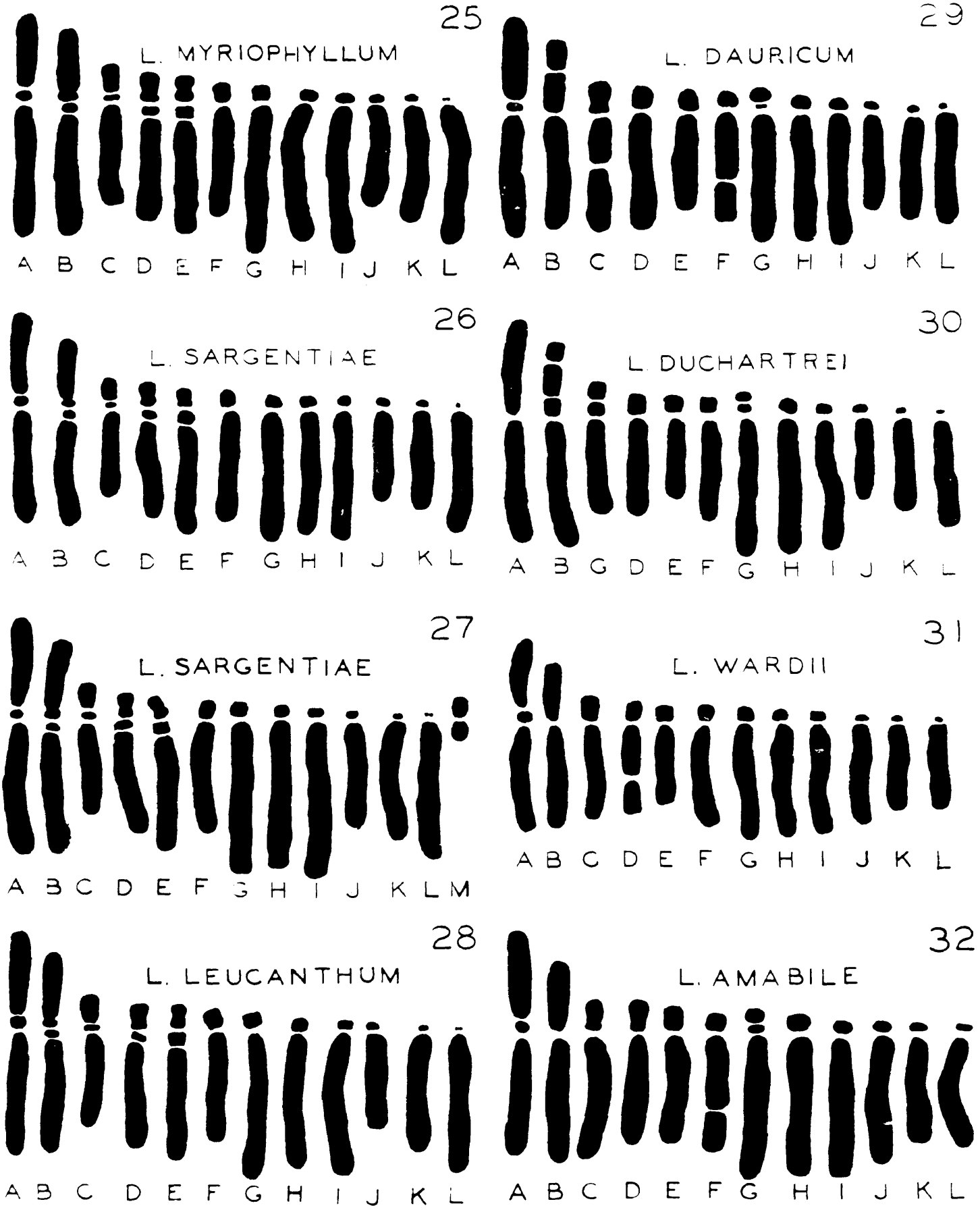
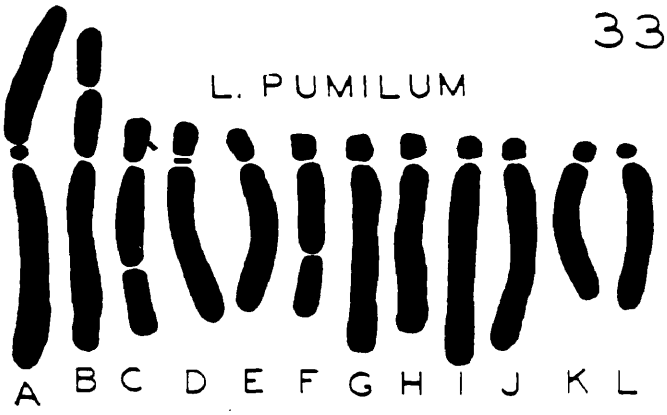

25
L. MYRIOPHYLLUM
A B C D E F G H I J K L
29
L. DAURICUM
A B C D E F G H I J K L
26
L. SARGENTIAE
A B C D E F G H I J K L
30
L. DUCHARTREI
A B C D E F G H I J K L
27
L. SARGENTIAE
A B C D E F G H I J K L M
31
L. WARDII
A B C D E F G H I J K L
28
L. LEUCANTHUM
A B C D E F G H I J K L
32
L. AMABILE
A B C D E F G H I J K L

PLATE II

33

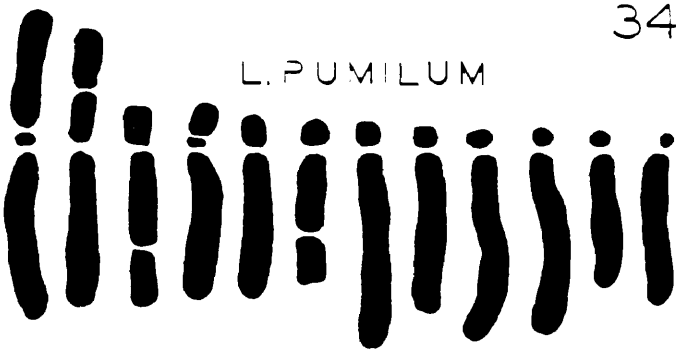
L. PUMILUM



A B C D E F G H I J K L

34

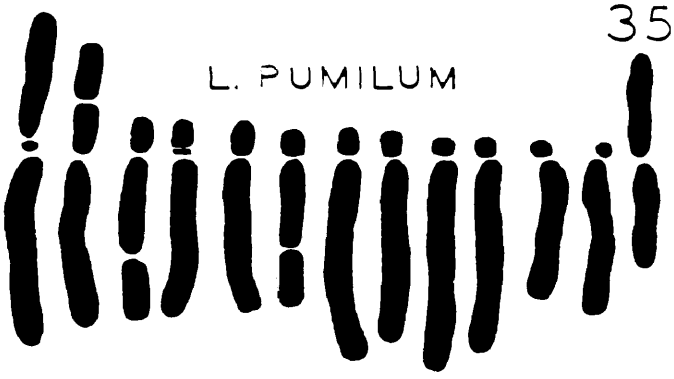
L. PUMILUM



A B C D E F G H I J K L

35

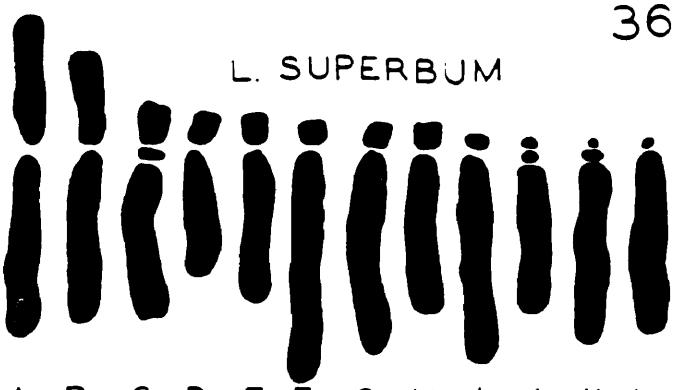
L. PUMILUM



A B C D E F G H I J K L M

36

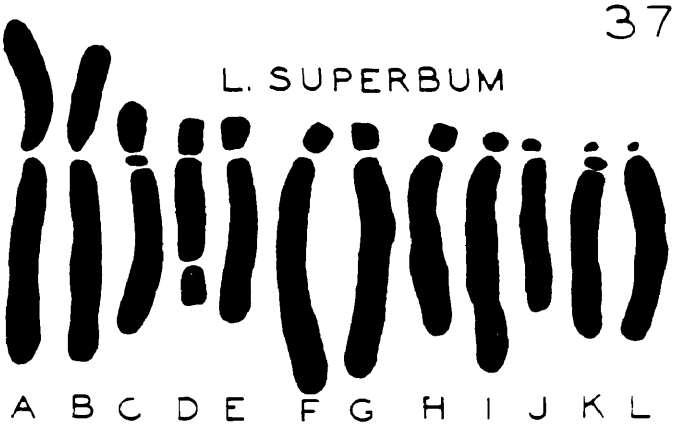
L. SUPERBUM



A B C D E F G H I J K L

37

L. SUPERBUM



A B C D E F G H I J K L

38

L. PHILADELPHICUM

A B C D E F G H I J K L

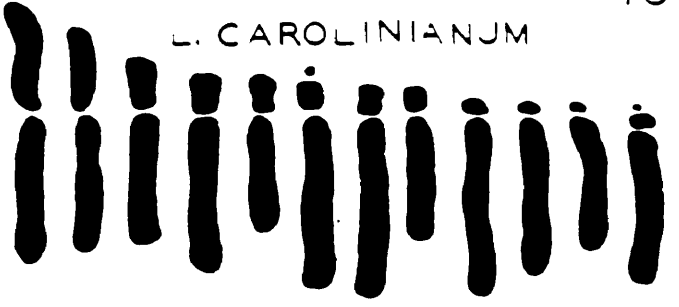
39

L. CATESBAEI.

A B C D E F G H I J K L

40

L. CAROLINIANUM



A B C D E F G H I J K L

PLATE 12

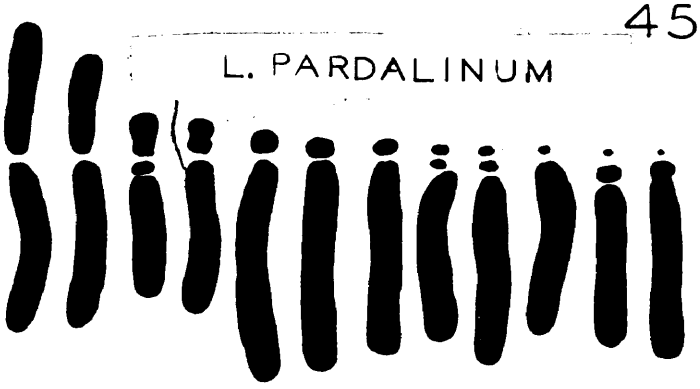
41

L. MICHIGANENSE

A B C D E F G H I J K L

45

L. PARDALINUM



A B C D E F G H I J K L

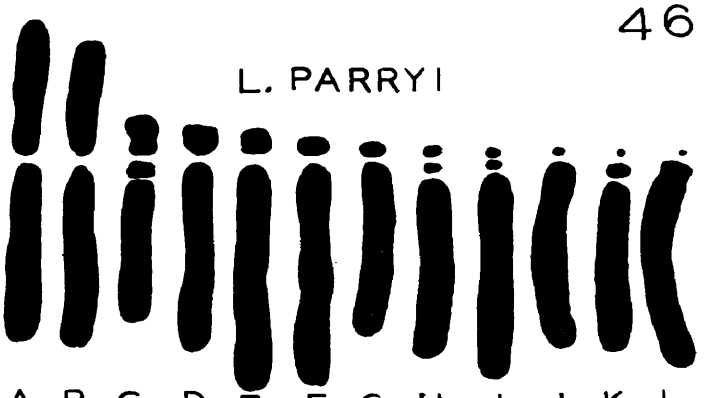
42

L. CANADENSE

A B C D E F G H I J K L

46

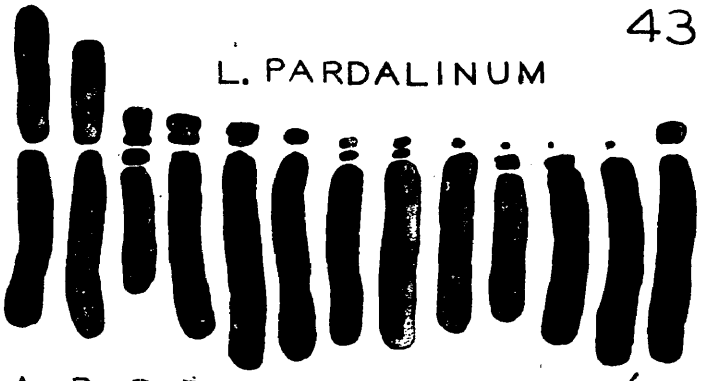
L. PARRYI



A B C D E F G H I J K L

43

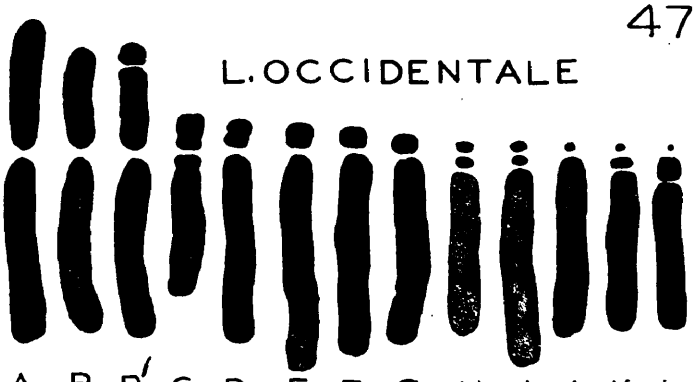
L. PARDALINUM



A B C D E G H I J K L F′ F

47

L. OCCIDENTALE



A B B′ C D E F G H I J K L

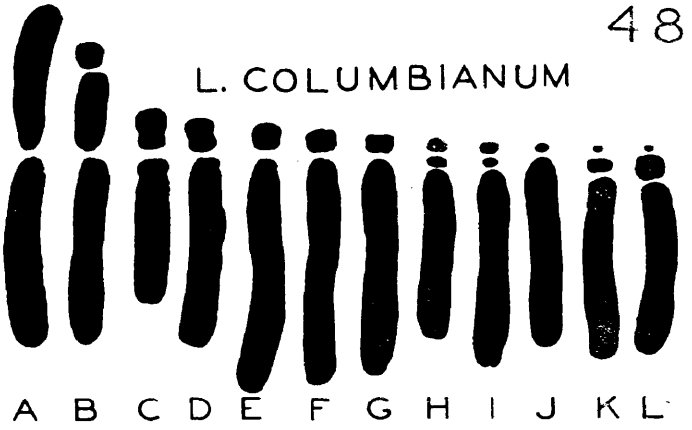
44

L. ROEZLII

A B C D E F G H I J K L

48

L. COLUMBIANUM



A B C D E F G H I J K L

PLATE 13