THE NUCLEOLUS IN TULIPA

BY

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Introduction

Nucleoli apparently occur in the nuclei of practically all living organisms (Sharp (21), Wilson (31)). The possible exceptions are the Myxophyceae and Schizomycetes. Even these may at least possess true nuclei (Smith (24), Tanner (26)) although it is still a question. The universal occurrence of nucleoli has resulted in an extensive examination of this structure in many diverse types of living organisms. In spite of many views that have resulted, the true function and significance of the nucleolus in the economy of the cell is today a matter of opinion. Much has been learned, however, as to the morphological characteristics of this body and its relationships with other nuclear constituents, particularly the chromosomes.

No attempt will be made in this paper to review all the literature on the nucleolus. Montgomery (14) thoroughly reviewed it up to 1897, Wager (30) reviewed a number of the important papers from 1897, and earlier, to 1904. Recent summaries have been presented by Yamaha and Sinoto (32), and Guilliermond and Mangenot (4). Sharp (21) has summarized many of the views regarding the nucleolus and particularly the relation of the chromosomes to this structure.

The significance of the satellited chromosomes in this connection has been a matter of debate since the work of S. Navaschin (17) who first discovered their existence and relation to the nucleolus in <u>Galtonia Candicans</u>. DeMol (13), Heitz (5,6) McClintock (11,12), and many others, have presented evidence that certain chromosomes have a specific nucleolus-forming function. The last two have shown quite convincingly that it is the chromosomes bearing satellites or certain other similar differentiations that possess this peculiar capacity. Dermen (1), however, seems to be somewhat skeptical of the constancy and importance of the relationship between specific chromosomes and the nucleolus. In evaluating the work in this field it is important to note, as Zirkle (33) has pointed out from the literature and, clearly demonstrated by his own work, that differences in micro-technical methods can lead to radically different fixation images for the nucleolus. Many differences of interpretation have undoubtedly arisen in this way.

The study of nucleoli in <u>Tulipa</u> was undertaken in connection with a morphological study of the chromosomes in this genus. Because of the variable nucleolar numbers, size of the chromosomes, and the occurence of auto-polyploids, unusually interesting material for an investigation of this kind was available. Nucleolar number, size and, the relation of certain chromosomes to the nucleolus have been considered at different stages of the life cycle.

Materials and Methods

Bulbs were obtained from growers in Europe or in this country. All of the material used had been flowered so its identity was checked. Root-tips were collected in the fall from bulbs grown in the experimental garden. Dual fixations with Flemming's medium solution or Allen and Wilson's Modification of Bouin's fluid were usually made. The ordinary paraffin technic was followed, and iron-alum haematoxylin was used exclusively as a stain for the root-tips. Pre-meiotic and meiotic stages were collected from bulbs kept in a cool storage cellar. The anthers were prepared by the paraffin method or smeared according to the general procedure of Taylor (28) except that ordinary Flemming's medium, and Navaschins fluids were used as fixatives. The crystal violet-iodine-picric acid schedule of Smith (22), or iron-alum haematoxylin were used to stain the smears. Paraffin sections were generally stained in the latter although Flemming's triple stain was occassionaly used. Meiotic material was necessarily limited because of the small amount (one flower bud) obtainable from each bulb.

Observations were made with a 2 mm. 90X apochromat oil immersion objective of n.a. 1.30. This was used in conjunction with a 15X compensating ocular. All drawings and

measurements were made with the aid of a camera lucida.
Volumetric determinations were recorded in arbitrary scale
units (one unit = ap.o.1587 microns). Measurements of
nuclei and cells have been recorded in cross-sectional
areas in square inches X 2000. A standard planimeter was
generally employed in determining these areas, although
they were sometimes based on diameter measurements. Photo-
micrographs were made with the combination described above.
Approximate actual magnifications are given for each figure.

Results

Nucleoli in root-tip mitosis

Certain events in the anaphase and, even meta-
phase, probably influence the development number of nucleoli
in daughter nuclei. The nucleolar matter or plastin
(Zirkle (13)), however, appears first in the telophase, and
usually disappears in the metaphase.

Anaphase. During late anaphase the chromosomes
become inter-and intra-connected by more or less chromatic
strands (figs. 1, A and 2, D). These strands, which appear to
arise from the matrix, may extend between the proximal and
distal arms of the same chromosome as well as between differ-
ent chromosomes.

Telophase. As the telophase proceeds the chromo-
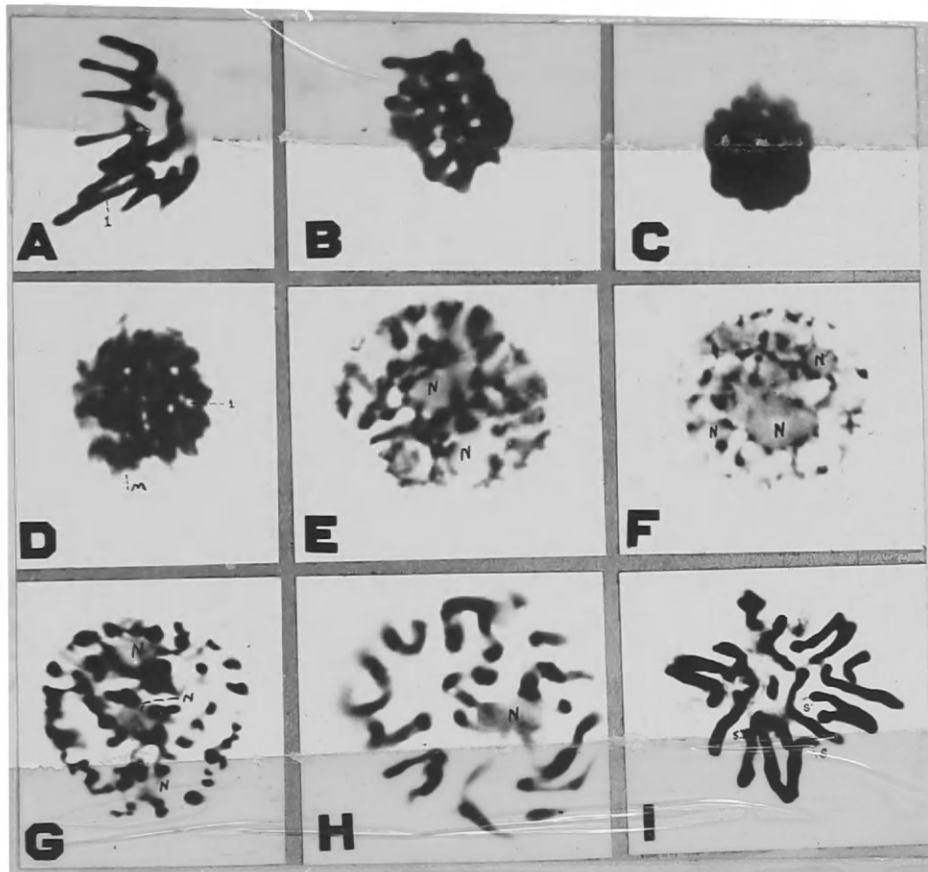
Figure 1. — Development of nucleoli in T. sprengeri during
mitosis in root-tip: A, Anaphase; B,C,D,E, telophase;
F, interphase; G,H, prophase; I, metaphase. In all figs.
N denotes plastin and S denotes satellites. X ap. 1800.
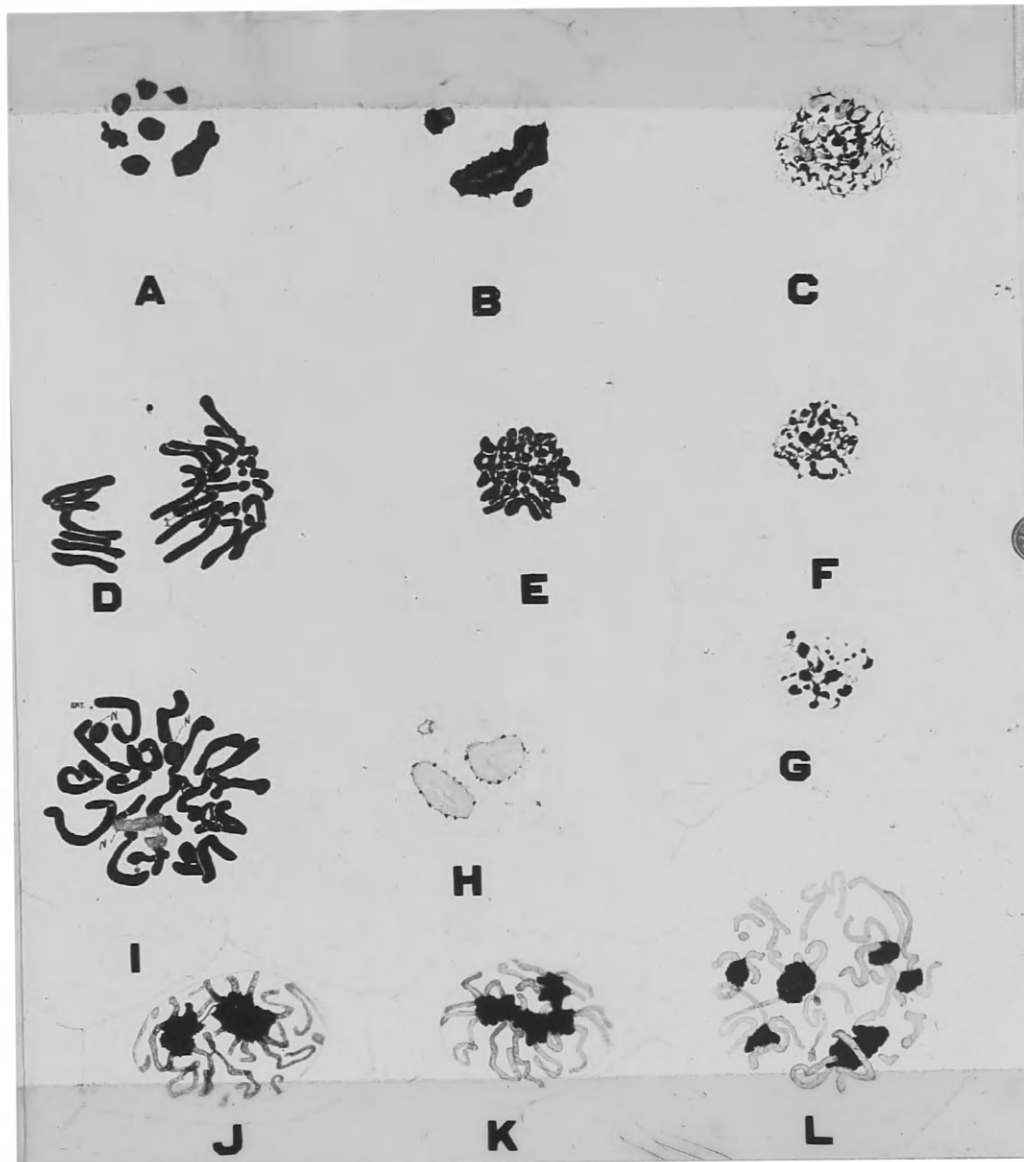
Figure 2. — Development of nucleoli during mitosis in root-tips: A to I, T. eichleri: A and B, interphase, Flemming's fixation; C, late telophase, Bouin's fixation; D, anaphase; E, early telophase; F, late telophase, Flemming's fixation; G, telophase later than F, Flemming's fixation; H, interphase, Bouin's fixation; I, early metaphase showing nucleolar remnants (N), Bouin's fixation; J and K, late prophase, T. linifolia, Flemming's fixation; L, Carrara, late prophase, Flemming's fixation. All camera lucida drawings X 2000. Reduced ap. ¼.

some connecting strands increase in number, and the chromo-
somes become very closely associated (figs. 1,B, 2,E and 1,
C). The first distinguishable plastin appears shortly after
the appearance of a membrane-like structure about the mass
of chromosomes. Bouin's fixation gives the best telophase
differentiation between chromatin and plastin. The latter
remains essentially unstained. Early telophase stages are
shown in figures 1,E, 2,C and 2 F. Careful studies of many
nuclei in different tulips has shown that the appearance of
plastin is always associated with the disappearance of
chromatin. In many preparations direct connections between
the matrix material and developing masses of plastin are
indicated. The latter appears to originate at a number
of points from the chromsome mass. These areas of collect-
ing plastin may be more numerous than the maximum nucleolar
number for the clon (see, nucleolar numbers in root tips,
this paper). The developing masses of plastin coalesce to
a varying extent as they increase in size. The occurrence
of a fixed maximum nucleolar number of each clon examined
strongly indicates that some actual flow of the plastin
itself must occur in the telophase. Preparations of telo-
phase nuclei frequently give this appearance. During early
telophase stages the nucleus is typically irregular in peri-
pheral outline. Toward the end of the telophase the chroma-
tin mass becomes very greatly reduced and the plastin mass

shows further increase. The latter material becomes organiz-
ed into definite nucleoli. Throughout the progression of
the telophase the whole nucleus increases in size, the
periphery becoming more regular in outline.

Interphase. During the interphase the chromati-
city of the chromonemata remains very low. The latter
appear to be arranged radiately about the nucleoli but it
is not possible to determine this definitely. The chromo-
nemata are greatly extended and form an interconnected
plexus of threads. Irregular small masses of chromatin are
usually associated with the chromonematic reticulum (figs.
1,F and 2,H). After fixation in the Bouin's fluid it can
be seen that aggregations of chromatin frequently occur on
the surfaces of the nucleoli (fig. 2, A and B). The latter
frequently show unmistakeable signs of their compoundness
(as the result of fusion of adjacent nucleoli).

Prophase. As the prophase proceeds the nuclei
swell, the chromonemata shorten and, gradually become
chromatic. In some tulips, T. sprengeri for example, the
chromonemata are very distinctly coiled in the early pro-
phase (fig. 1,G). It soon becomes evident that the develop-
ing chromosomes are arranged in bouquet-like fashion about
the nucleoli. In the earlier stages of the prophase the
doubleness of each chromosome can often be seen. Distinct
pairs of chromomeres are also evident. In fig. 20, plate 1,

pairs of chromomeres can be seen in two of the chromosomes,
but at one or two points it appears as though certain of
the former have not divided. The distinctly radiate arrange-
ment of the chromosomes about the nucleoli is a very charac-
teristic feature of the prophase (figs. 1,H and 2, J,K and L).
All of the chromosomes are not always in direct contact with
nucleoli. In the nucleus shown in figure 2,J one chromosome
was not in contact with a nucleolus but, it was connected
to a chromosome that was, by a distinct chromatic strand.
This figure shows several other apparently free chromosomes
but these were attached at levels not recorded in the draw-
ing. Even though all of the chromosomes do not always
touch the nucleoli directly they apparently form a completely
interconnected system by virtue of the connecting strands.
This seems to be the case in all nuclei examined.

The relations of the chromosome arms to the nucleo-
li are best studied in preparations fixed in the Bouin's
fixative. In figure 22, plate 1, the arrangement of the
chromosomes about a prophase nucleolus is shown. Some of
the chromosomes are attached to the nucleolus by both of
their ends. The small mass of plastin at N, is connected
to the distal end of one chromosome and the middle of
another chromosome which is also attached to the large
nucleolus. It is at similar stages of the prophase that
the satellites can sometimes be seen although these structures

are usually hard to demonstrate. Figure 20, plate 1, shows
a pair of satellites lying on the surface of a nucleolus
although the connecting threads to the end of the chromosome
arm could not be distinguished. During the early and middle
prophase the satellites appear to be located on the surface
of the nucleoli. The chromosomes, whether satellite-bear-
ing or not, also come into contact with the surface or near-
surface regions of the nucleolus. In very late prophase or
early metaphase the chromosomes may become free of the nucleo-
lus. This seems to be associated with a breaking down or
disappearance of the nucleolar matter. In most cases the
nucleoli do not persist into metaphase. Sometimes, however,
masses of nucleolar matter adhere to the arms of the chromo-
somes even into metaphase (figs. 15, 16 and 19, pl. 1). The
nucleoli appear to remain undiminished in size and staining
density up to very late prophase. The final breakdown or
disappearance of most of the nucleoli between prophase and
metaphase apparently is accomplished rapidly. It does not
occur until the chromosome matrices appear to be fully or
almost fully developed.

Metaphase. During the metaphase the chromosomes
undergo further contraction finally becoming arranged into
a definite equitorial plate formation. The satellites are
most easily seen during the metaphase but may sometimes be
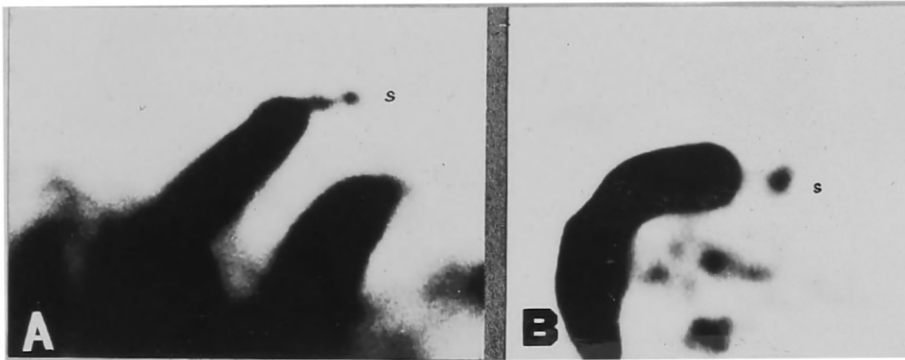clearly seen in anaphase. In all of the Leiostemones

Figure 3. - Metaphase satellites, Darwin: Allard Pierson;
A, tandem satellites borne by one J-shaped chromosome; B,
normal type of satellite in divided condition at late meta-
phase. (only distal ends of chromosomes show). Photomicro-
graphs X ap. 6000.

reported in this paper, except _T. sprengeri_, they occur on the ends of the long or distal arms of the chromosomes. In _T. sprengeri_ (fig. 1, J) the statellites are unusually distinct and are borne only on the short or proximal arms of the chromosomes. In species of the Eriostemones both types of attachment occur. In one Darwin tulip, Allard Pierson, one chromosome bears two satellites in tandem (figs. 3,D and 23, 24, pl. 1). These are apparently very similar to tandem satellites reported in _Allium_ by Taylor (27). Satellites may appear divided before or after the visible separation of the daughter chromatids in the metaphase. In certain tulips demonstration of the satellites in root tip chromosomes is very difficult.

Nucleolar Numbers in Root-tips

Because of the difficulty in demonstrating directly the satellited chromosomes in root-tip nuclei another method of attacking the problem was undertaken. If the maximum number of nucleoli in a given clon is equal to the number of nucleolus-forming chromosomes a mathematical study of nucleolar frequencies should be suggestive. These were determined by random selection of cortical nuclei. Fused nucleoli were counted as single units. Thus the nucleus shown in figure 2, K has but one nucleolus, that in figure 2, A, seven. In determining the characteristic root tip

nucleolar number of any given clon certain precautions were
found to be necessary. The characteristic number for a clon
can be expressed as the average number of nucleoli per
nucleus. In some roots, however, the minimum and maximum
nucleolar numbers remain fixed, but the average number per
nucleus may show considerable variation at different levels
in the same root. In some cases the mean number of nucleoli
in the apical region of the root tip is less than in areas
farther back from the apex. In some roots the rate of in-
crease appears as an approximately straight line when the
mean is plotted against the level in the root in microns.
In other cases there is no shift in the mean at successive
levels and the graphed line is horizontal rather than in-
clined. In some roots of T. sprengeri the highest mean
occurs nearest the apex.

In order to determine accurately the shift in
average nucleolar numbers it was found necessary to average
a large number of counts at each level in the root. One
section of 20 microns thickness was found insufficient for
an accurate determination so two to four sections were
counted and the mean level in microns computed for the
regions examined. The presence of "false nucleoli"
(Zirkle (33)) or non-nucleolar chromatic masses in certain
regions of some roots occassionaly made accurate counting
difficult. In such cases fixation with Allen and Wilson's

Levels on graph (fig.4)	Range in microns in root used in making determinations. The meristematic apex equals 0.	Mean level in microns plotted on graph	Mean no. of nucleoli per nucleus.	Number of nuclei observed
I	0 – 54	27	3.270	100
II	162 – 216	189	3.410	200
III	396 – 450	423	3.660	200
IV	504 – 558	536	3.762	400 (1)
V	630 – 684	659	3.995	200
VI	756 – 810	783	4.165	400 (1)
VII	864 – 918	891	4.247	400 (1)
VIII	990 – 1044	1017	4.410	400 (1)
IX	1098 – 1152	1125	4.500	200
X	1332 – 1386	1359	4.700	200

Table 1. Variations in average number of nucleoli per nucleus in one root of S. L. Carrara at ten different levels.

(1) counts made by counting 100 nuclei in each of two pairs of adjacent sections and then making recounts in same sections and summing both sets of data.
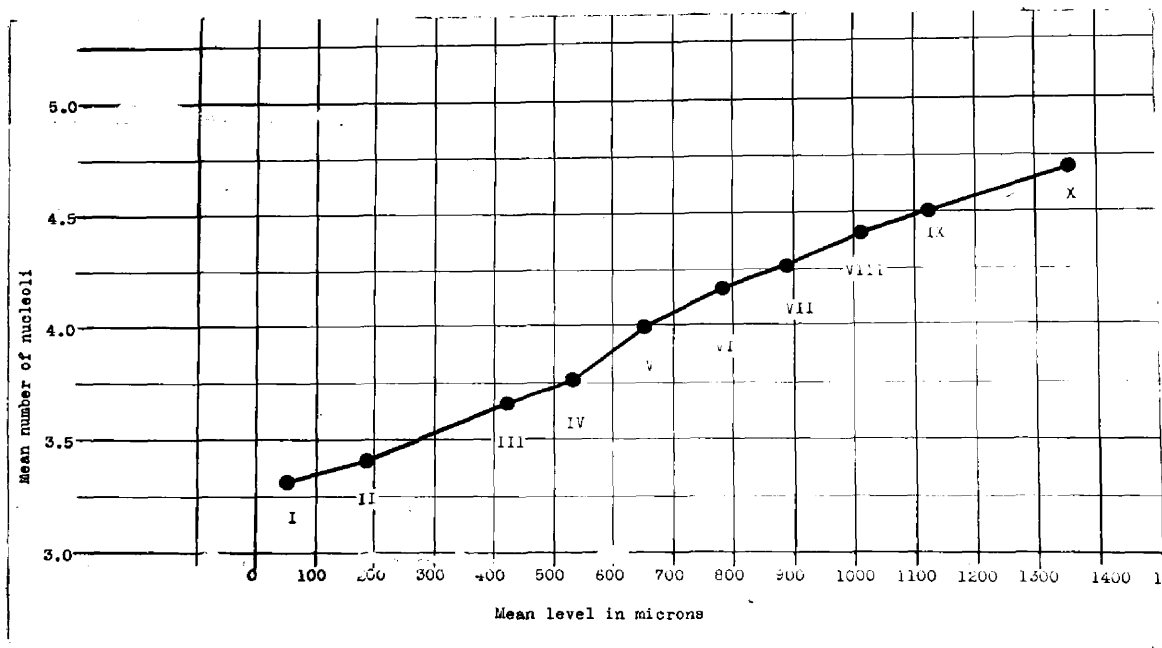
Figure 4. - Relation of level in the root-tip to nucleolar frequency in Carrara. Approximate position of apical histogens equal zero. (See table 1 and text for complete explanation),

modification of Bouin's resulted in a clear differentiation between the true and "false nucleoli". The former were often so lightly stained, however, that the preparations were not used for counting as very small nucleoli might easily have been overlooked. The material used in this investigation was selected with special reference to its desirability for numerical studies. Figure 4 illustrates graphically the gradient in nucleolar mean number obtained at ten different mean levels in a single root of the cottage tulip, Carrara. Table 1 gives the data upon which this graph is based. Extra nucleolar masses of chromatin at level I, made accurate counting difficult. The range of probable greatest accuracy runs from level II to level X. The latter is equal to the "characteristic maximum area".

In figure 5 the nucleolar gradients for several species and varieties are compared. Only a few points have been determined in most cases and the exact slopes are therefore not shown on this graph. The data, however, illustrates the necessity of considering nucleolar number gradients in attempting to determine the characteristic mean nucleolar frequency per nucleus for a clon.

In the tulips examined in this study it was found that if successive nucleolar counts were made in 100 or more nuclei at any one level, or closely adjacent levels, there was often an unmistakable regularity in the frequencies for

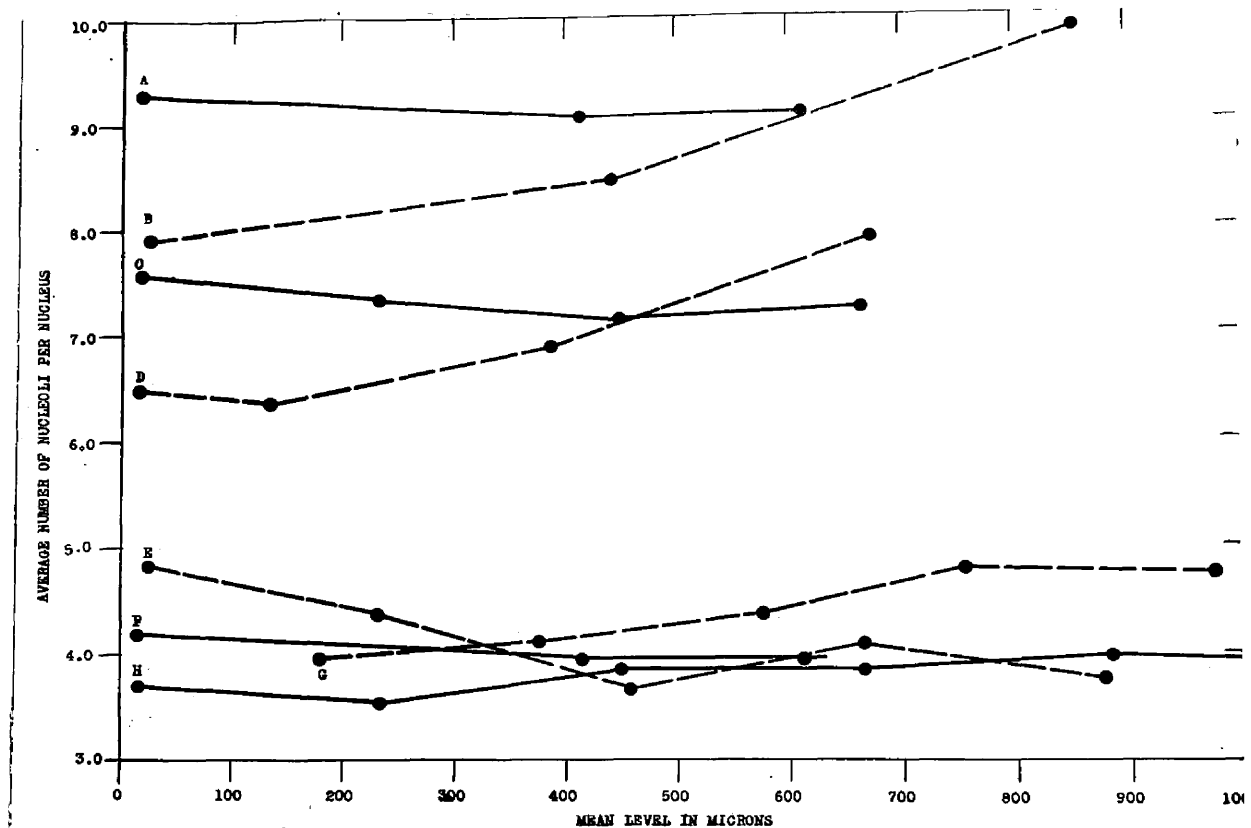Figure 5. — Relation of level in the root-tip to nucleolar frequency in several species and varieties of Tulipa: A, T. orphanidea 4n (238 nuclei); B, T. clusiana 5n (300 nuclei); C, T. saxatilis 3n (273 nuclei); D, Inglescombe Yellow 3n (350 nuclei); E, T. sprengeri 2n (580 nuclei); F, T. hageri 2n (228 nuclei); G, Mrs. Moon 2n (1000 nuclei); H, T. fosteriana 2n (709 nuclei). One root-tip was used in each case.

the different numbers of nucleoli per nucleus. From the distributions obtained it is certain that the nucleolar numbers of at least some of the investigated clons are normally fixed within definite limits in the root tips. This is probably the case in all of the tulips investigated in this study. Even though the mean number of nucleoli per nucleus may shift at different levels in the roots of a given clon the maximum and minimum numbers remain fixed. This is best evidenced by the shapes of the curves obtained when the number of nucleoli are plotted against the percentage of nuclei obtained for each number class. Figures 6 and 7 show the curves obtained for the two diploid cottage tulips Mrs. Moon and John Ruskin. In each figure curves have been plotted for two different levels in the root. Figure 7 also shows the curve obtained by combining the nucleolar counts in 1000 nuclei from two other roots of John Ruskin. Figure 8 shows the curves obtained by plotting the nucleolar frequencies at four of the levels in the root of Carrara used in plotting figure 4. The nucleolar frequency curve for 1665 nuclei, included in both minimal and maximal nucleolar levels of five roots of T. sprengeri, is shown in figure 9.

In each of the above mentioned four tulips the somatic chromosome number is 24. The maximum root-tip nucleolar number in each is obviously eight. In John Ruskin
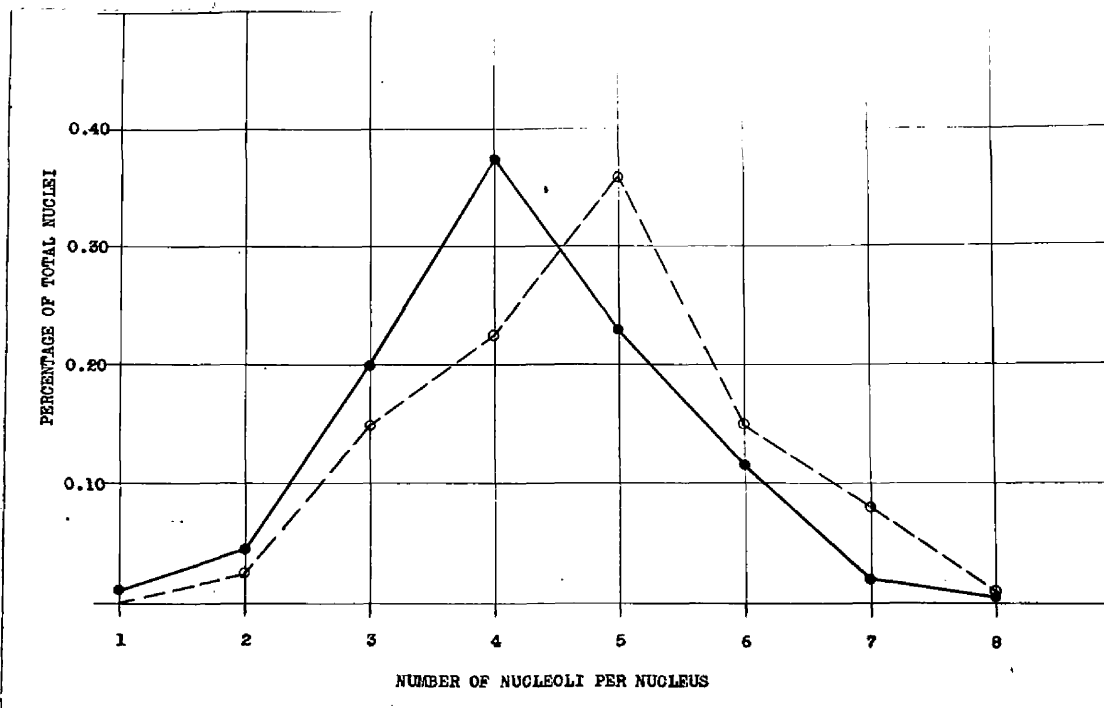
Figure 6. - Nucleolar frequencies at two different levels in a root-tip of Mrs. Moon. Solid line, mean level of 378 microns; broken line, mean level of 972 microns. Each distribution is based on counts of 200 nuclei.
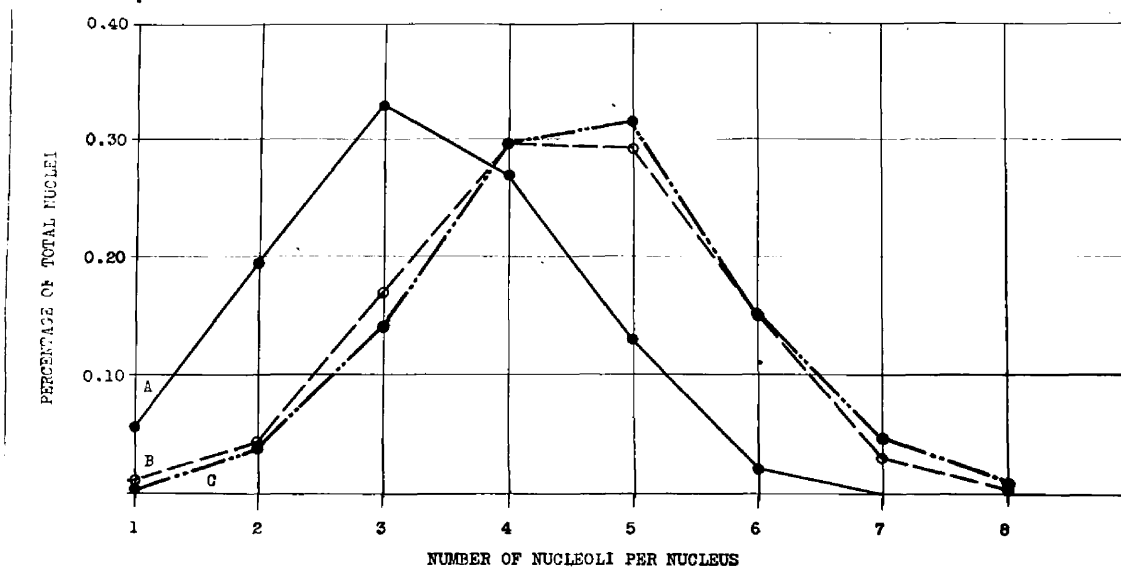
Figure 7. - Nucleolar frequencies in root-tips of John Ruskin: A, root l.mean level of approximately 380 microns, 200 nuclei; B, root l.mean level of approximately 980 microns, 300 nuclei; C, based on 1000 nuclei from two roots, counts made at or near levels having maximum characteristic nucleolar averages.

Figure 8. — Nucleolar frequencies at four different levels in a root-tip of Carrara. Roman numerals refer to levels and correspond to table 1.

nucleolar counts have been made in a total of 4585 nuclei,
in Carrara at least 4000 and, in Mrs. Moon 2650. The maxi-
mum nucleolar number given above, was never exceeded. In
addition to the counts actually recorded many others have
been made in connection with other studies in the same
tulips. No exceptions to the rule just given were found.
Other tulips were found to be characterized by maximum
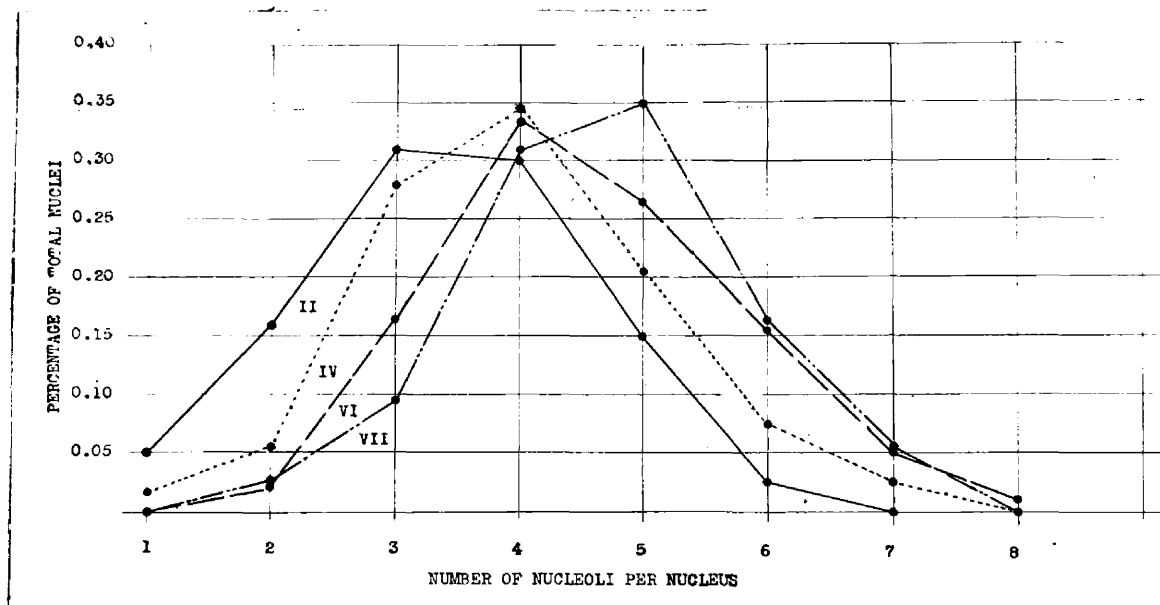nucleolar numbers greater or less than eight. Figure 10
shows nucleolar distribution curves for the triploid tulips
Inglescombe yellow, Inglescombe pink and T. lanata. Note
that the rates of slope indicate a nucleolar maximum near 13.

It was found by experiment .that merely by inspec-
tion of several levels in a root the areas having the highest
mean numbers per nucleus can be determined with considerable
accuracy. If counts are made in such areas in different
roots a characteristic average nucleolar number for any given
clon of a species can be determined. Each of the named
"garden varieties" represents a clon derived from a single
seed or bulb so a characteristic nucleolar number can be cal-
culated for each one of these. While there is some variation,
enough counts at comparable levels in different roots give
surprisingly uniform values. Table 2 lists the observed
nucleolar numerical characteristics for about 30 species,
varieties and aneuploid hybrids of Tulipa. One heteroploid
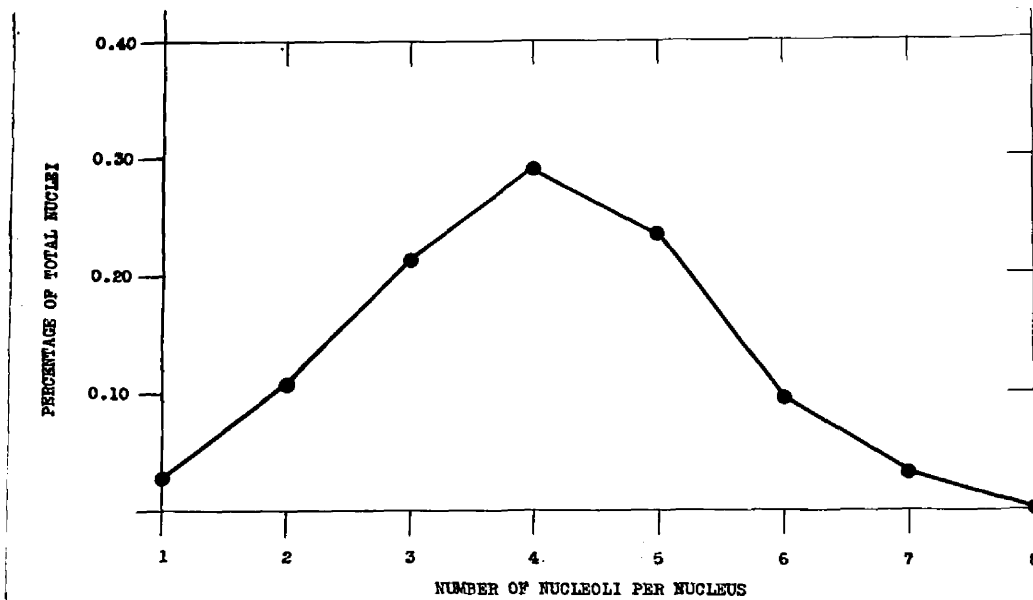
Figure 9. - Nucleolar frequencies in four root-tips of
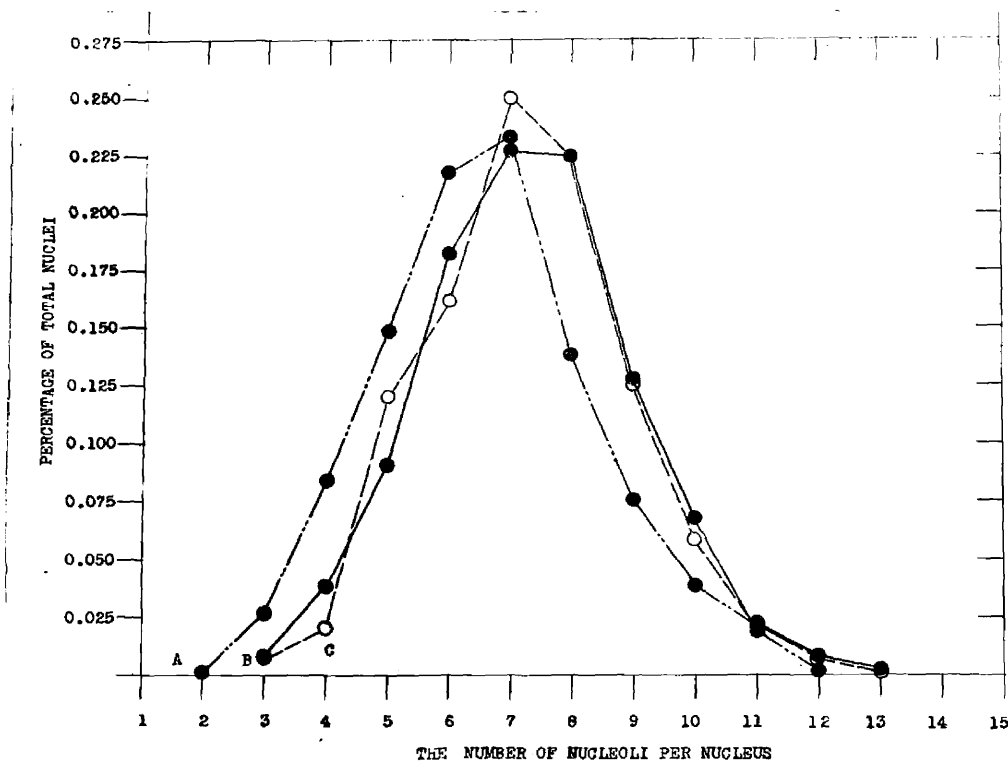T. sprengeri. Based on 1665 nuclei from different levels.

Figure 10. - Nucleolar frequencies in root-tips of three
triploid tulips: A, Inglescombe Pink, 488 nuclei from
four roots; B, Inglescombe Yellow, 1000 nuclei from two
roots; C, T. lanata, 391 nuclei from four roots. (All
counts made at levels in the roots having maximum character-
istic nucleolar averages for the clons concerned.)

Name and Chromosome number		Avg. number nucleoli each root (roots 1 to 6)						Avg. all roots	Max. no.	No. nuclei observed
		No.1	No.2	No.3	No.4	No.5	No.6			
T. linifolia Regel	(24)	4.07	3.88	4.01	3.76	--	--	3.93	5	442
T. stellata Hook	(24)	4.77	4.47	4.50	4.92	4.52	4.5.	.52	5	604
T. fosteriana Hoog	(24)	4.91	4.75	4.41	4.63	--	--	.25		409
T. sprengeri Baker	(24)	3.72	3.78	4.00	---	--	--	3.83		1222
T. kageri Heldr.	(24)	4.00	3.91	--	--	--	--	3.95	8	531
T. violacea Boiss.	(24)	3.45	3.36	--	--	--	--	3.7	7	552
S. L. Carrara	(24)	4.66	4.51	4.66	4.55	4.70	--	4.60	8	642
S. L. Mrs. Moon	(24)	4.74	4.71	4.73	4.5	--	--	4.73		540
S. L. John Ruskin	(24)	4.24	4.7	4.66	4.55	--	--	4.		4.5
S. L. Adonis	(24)	3.97	3.90	3.80	7.31	3.70	3.5	3.7	6	1500
New Mendel: Herman Waller	(24)	4.71	4.73	3.5	4.13	--	--	4.05	7	1041
Breeder: Louis XIV	(24)	5.34	5.50	4.	5.20	--	--	5.27	9	552
New Mendel: Gipsy	(24)	4.60	5.00	4.51	5.75	--	--	4.55		5
S.E.La Reine Maximus	(24)	5.44	--	--	--	--	--	5.44		150
DO 72 chromosome area		22.35	--	--	--	--	--	22.35	5	17
T. lanata	(36)	7.13	7.62	7.34	7.07	--	--	7.	15	501
T. saxatilis Sieber	(36)	7.35	7.53	7.03	---	--	--	7.30	55	551
S. L. Inglescombe Pink	(36)	6.32	6.51	6.81	6.71	--	--	6.56	12	480
S. L. Inglescombe Yellow	(36)	7.91	7.51	7.52	7.43	--	--	7.50	13	4.75
S. s. antha Boiss.	(48)	5.28	5.57	5.27	5.22	--	--	5.35	15	550
T. estremnica Regel	(48)	7.05	--	--	--	--	--	7.05	11	201
T. orphanides Boiss. ?	(48)	6.14	6.32	--	--	--	--	6.06	15	315
T. clusiana DC.	(60)	9.20	9.53	9.54	10.6	--	--	.97	17	554
Louis XIV x Mrs. Moon	(24)	4.40	--	--	--	--	--	4.40	5	201
Louis XIV x Mrs. Moon	(24)	4.35	--	--	--	--	--	4.5		150
Carrara x John Ruskin	(24)	4.76	--	--	--	--	--	4.76		149
Carrara x John Ruskin	(24)	4.43	--	--	--	--	--	4.43		150
Inglescombe Yel. x John Ruskin	(27)	4.50	--	--	--	--	--	4. 5	15	550
Ingles. Yell. x John Ruskin	(27)	4.45	--	--	--	--	--	4. 5		55
Ingles. Yell. x John Ruskin	(30)	5.32	--	--	--	--	--	5.70	10	17
Inglescombe Y. x John Ruskin	(30)	5.45	--	--	--	--	--	5.6	10	

Table 2. Nucleolar numbers in Tulipa.

For graphical representation of some of this data see figures 10b and 10c.
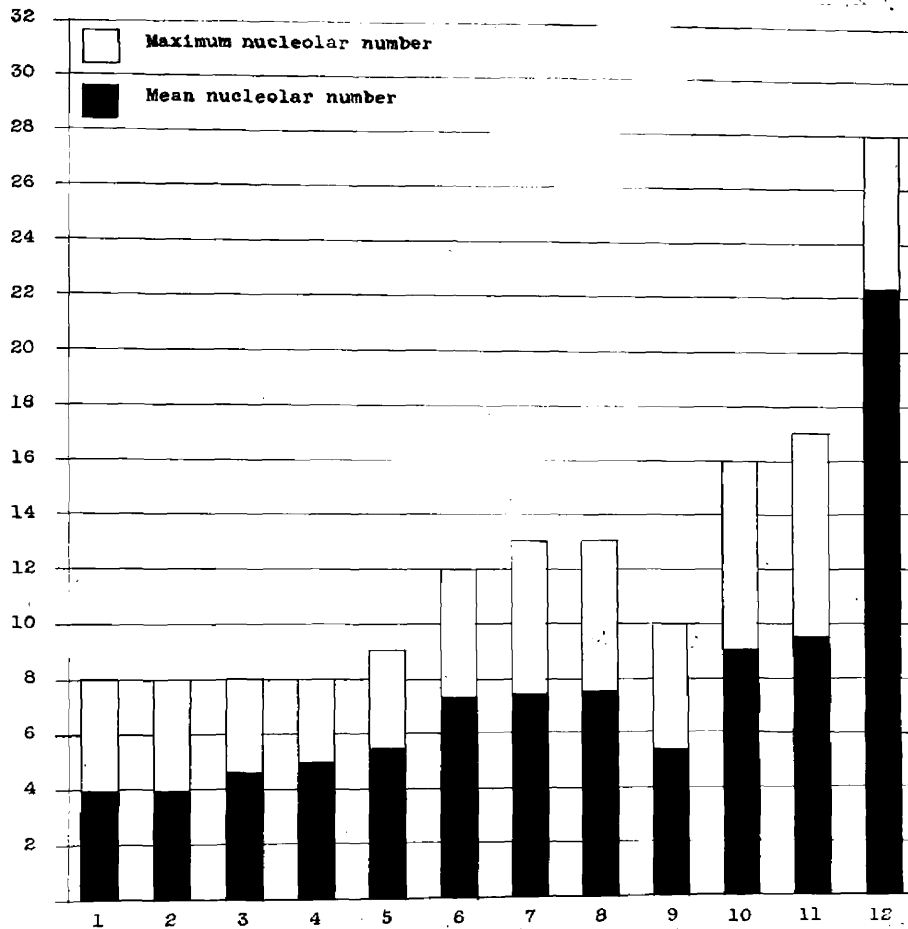
Figure 10b. — Nucleolar numbers in species and varieties
of Tulipa:

1-T.linifolia   (24)   7-T.lanata   (36)
2-T.hageri   (24)   8-Inglescombe Yellow (36)
3-T.fosteriana   (24)   9-T.chrysantha   (48)
4-John Ruskin   (24)   10-T.orphanidea   (48)
5-La Reine Maximus (24)   11-T.clusiana   (60)
6-T.saxatilis   (36)   12-La Reine maximus (72 ?
                                   chromosome chimera)
     (somatic chromosome numbers in brackets after names)
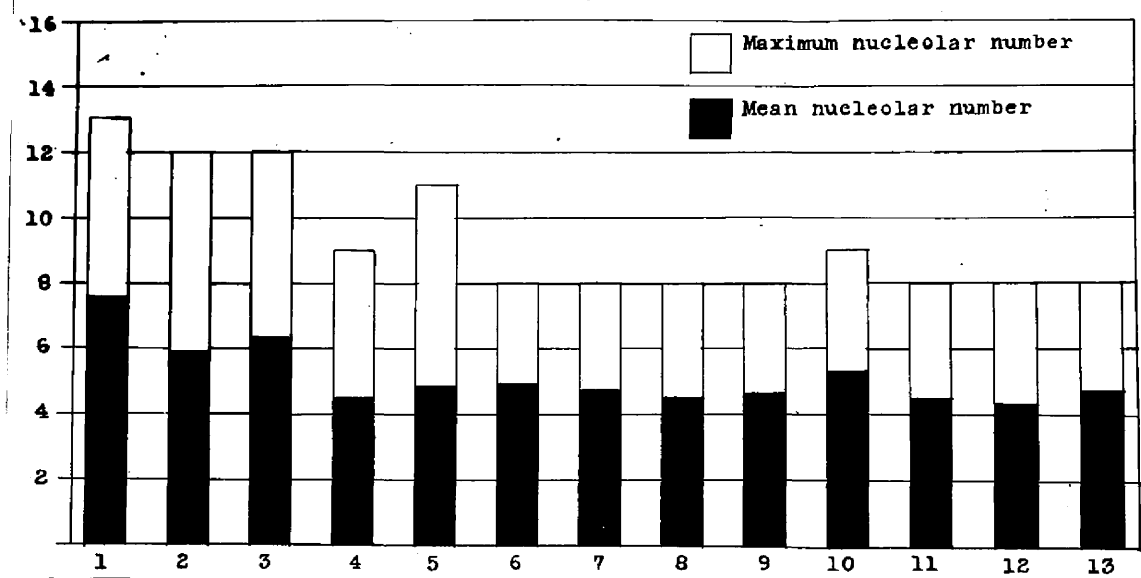
Figure 10c.- Nucleolar numbers in tulip varieties and their hybrids:

1 - Inglescombe Yellow (36)
2 - Inglescombe Yellow X John Ruskin, hybrid no.1   (32)
3 - Inglescombe Yellow X John Ruskin, hybrid no.2   (30)
4 - Inglescombe Yellow X John Ruskin, hybrid no.3   (28)
5 - Inglescombe Yellow X John Ruskin, hybrid no.4   (27)
6 - John Ruskin (24)
7 - Carrara X John Ruskin, hybrid no.1   (24)
8 - Carrara X John Ruskin, hybrid no.2   (24)
9 - Carrara (24)
10--Louis XIV   (24)
11- Louis XIV X Mrs. Moon, hybrid no.1   (24)
12- Louis XIV X Mrs. Moon, hybrid no.2   (24)
13- Mrs. Moon (24).
    (Somatic chromosome numbers are given in the brackets)

chimera of a diploid root of La Reine Maximus is also in-
cluded. In this abnormal case one excellent metaphase plate
contained 72 chromosomes (fig. 11,). The nucleolar counts
were made in cells closely adjacent. The autoheteroploid
nuclei were readily distinguished (fig. 11,) by their great
size. They probably had the same chromosome number as the
one cell in which the count was made but, in any case it
must have been considerably more than 24.

Volumetric Relationships in the Root-tip

Measurements were made of cell, nuclear, and
nucleolar sizes to determine their relation, if any, to
nucleolar number. It was thought the nucleolar number
gradients, previously referred to, might be explained in
part on this basis. Measurements were confined to the mitotic
regions although in the upper limits very few cell divisions
occurred.

Nucleolar number in relation to cell and nuclear
size. Two methods were employed. First an attempt was made
to correlate nuclear and cell size with nucleolar number at
a given level in the root, and second to make comparisons of
these factors between several different levels in the same
root. Roots of Carrara and T. sprengeri were studied in de-
tail because of the radically different types of nucleolar
number gradients obtained in each. In Carrara the highest
mean number of nucleoli occurred farthest from the apex.
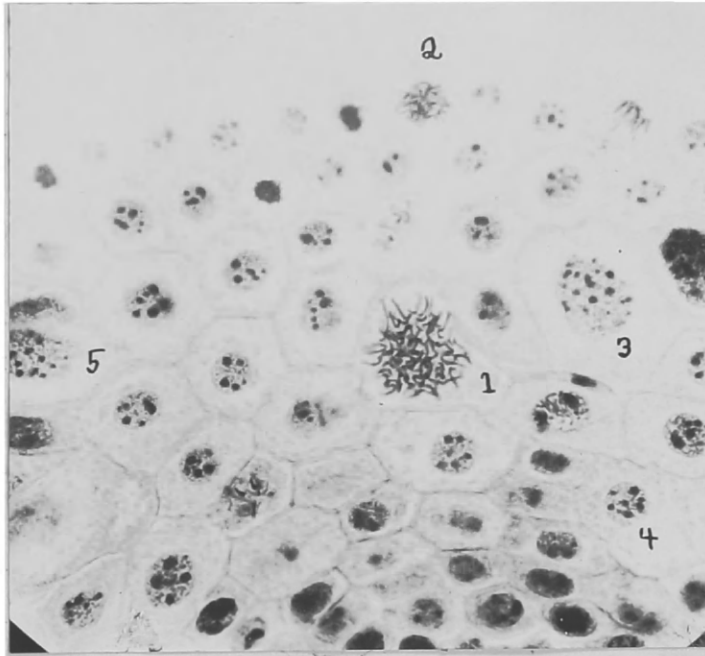
Figure 11. — Sector of a root-tip of La Reine Maximus showing
chimeral areas. The metaphase plate at (1) has 72 chromosomes,
the plate at (2) has 24 chromosomes. Heteroploid nuclei (with
72? chromosomes) are shown at (3) and (5). A diploid nucleus
is shown at (4) and elsewhere. Nucleolar counts were made in
nuclei above and below the regions marked (1) and (3). Photo-
micrograph X ap. 500.

In T. sprengeri the highest nucleolar frequencies occur near
the apex, the rest of the mitotic region having a lower, and
fairly constant average number of nucleoli. In table 3 the
measurements of 432 nuclei in one root of Carrara are record-
ed with their nucleolar numbers. From this data there seems
to be little if any correllation between nuclear size and
nucleolar number. If the mean nuclear sizes for all levels
are considered, however, there does appear to be some slight
increase in nuclear size as the nucleolar number increases
(fig. 12). The average size for nuclei containing six
nucleoli is approximately the same as that for two nucleolate
nuclei, however. This data clearly shows that the nuclei
are larger at the 423 micron level than at either the 189 or
891 micron levels. A similar situation seems to hold in the
other roots examined i.e., the maximum average nuclear size
occurs somewhere between the apex and the upper limits of the
mitotic region.

The situation with respect to nuclear size and
nucleolar number relationships in T. sprengeri is somewhat
different than in Carrara. In the former the smaller nuclei
tend to have the higher nucleolar numbers. There is some
over-lapping, however, in sizes of individual nuclei between
the different nucleolar number classes. The maximum nucleolar
number may occur in a large nucleus or a small one. The same
is true for the lower nucleolar numbers. That the smaller
nuclei generally contain the largest number of nucleoli,

Nucleolar Number	Nuclear size (average)[1] at three different mean levels			Avg. nuclear size all levels	No. of nuclei measured
	189 microns	423 microns	891 microns		
1	0.93	1.18	-------	1.05	15
2	1.03	1.46	0.97	1.15	65
3	1.14	1.46	1.09	1.23	88
4	1.10	1.49	1.13	1.24	95
5	1.24	1.46	1.09	1.26	95
6	1.10	1.34	1.07	1.17	56
7 & 8	-------	1.47	1.07	1.27	18
Aug.	1.09	1.40	1.08	1.19	432

Table 3. Nuclear size in relation to nucleolar number in one root of S.L. Carrara (See table 1.) [1] Nuclear sizes expressed as square inches X 2000.

Cell size[1]	Nuclear size	No. of nucleoli	Total nucleolar volume [2]	No. cells measured	Mean Level in root in microns	Minimum nucleolar volume	Maximum nucleolar volume	Size of cell with minimum nucleolar volume	Size of nucleus with maximum nucleolar volume	Size of cell with maximum nucleolar volume	Size of nucleus with maximum nucleolar volume
4.30	1.63	2	37,577.1	10	423	26,437.6	49,998.6	3.46	1.27	5.31	2.00
4.66	1.70	4	35,941.2	10	423	24,844.5	50,507.1	4.39	1.62	4.19	1.79
4.69	1.60	6	29,086.8	10	423	18,543.0	50,999.7	2.58	1.30	6.43	2.38
6.20	1.07	2	14,140.7	5	891	7,726.3	22,194.0	5.91	1.12	6.98	0.98
7.51	1.26	4	13.972.7	10	891	10,041.6	19,526.6	8.27	1.31	9.20	1.46
7.93	1.14	6	9,967.6	10	891	6,797.2	14,458.2	7.05	1.00	9.70	1.35

Table 4. Nucleolar number and volume in relation to nuclear and cell size at two
different levels in one root (see table 1.)
(1) Cell and nuclear sizes expressed as square inches X 2000.
(2) Nucleolar volumes expressed in cubic scale units.

Nuclear sizes [1] at different levels in two roots

Nucleolar Number	Mean levels in Microns root no. 1				Mean levels in microns root no. 2.		Root No. 1		Root No. 2	
							Avg. nuclear size all levels	Number of nuclei measured	Avg. nuclear size all levels	Number of nuclei measured
	27	243	459	891	27	441				
1	-----	1.03	1.05	0.95	0.93	1.09	1.01	21	1.01	18
2	0.83	1.02	1.03	1.07	1.02	1.09	0.98	50	1.05	43
3	0.80	0.99	1.03	0.98	0.97	1.10	0.95	78	1.03	70
4	0.79	0.93	0.99	0.95	0.93	1.03	0.91	65	0.98	57
5	0.72	0.88	0.89	0.86	0.95	0.99	0.83	83	0.97	50
6	0.72	0.91	0.91	0.80	0.89	0.99	0.83	57	0.94	21
7	0.68	0.79	0.83	0.80	0.88	0.97	0.83	37	0.92	15
8	0.67	-----	-----	0.80	0.82	-----	0.73	8	0.82	2
Avg.	0.74	0.93	0.96	0.90	0.92	1.03	0.88	399	0.97	276

Table 5. Nuclear size in relation to nucleolar number at different levels in two roots of T. sprengeri.

(1) Nuclear sizes expressed as square inches X 2000.

Avg. level in the root in microns	No. of nucleoli	Avg. cell size (1)	Avg. nuclear size (1)	Avg. total nucleolar volume (2)	Maximum nucleolar volume	Minimum nucleolar volume	Size of cell with maximum nucleolar volume	Size of nucleus with maximum nucleolar volume	Size of cell with minimum nucleolar volume	Size of nucleus with minimum nucleolar volume	No. of cells measured
27	3	2.21	0.83	9,236.8	12,782.9	4,374.1	2.24	0.97	1.70	0.61	10
DO	7	1.91	0.77	6,098.6	10,733.6	3,352.7	2.54	0.79	1.70	0.60	10
459	1	4.33	1.16	33,609.8	47,840.6	28,807.8	4.20	1.24	3.78 & 5.45	1.11 & 1.22	8
DO	2	4.08	1.18	25,532.2	46,842.2	18,219.5	4.46	1.35	3.41	1.10	13
DO	3	3.43	1.04	16,532.8	22,519.2	8,330.1	4.39	1.13	2.89	0.98	10
DO	4	3.95	1.14	21,960.7	33,690.2	12,301.9	6.11	1.47	2.45	1.07	10
DO	5	3.18	0.93	14,967.6	27,528.4	9,951.3	2.80	1.05	3.02	0.80	10
DO	6	2.77	0.90	12,879.3	21,740.6	7,306.4	3.46	1.22	2.00	0.72	10
1368	1	5.75	0.93	17,407.9	36,835.2	11,777.0	7.49	1.04	8.18	0.84	10
DO	2	5.92	0.90	13,406.0	17,721.2	6,617.3	4.17	0.92	5.30	0.75	10
DO	3	4.92	0.87	11,514.6	20,441.6	4,873.2	5.08	1.07	2.16	0.90	10
DO	4	5.81	0.91	11,401.0	13,998.6	8,828.6	4.70	0.92	4.07	1.00	10
DO	5	5.04	0.82	8,661.1	11,363.5	5,515.6	8.23	0.92	3.00	0.88	11
DO	6	4.31	0.84	8,223.9	13,708.8	4,749.7	6.07	0.99	4.78	0.70	10

Table 6.   Nucleolar number and volume in relation to nuclear and cell size at three different levels in one root of _T. sprengeri_.

(1) Size expressed as square inches X 2000.
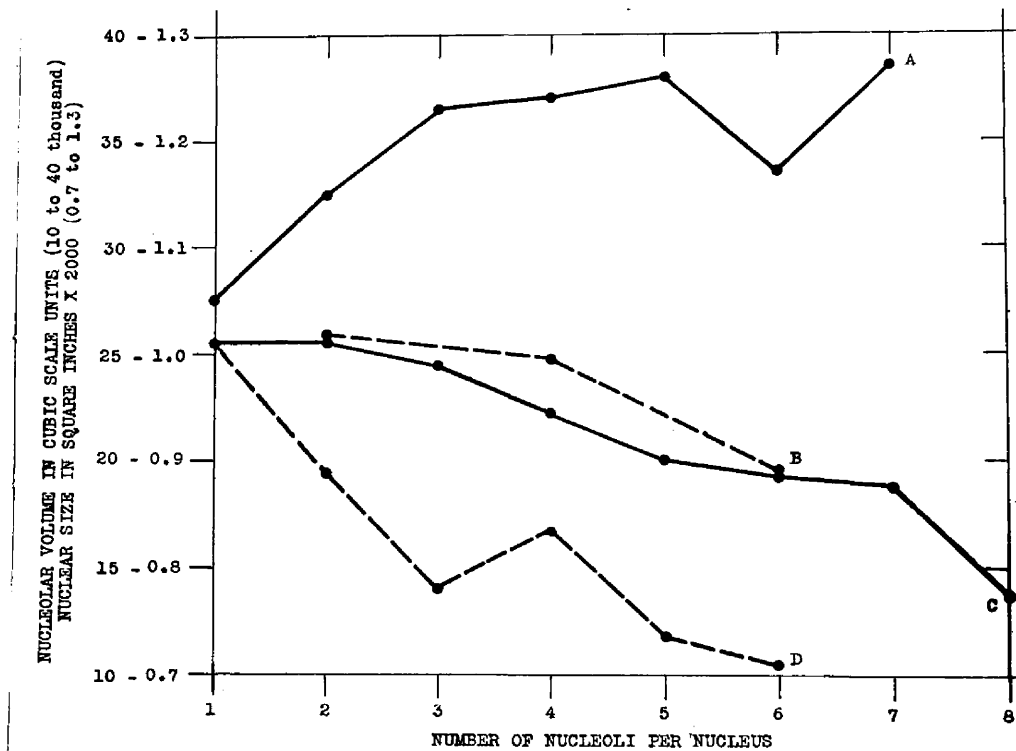(2) Volume in cubic scale units.

Figure 12. — Relation between nuclear size, nucleolar volume
and nucleolar number in root-tips of <u>T. sprengeri</u> and
<u>Carrara</u>: A, Nuclear size in Carrara; B, nucleolar volume
in Carrara; C, nuclear size in <u>T. sprengeri</u>; D, nucleolar
volume in <u>T. sprengeri</u>.

however, is clearly shown by the data recorded in tables 5
and 6. In figure 12 the average nuclear sizes for all levels
in both roots of T. sprengeri recorded in table 5 have been
plotted against the number of nucleoli. It can be seen that
the rate of decrease in nuclear size is not the same between
all intervals.

Nucleolar, Nuclear and cell volumes. Measurements
made in roots of Carrara and T. sprengeri show that large
nuclei generally have larger nucleolar volumes than small
nuclei in the same sections. Tables 4 and 6 give the nuclear
and cell sizes together with the nucleolar volumes for several
levels in one root for each tulip. In each case there is only
one exception to the rule that the highest observed nucleolar
volumes occur in nuclei larger than those containing the
corresponding minimum nucleolar volumes. The large nuclei
usually occur in cells of greater cross-sectional area than do
the small nuclei but, several excpetions are recorded in the
tables. In the levels of the roots farthest from the apex
the increase in size in the areas measured is due chiefly to
an increase in radial dimensions of the cells.

Nucleolar volumes at different levels in the root.
In table 6 it can be seen that in the root of T. sprengeri
the highest nucleolar volumes occur at the 459 micron level.
It is at this level that the average nuclear size is the
greatest. The lowest nucleolar volumes occur at the 27 micron

level where the average nuclear size is also the lowest.
At the 1368 micron level the average nuclear size is
slightly higher than at the 27 micron level as is also the
average nucleolar volume. At the 27 micron level the mean
number of nucleoli per nucleus for 101 nuclei was 4.82; at the
459 micron level it was 3.66 for 114 nuclei; at the 1368
micron level it was 3.41 for 140 nuclei counted.

The data in table 6 shows that the higher the
nucleolar number the lower the total nucleolar volume. For
example, at the 459 micron level the one-nucleolate nuclei
have a total average nucleolar volume of 33,609.8 cubic
scale units whereas the nuclei with six nucleoli at the same
level have a total average nucleolar volume of only 12,879.3
cubic scale units - a reduction of over fifty percent.

In the root of Carrara the same type of deter-
minations (table 4) were made at 423 and 891 micron levels.
The nuclei at the former level average decidedly larger than
at the latter level. The same relationship holds for the
nucleolar volumes. At the 891 micron level the average total
nucleolar volume for two-nucleolate nuclei is less than one-
third the volume at the 423 micron level.

From the maximum and minimum nucleolar volumes
listed in tables 4 and 6 it can be seen that while the average
total nucleolar volumes show the gradations just referred to,
in passing in the roots from one level to the next, the ob-
served minimum and maximum total nucleolar volumes for indi-

vidual nuclei do not necessarily show any such gradations.
The maximum nucleolar volumes observed nearly always occur
in nuclei larger than the average whereas the minimum
nucleolar volumes usually occur in nuclei smaller than the
average.

Volumetric measurements above the levels indi-
cated in the tables have not been made. However, by direct
inspection of serial cross sections, and serial longitudinal
sections of several different tulips, it was clear that the
nucleolar volumes are less in regions above the active
mitotic region than in regions nearer the growing point.
The largest nucleoli occur in or very near to the region of
most active mitosis. It is in this region that the nuclei
attain their greatest dimensions.

Nucleolar volume in relation to chromosome number.
Table 7 summarizes some measurements from diploid and heter-
oploid cells in a root of La Reine Maximus. Measurements
from a root of Carrara (data from table 4) and from one root
of the triploid Inglescombe Yellow (data from table 1) are
also included. The measurements are not strictly comparable
due to differences in relative levels in the roots concerned.
To make them so would have required more measurements than
were possible in this study. The diploid and heteroploid
areas in the one root, however, offered excellent material
for comparisons. The normal nuclei were measured in an area

Material Determined	Avg. cell size (1)	Avg. nuclear size (3)	Avg. total nucleolar volume (2)	Avg. nucleolar no. for nuclei measured	Minimum nucleolar volume observed	Maximum nucleolar volume observed
24 chromosome cells in La Reine Maximus.	5.79	1.15	20,903.9	4.8	14,023.6	25,171.0
DO but 72 ? chromosome cells.	14.70	3.63	43,419.6	23.0	35,446.1	48,380.8
Carrara 24 chromosomes	4.66	1.70	35,941.2	4.0	24,844.5	50,507.1
Inglescombe yellow 36 chromosomes	not det.	1.70	32,160.0	6.5	21,075.6	40,379.6

Table 7.   Nucleolar volume in relation to chromosome number.

(1) Nuclear size in square inches X 2000;
(2) Nucleolar volume in cubic scale units.

immediately surrounding the chimeral region. In the case of Carrare the data was obtained from an area likely to have relatively higher nucleolar volumes than the region used in Inglescombe yellow. The data in table 7 shows that in increase in chromosome number is accompanied by an increase in total nucleolar volume in the case of the root of La Reine maximus but, that there is little if any significant difference between the diploid Carrara and the triploid Inglescombe yellow. The former even had a slightly higher nucleolar volume. These two tulips were selected because of their similarity. To make accurate comparisons of this type it is obvious that all of the clons should be of the same genetic origin i.e., absolute auto-polyploids derived by some means of vegetative reproduction. Such tulips of absolutely certain origin, were not available.

Pre-Meiotic stages

Material has been very limited for studies of pre-meiotic stages. The data here presented was obtained from longitudinal sections of anthers. It was difficult to get a fixation and stain that differentiated the nucleoli clearly. This portion of the investigation was undertaken to determine the nucleolar changes, if any, that occur between the last pre-meiotic interphase and the heterotype prophase.

Nucleolar number. Nucleolar counts have been made in three tulips, i.e., the two diploids Mrs. Moon and John
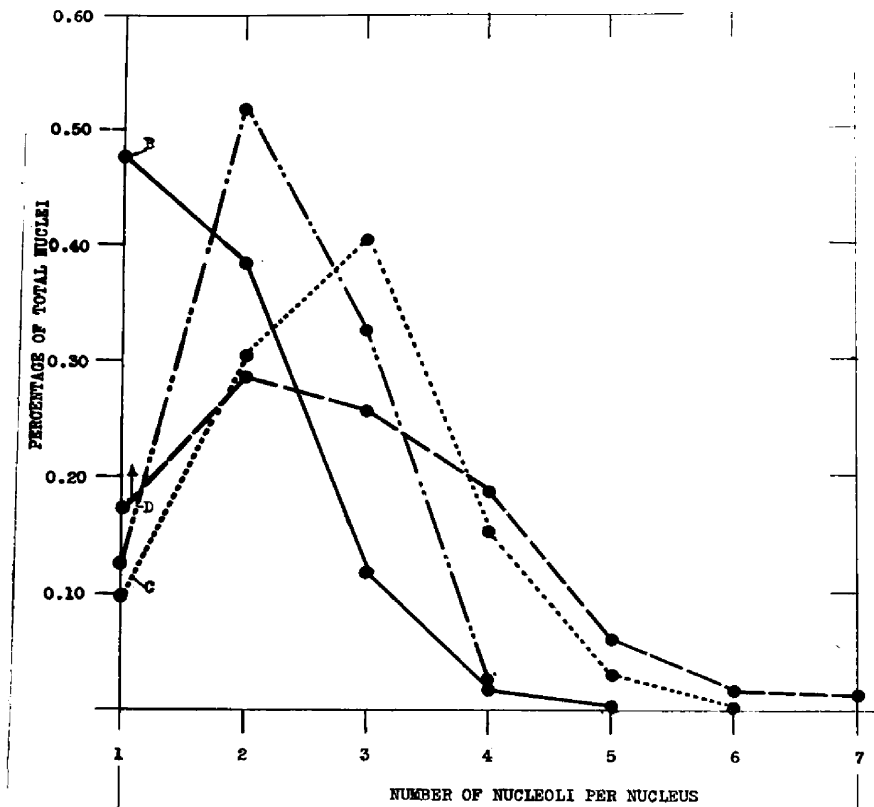
Figure 13. - Nucleolar frequencies in pre-meiotic interphases
and first meiotic prophase in anthers of Mrs. Moon and John
Ruskin: A, Mrs. Moon, pre-meiotic interphase, divisions still
occurring, from 263 nuclei with an average nucleolar number of
2.77; B, Mrs. Moon, first meiotic prophase, 105 nuclei with
average nucleolar number of 1,70; C, John Ruskin, last pre-
meiotic interphase, from 507 nuclei with an average nucleolar
number of 2.72; D, John Ruskin, first meiotic prophase, from
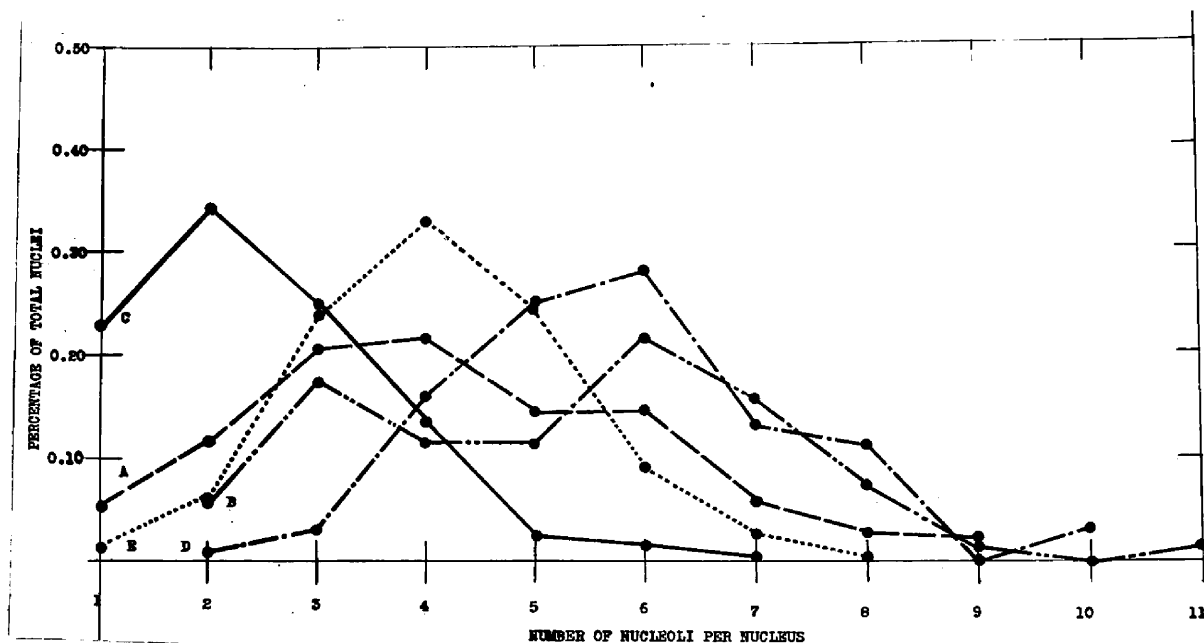224 nuclei with an average nucleolar number of 2.25.

Figure 14. - Nucleolar frequencies in pre-meiotic and meiotic
stages in anthers of the triploid Inglescombe Yellow:
A, Pre-meiotic interphase, divisions still occurring in anther,
from 207 nuclei with an average nucleolar number of 4.25:
B, last pre-meiotic interphase, from 69 nuclei with an average
nucleolar number of 5.02;
C, first meiotic prophase, from 204 nuclei with an average nucleo-
lar number of 2.44;
D, dyad nuclei, from 100 nuclei with an average nucleolar number
of 5.77;
E, microspores in tetrad stage, from 400 nuclei with an average
nucleolar number of 4.13.

(Note: small nucleolar granules in dyad and tetrad nuclei not
       counted. See text)

Ruskin and the triploid Inglescombe yellow. In figure 13 the
nucleolar numbers per nucleus have been plotted against their
frequencies for pre-meiotic interphases and the first meiotic
prophase stages in the two diploids. The former stages ob-
served in Mrs. Moon represent a condition found several weeks
before the advent of the meiotic prophase. At this time
nuclear divisions were still frequent in the microsporogenous
tissue. The same stages for Inglescombe Yellow are represent-
ed graphically in figure 14.

In the pre-meiotic stages in Mrs. Moon most of the
nuclei possessed one large nucleolus with several small addi-
tional ones. Nuclei with five, or less, nearly equal-sized
nucleoli, however, were fairly common. The graphs do not
take these size differences into account. The mean number
of nucleoli per nucleus at this stage in Mrs. Moon was
approximately 2.8, whereas the first meiotic prophase was
characterized by a mean nucleolar number of about 1.7. Nuclei
with seven nucleoli were found at the former stage but, none
with more than five at the latter. The pre-meiotic nucleoli
were typically much more uniformly spherical in shape than
root-tip nucleoli. They also lacked the distinct and large
"bud"-like structure so characteristic of the first meiotic
prophase in all tulips examined. Somewhat similar, though
smaller, structures have been observed on pre-meiotic nucleoli.
These are probably satellites.
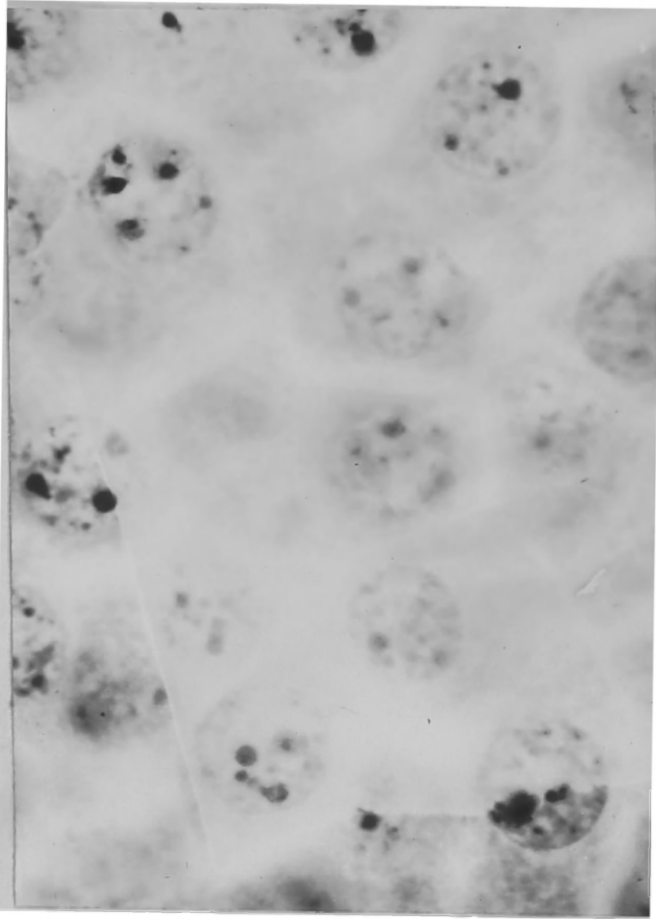
A
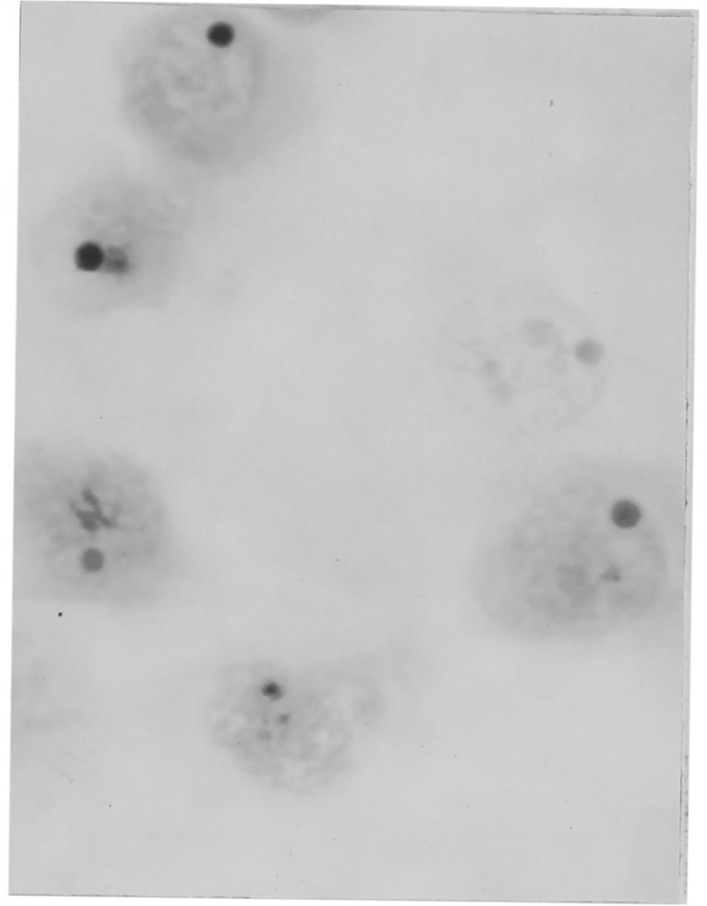
B

Figure 15. — Inglescombe Yellow: A, Pollen mother cells in
last pre-meiotic interphase; B, pollen mother cells in first
meiotic prophase. Photomicrographs X ap. 600.

Material was obtained from John Ruskin and
Inglescombe Yellow that unquestionably represented the last
pre-meiotic interphase. It was collected in September about
ten days before the heterotype prophase was observed in any
bulbs of these varieties. The nuclei in the last pre-meiotic
interphase were all very uniform in size and appearance
(fig. 15,A). All of the nuclei in the sporogenous tracts
were in the interphase condition. Some anthers of John
Ruskin, collected just before the cessation of divisions were
very similar in appearance except for slightly smaller nuclei
and the presence of a few nuclei still in the process of divi-
sion. The bud-like formations typical of the meiotic prophase
were not observed on pre-meiotic nucleoli of John Ruskin but,
small stalked spheres resumbling satellites were sometimes
found attached to nucleoli of Inglescombe Yellow at these
stages. In both tulips the average and maximum numbers of
nucleoli were higher in the interphase preceding the hetero-
type prophase than at the latter stage (figs. 13,14,15).

Meiotic Stages

The nucleolar numbers and volumes remain fairly
constant from leptonema to late diakinesis. The nucleolar
counts and measurements here reported were made chiefly from
late zygonema or early pachynema to early diakinesis. The
nucleoli and the relation of the chromosomes to them were
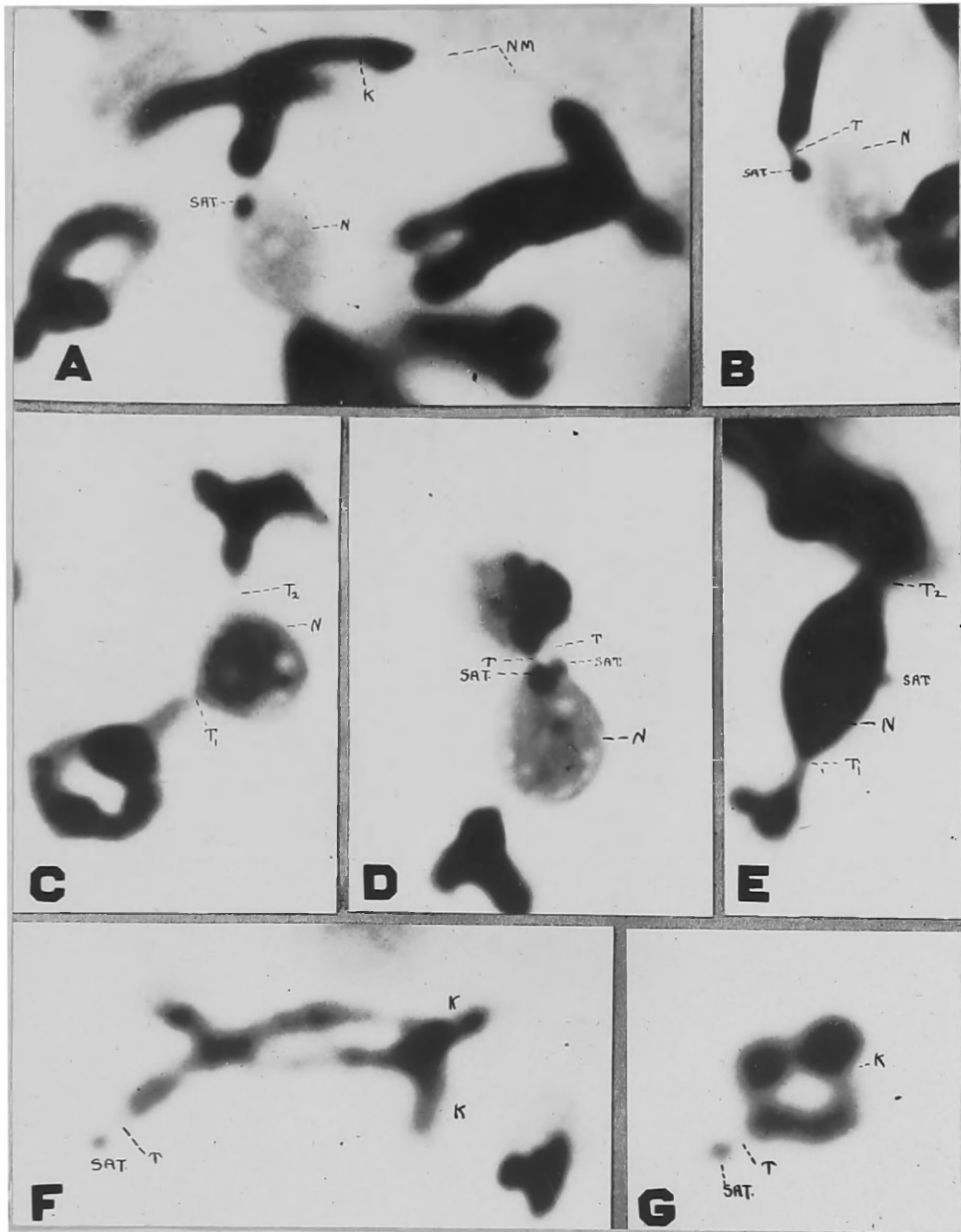
Figure 17. — Nucleoli and satellited chromosomes at diakinesis:
(In all the figures SAT refers to the satellite or "bud" (see
text), N refers to the nucleolus, T denotes the satellite
attachment thread, K marks the position of the kinetochore and
in A the nucleolar membrane is labled NM) A, showing attach-
ment to the nucleolus of one of the chromosome pairs hetero-
morphic for satellites; B, another nucleus showing same type of
attachment as in A except for distinct connecting thread (T);
C, nucleolus attached to two heteromorphic pairs of chromosomes;
D, showing attachment of both satellites of homomorphic chromo-
some pair to nucleolus; E, nucleolus attached to two hetero-
morphic pairs of chromosomes; F, one of heteromorphic chromo-
some pairs showing satellite; G, homomorphic chromosome pair,
satellites paired. All from the variety.Adonis. Photo. X
ap. 4000.

most readily studied at these stages. The first meiotic
prophase was remarkably uniform in appearance with respect
to nucleoli.   In the diploids each pollen mother cell
nucleus generally possessed one or two large nucleoli, and
one or more additional small ones.   Rarely three nearly
equal-sized ones occurred.   The mean numbers of nucleoli
per pollen mother cell nucleus in the forms counted lay be-
tween 1.5 and 2.5.   The numerical characteristics of meiotic
nucleoli are discussed below.

Relation of the chromosomes to the nucleoli.   It
was not possible to accurately trace the nucleolar-chromosome
relationships during the contraction.   During late zygonema
and pachynema, however, it was found that each nucleolus was
attached to at least one pair of chromosomes.   A fairly typi-
cal situation is illustrated in figure 12, plate 1.   Three
chromosomes were attached to the largest nucleolus and one to
the smaller.   There were only two nucleoli present in this
nucleus.   Satellites or "buds" were not visible.

Whereas all, or practically all, of the chromosomes
in root-tip nuclei are attached directly to nucleoli in the
prophase only a few are so attached in the meiotic prophase
from leptonema to late diakinesis.   The nucleoli disappear at
the latter stage.   In the somatic prophase the chromosomes may
be attached terminally or laterally to the nucleoli (fig. 22,
pl. 1).   In first meiotic prophase, however, the chromosomes
are apparently always attached terminally.   The nucleoli during

these stages are generally characterized by a peculiar bud-
like structure situated on the surface of the nucleolus
(figs. 3, 9 and 11, pl. 1 also fig. 16,B, F and J). Rarely
a very finely stalked small satellite-like structure was seen
projecting from the "bud".   As the result of a study of many
nuclei from early diplotene to late diakinesis it was conclud-
ed that the nucleolar "bud" represents the place of attachment,
or the approximate place of attachment of the satellites to
the nucleolus. This view is in keeping with the occasional
observance of smaller satellite-like structures projecting
from or lying near the "bud".   That the association between
satellite and "bud" is usually extremely close, however, is
evidenced by the condition found in most nucleoli (fig. 3, pl.
1). With sufficient destaining the "bud" usually appears as
a partly projecting sphere with a darkly stained center, no
other projections or satellite-like bodies being visible.   In
siutations like those illustrated in figure 17, A, B, D,and
figs. 1, 6 and 7, pl. 1, it is difficult to distinguish between
"bud" and satellite. The chromatic connecting thread to the
chromosome arm is often very distinct (fig. 17, B), the large
darkly staining sphere (the "bud"?) appearing like a true
satellite.   In a few cases a second, smaller darkly staining
body, was observed close to the larger sphere but the chromatic
connecting thread occurred between the larger body and the
chromosome arm. The different aspects of nucleolar structure

observed during the first prophase are illustrated in figure 17 and figures 1 to 12, plate 1. It seems likely that the "bud" represents a nucleolar structure with which the satellite or satellites are very closely associated or even fused. However, it also seems likely that in some cases the bud-like differentiation is absent in which case typical satellites alone are located on the region the "bud" would have occupied (fig. 1, pl. 1 and fig. 17, D). That the chromosomes themselves are firmly attached to the nucleoli is shown by the conditions illustrated in figures 4 and 9, plate 1 and, figure 17, E. The nucleolar matter must be considerably plastic to account for the spindle-shaped form frequently observed. In such cases the nucleolus and attached chromosomes look as though they had been under tension at the time of fixation.

In the single late tulip, Adonis, exceptionally good material was obtained for a study of satellites. In this tulip it was possible to accurately determine the number of satellited chromosomes. By observing a large number of nuclei at diakinosis it was found that there are six, and only six, of these. These are unquestionably the chromosomes that are associated directly with the nucleoli. There are four pairs of chromosomes in which one, and only one, homologue of each pair bears a satellite (figs. 5 and 8, plate 1 and fig. 17, F). There is a fifth pair in which each homologue bears a satellite. In this latter pair the satellites are usually, though not

always, closely paired in the first meiotic prophase (figs.
1 and 2, pl. 1 and fig. 17, D and G).   All of the satellite-
bearing chromosomes are not necessarily associated with
nucleoli during diakinesis or are all of the same ones always
associated with these structures. (figs. 10 and 11, pl. 1).

The satellites occur on the ends of the distal
(long) arms of the chromosomes.   In some cases they have been
observed clearly on metaphase chromosomes in the equitorial
plate stage.   Two typical diakinetic nucleoli are illustrated
in figures 10 and 11, plate 1.   In each nucleus 12 pairs of
chromosomes can be readily counted.   These have been numbered
more or less arbitrarily as it has not been possible to
accurately distinguish all of the different morphological
types characteristic of the tulip karyotype.   The satellite-
bearing chromosomes have also been given the same numbers in
each nucleus, but the only pair which can be definitely determined
is the pair morphologically homologous for satellites (No. 7).

In the nucleus shown in figure 10, plate 1, the
pair morphologically homologous for satellites (number 7) is
not attached to any nucleolus.   Two satellite attachment
threads were apparently present, but the satellites (marked
S in the figure) themselves were very closely paired. Chromo-
some pair 12 is attached to a small spherical nucleolus (N).
The large spindle-shaped nucleolus is attached to one homo-
logue of each of two pairs of chromosomes (5 and 11). Chromo-

some pair 4 is not attached to a nucleolus but the satellite

(S) on one of the homologues is distinctly visible.   In

figure 11, plate 1, a very different situation obtains.   The

chromosome pair consisting of two satellite-bearing chromo-

somes is here attached by one member to the principal nucleo-

lus (note distinct "bud") and, by the other homologue to a

much smaller nucleolus.   These nucleoli are each attached to

pairs of chromosomes morphologically non-homologous for

satellites.   The small nucleolus is attached to chromosome

number 5 and the large one to number 4.   The other two satel-

lite-bearing chromosomes, numbers 11 and 12, are each attached

to a single nucleolus.   Apparently all possible combinations

of association of the satellite-bearing chromosomes with the

nucleoli may occur.   The pollen mother cells of Adonis most

often contained but one nucleolus (fig. 18).

In the triploid, Inglescombe Yellow, trivalent

chromosome groups have been observed at diakinesis in which

each homologue possessed a satellite.   Others were apparently

non-homologous with respect to satellites.

Definite connecting strands, similar to those

observed in root-tip mitosis, have been observed at zygotene

and diplotene of the first meiotic prophase.   These may

occur both laterally and terminally between the chromosomes.

The inter-connections or anastomoses are apparently reduced

in number as the diplonema stages move into diakinesis.
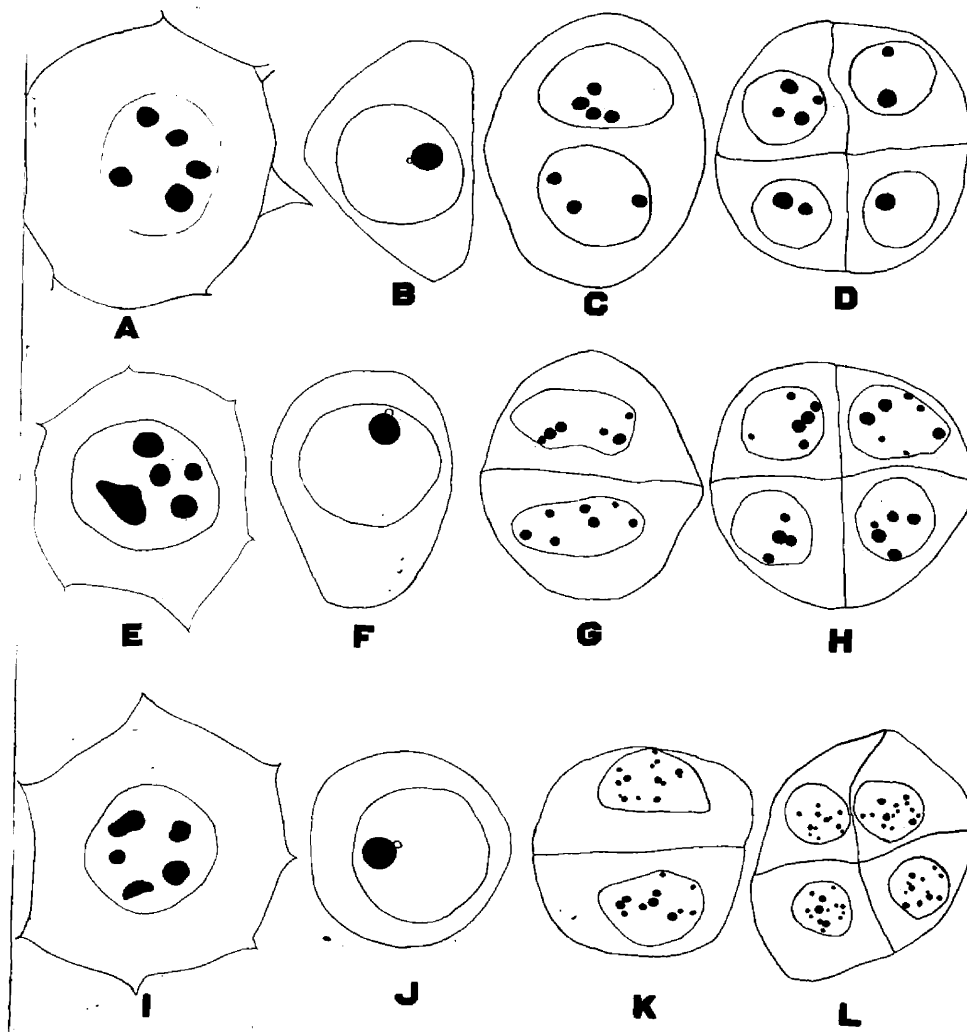
Figure 16. — Types of nucleolar organization in diploid and haploid stages of several tulip varieties: A to D, Adonis; E to H, Herman Waller; I to L, Mrs. Moon. All nuclei in interphase condition. Five, and single nucleolate nuclei were arbitrarily selected to represent root-tip and first meiotic stages. In each case the stages are arranged in the following order from left to right: root-tip, first meiotic prophase, dyad stage, and tetrad stage. Camera lucida drawings X 2000, reduced abt. ½.

Whereas the nucleolar matter disappears in late diakinesis or early metaphase, the satellites may still be visible in the equatorial plate stage. Usually, however, they too cannot be seen after diakinesis.

Numerical relations. All of the tulips examined are characterized by remarkably low nucleolar frequencies during the first meiotic prophase. The observed frequencies are represented graphically in figures 13, 14, 18, 19 and 20. The frequencies at the second interphase or tetrad stage are also given in these figures together with the root-tip frequency for comparison.

Whereas all of the tulips examined were very similar in the first meiotic prophase striking differences occurred in the haploid or reduced phases. The three principal types observed are illustrated semi-diagrammatically by camera lucida drawings in figure 16. The first vertical column on the left, A, E and I, show typical root-tip nuclei. Five-nucleolate nuclei were arbitrarily selected. The second column, B, F and J, illustrates typical nuclei of the first meiotic prophase. Single-nucleolate nuclei were also arbitrarily chosen. The differences in nucleolar behavior responsible for this classification are illustrated in the remaining two columns.

In the first type of nucleolar organization (C and D) the number of nucleoli at first interphase and in the

Figure 18. – Nucleolar frequencies in Adonis:
A, first meiotic prophase, 400 pollen mother cell nuclei
with average nucleolar number of 1.49;
B, microspores in tetrad stage, 400 nuclei with average
nucleolar number of 2.10;
C, root-tip, 1000 nuclei with average nucleolar number of
3.57.

Figure 19. — Nucleolar frequencies in Gipsy:
A, first meiotic prophase, 203 pollen mother cell nuclei with average nucleolar number of 2.33;
B, microspores in tetrad stage, 400 nuclei with average nucleolar number of 2.53;
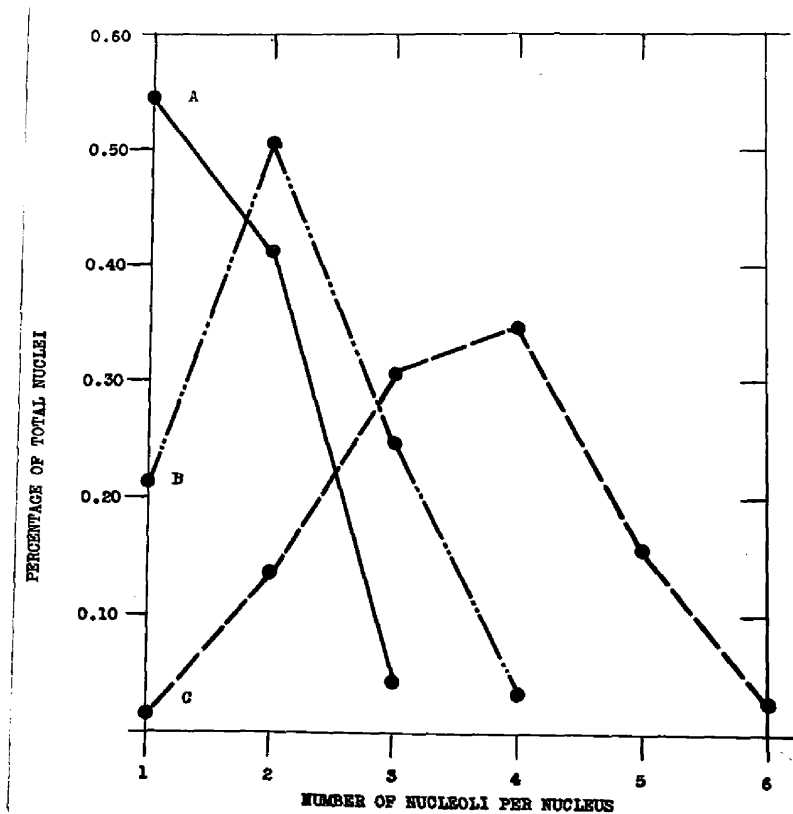C, root-tip, 500 nuclei with average nucleolar number of 4.90.

Figure 20. — Nucleolar frequencies in Herman Waller:
A, first meiotic prophase, 200 pollen mother cell nuclei with
average nucleolar number of 1.91;
B, microspores in tetrad stage, 100 nuclei with average nucleo-
lar number of 3.10;
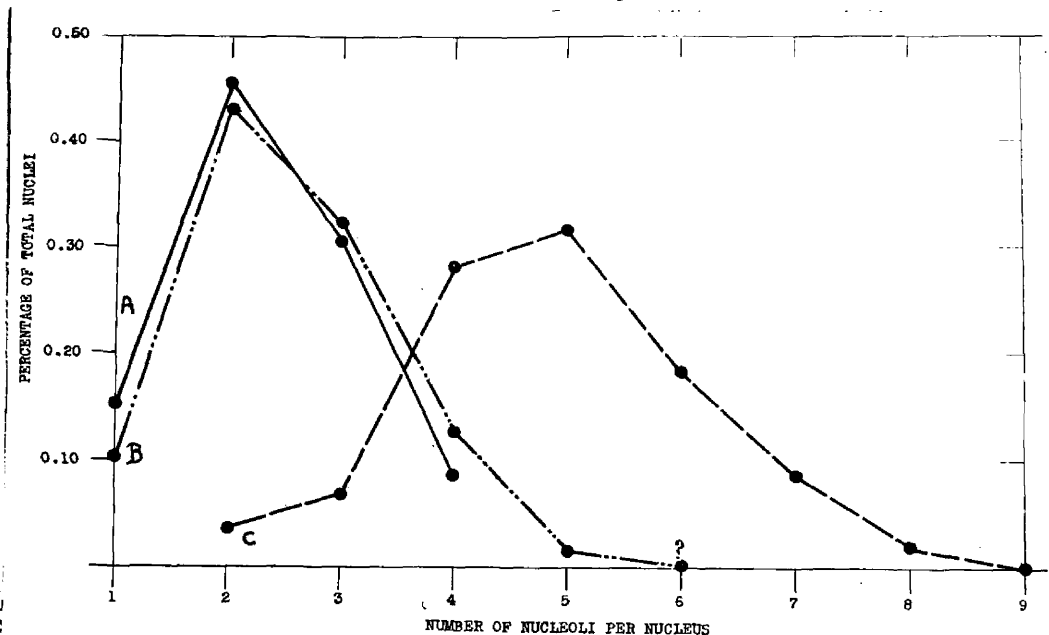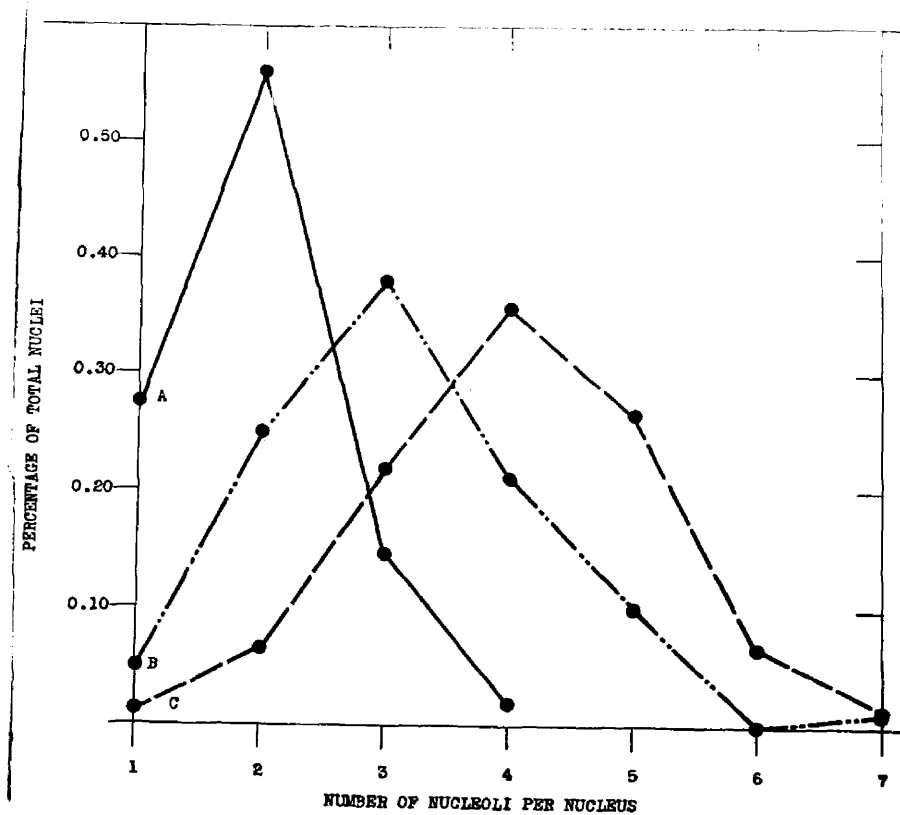C, root-tip, 1231 nuclei with average nucleolar number of 4.04.
(Note: nucleolar granules in microspores not counted.
See text)

tetrad stage is always, or nearly always, fixed within definite limits. The nucleolar numbers occurring in the haploid nuclei nearly always fall within the limits possible on the theory that the maximum number of nucleoli for the root-tip is equal to the number of nucleolus-collecting or forming chromosomes. There occurs, therefore, a characteristic maximum nucleolar number for haploid nuclei. The dyad illustrated in figure 16 C constitutes the only observed exception in the tulip Adonis. There are a total of seven nucleoli present whereas the maximum number of nucleolus collecting chromosomes in the diploid soma is six. No exceptions were noted in the microspores, however.

In the second type of organization the nucleolar number is much less regular. Numbers of nucleoli are found at the dyad and tetrad stage that could not possibly occur if the nucleolar chromosomes all continued to function completely in the haploid cells. The extra nucleoli are usually small, however, and the larger nucleoli do follow a somewhat regular distribution (fig. 19).

The third type shows no definite nucleolar organization whatever in the haploid nuclei. The number of nucleolar bodies (or granules) frequently is above the number of chromosomes. All of the dyad and tetrad nuclei show the same lack of nucleolar organization i.e., there is no segregation of this factor.

Name and the somatic chromosome number (Det. in root-tip)	Root-tip "characteristic area"			Anther Last pre-meiotic inter-phase			Anther First meiotic prophase			Microspores Tetrad stage (2)		
	Avg.	Max.	No. Det. (1)	Avg.	Max.	No. Det.	Avg.	Max.	No. Det.	Avg.	Max.	No. Det.
S.L.Adonis (24)	3.60	6	1000	not det.	not det.	-----	1.49	3	400	2.10	4	400
N.M.Herman Waller (24)	4.05	7	1231	DO	DO	-----	1.91	4	200	3.10 plus n	7 plus n	100
S.L.Mrs.Moon (24)	4.73	8	2640	DO	DO	-----	1.70	5	105	n	n	100 plus
S.L. John Ruskin (24)	4.89	8	4585	2,72	6	507	2.25	4	224	not det.	not det.	-----
N.M.Gipsy (24)	4.65	9	500	not det.	not det.	-----	2.33	4	203	2.53	5 or 6?	400
S.L.Inglescombe yellow (36)	7.59	13	1475	5.02	11	69	2.44	7	204	4.13 plus n	8 plus n	500

Table 8.   Nucleolar numbers in certain garden tulips. Average and maximum numbers per nucleus are listed. Note: (1) total numbers of root tip nuclei counted to obtain maximum nucleolar number. Average values based on data of table 1. (2) symbol (n) refers to an indefinite number of nucleolar granules.

The above described types of nucleolar organiza-
tion have been checked in both smear and paraffin-sectioned
material. They are unquestionably characteristic of the
particular clons investigated and do not represent the re-
sults of fixation, pre-treatment, etc. The single late or,
cottage tulip, Adonis and the New Mendel tulip Gipsy belong
in the first class whereas the New Mendel, Herman Waller and,
the single late Ingelscombe Yellow belong in the second class.
Mrs. Moon is the only representative of the third class found
thus far. Other tulips have not yet been classified on this
basis.

For the sake of comparison the nucleolar numerical
characteristics at different stages in the life cycle have
been brought together in table 8.

Volumetric relations. The nucleolar volumes at
any given stage are more constant during the phases of
meiosis in the anther than in root-tip nucleoli. Some varia-
tion was observed in average total nucleolar volumes following
different fixatives and methods of preparation, however. For
example in a permanent smear fixed in Flemming's medium fluid
from one anther of a bulb of Inglescombe Yellow the average
total nucleolar volume for ten pollen mother cell nuclei in
first meiotic prophase was 11,177.9 cubic scale units. In
the same type of material from another bulb fixed in Flemming's
fluid and prepared by the paraffin technic the average total
volume for ten nuclei was 10,581.0 cubic scale units. In the

former determination the maximum total volume observed was
13,645.2 cubic scale units and, the minimum 7,030.8 cubic
units whereas in the latter the maximum was 14,576.0 cubic
units and the minimum 7,257.6. In a third determination
from another bulb the average total volume for the first
prophase was 8,316.6 cubic scale units for 51 pollen mother
cell nuclei. The maximum value obtained was 11,497.9 cubic
units and the minimum 5,636.2 cubic units. Whereas the first
two determinations were essentially the same the third was
quite different.

Ten last pre-meiotic interphase nuclei in an
anther of Inglescombe Yellow had an average total nucleolar
volume of 12,337.4 cubic scale units. The highest volume was
16,961.6 cubic units and the lowest 8,625.7 cubic scale units.
The average nuclear size was approximately 1.6 (square inches
X 2000). Table 9 presents volumetric data for the nucleoli
of three tulips in which measurements were made in both root-
tips and sporogenous tracts of anthers. The data is intended
to give only a very approximate idea of the relative volumes
of nucleolar matter at different stages of the life cycle.

Stage of life cycle	Measurements	Name of Tulip and Chromosome number		
		Single late ADONIS (24)	New Mendel GIPSY (24)	Single late INGLESCOMBE YELLOW (36)
SOMATIC INTERPHASE (root tip)	Avg. Nuclear size (1)	1.33	1.77	1.70
	Max. Nucleolar volume (2)	32,131.9	55,016.2	40,879.6
	Avg. Nucleolar volume	18,386.4	37,819.7	32,160.0
	Min. Nucleolar volume	8,796.4	29,102.7	21,075.6
	Avg. no. Nucleoli in nuclei measured (3)	3.6	5.8	6.5
	Number of Nuclei measured	10	10	10
FIRST MEIOTIC PROPHASE (anther)	Avg. Nuclear size	1.44	1.42	2.54
	Max. Nucleolar volume	12,804.2	14,973.4	13,645.2
	Avg. Nucleolar volume	10,055.5	8,800.8	11,177.9
	Min. Nucleolar volume	6,633.8	5,662.1	7,030.8
	Avg. no. Nucleoli in nuclei measured	1.5	2,2	1.7
	Number of Nuclei measured	20	10	10
MICROSPORES IN TETRADS (anther)	Avg. Nuclear size	0.51	0.73	0.60 (a)
	Max. Nucleolar volume	2,358.3	3,175.2	4,258.5
	Avg. Nucleolar volume	1,323.2	2,092.0	2,597.7 (b)
	Min. Nucleolar volume	742.7	1,584.3	1,828.6
	Avg. no. Nucleoli in nuclei measured	2.0	2.5	3.9
	Number of Nuclei measured	40	20	a --- 52 b --- 88

Table 9. Nucleolar volumes compared in root tips, first meiotic prophase and microspores. Note: (1) avg. nuclear size expressed in cross-sectional areas as square inches X 2000; (2) volumes in cubic scale units: (3) representative nuclei arbitrarily selected for measurement. For characteristic means see Table. 1.

DISCUSSION

There can be little doubt that the nucleolar material (plastin) is developed by or on the chromosomes in telophase. The observations of many workers, Van Camp (29), Dermen (1), Sorokin (25), Zirkle (33), Wager (30), Heitz (5), McClintock (11), etc. all support this view. There has been considerable divergence of opinion, however, as to the exact method of origin. Heitz (5), for example, as a result of work on a number of monocots and dicots came to the conclusion (5 pg.779) that "Die Nukleolen enstehen an (nicht aus) bestimmten chromosomen". These chromosomes he designated as nucleolus chromosomes or "SAT Chromosomen" because they possessed trabants or satellites set off by achromatic threads or constrictions the latter being "Thymonukleinseure-frein Stellen" (i.e., "sine acido thymonucleinico"). The nucleolus, according to him, develops in this achromatic region behind the satellite. It is important to stress his use of the word "an" in contrast to "aus" to denote the origin of the nucleolus. Heitz also observed that the nucleolar number in telophase ("primarzahl") was equivalent to the number of SAT-chromosomes characteristic of the species. He (6) later showed, however, that when small extra nuclei were formed by lagging or fragmented chromosomes these might form a nucleolus even when the SAT-region was absent. From this he concluded that all chromosomes give off the nucleolar

material but that the SAT-chromosomes perform the function

of organizing this into a definite number of bodies.

McClintock (12) has shown in Zea that it is not the

achromatic stalk of the satellite but a specialized region

just behind this that is the real nucleolus-forming region.

In contrast to the above views Schaede (20), working with

Allium, Lilium, etc., came to the conclusion that the nucleo-

li form not from the telophase chromosomes but rather result

from a condensation of small vacuole-like particles that ori-

ginate in the nuclear sap. Sorokin (25) found that the

nucleoli contain at least part of the actual chromatin of the

chromosomes in Ranunculus, Anemonella, and Isopyrum. Accord-

ing to Dermen (1) the nucleolus may represent a by-product of

the chromosome matrix and the nuclear sap formed as a surface

phenomenon which can be explained by the assumption that a

chemical reaction takes place between the surface substance of

the chromosomes and the surrounding medium. Zirkle (33, pg.413)

as a result of his studies on Zea concluded that "as the chromo-

somes unite to form the daughter nuclei, and as the nuclear

membranes are formed, the nucleolar material collects into

several droplets. These droplets, united by stainable threads

flow together and form the nucleolus of the resting cell".

Zirkle's description for the telophase in Zea seems

to fit the situation in Tulipa.   In the latter genus the

chromosomes establish inter- and intra-connections between

their arms during anaphase and telophase.   These anastomoses

appear to originate from the matrix substance and may well facilitate the aggregation of plastin in the telophase. Inter-connected masses of the latter material as described by Zirkle (33) for Zea also occur in Tulipa (fig.2 C, E, F, G). The plastin is apparently matrical in origin, originating from the mass of anastomosed telophase chromosomes. There are present in each telophase nucleus a definite number of nucleolus-organizing centers which eventually, with or without fusion of adjacent centers, become the interphase nucleoli. It is believed that these plastin-collecting centers represent the positions occupied by the nucleolus-forming regions of certain chromosomes of the complement although this cannot be observed in mitosis. However, from evidence obtained at meiotic stages it seems very probable that it is the satellite-bearing ends of certain chromosomes that so function.

Much has been written as to the relation between the prophase chromosomes and the nucleolus. The majority of workers in this field have described the definite attachment of the chromosomes to the nucleolus in somatic prophases. Zirkle (33, fig. 2) in Zea figures distinct attachment of the "spireme" to the nucleolus. His figures for Pinus (34, fig. 27, pl.6, figs. 1,2,3, etc., pl.3) are very clear and definite. Wager (30) presents illustrations showing clearly the attachment of the prophase chromosomes to the nucleolus. Such attachments to the nucleolus are figured abundantly in

both nucleolar and other cytological literature. These
cover a wide range of micro-technical methods and material.
There can be no doubt that the chromosomes of many organisms
are regularly attached to the nucleoli in somatic prophase.
There cannot be the slightest doubt of this in _Tulipa_. In
this genus, at least, it seems possible that the actual
attachment of the chromosomes to the nucleolus occurs in early
telophase. This has not been substantiated, but there is an
indication of nucleolar-chromonema attachments in the inter-
phase. Zirkle (33), however, was unable to definitely demon-
strate connection of the chromosomes to the nucleolus at
interphase in _Zea_. The characteristic "bouquet" arrangement
of all or most of the chromosomes (fig.1, H and 2, J,K,L).
about the nucleoli suggests that some sort of an attraction
may exist between the nucleolus-forming centers and the
chromosomes. It is possible that the activities of nucleolus
organization accomplished by the nucleolus-forming chromosomes
brings this about.

Dermen (1) seems to attach little significance to the
attachment of the chromosomes to the nucleolus. In fact (pg.
314) he " ---found no connection between the nucleolus and
chromosomes in _Yucca_." As a result of applying reagents to
living stigma hair cells of _Callisia_ Dermen (1 pg.305) came
to the conclusion "---- that even at early stages there is
no complete direct association between all the chromosomes
and the nucleolus and that a single attachment point between

a chromosome and a nucleolus can not be considered of any physiological importance".

As already mentioned, Zirkle's (34) drawings and photomicrographs of nuclei in Pinus are unusually clear and convincing with respect to the attachment of the chromosomes to the nucleolus. Dermen (1) likewise worked with Pinus but, due to technical difficulties did not figure the chromosomes in any of his illustrations of this genus in which nucleoli were involved. Two of his figures are of cells in Pinus strobus, the same species used by Zirkle. Frew and Bowen (3), however, like Dermen were unable to establish nucleolar-chromosomal connections. In regard to Dermen's (1) view that the attachment of only one chromosome to the nucleolus could have no physiological significance the apparently general occurrence of anastomoses between chromosomes (see Sharp (21, pg.135) should be considered. While these may have no physiological importance whatever, it should be noted that in Tulipa at least, they may persist in the root-tip until well into metaphase. If the chromosomes form an inter-connected plexus in the prophase it would be at least unwise to assume that the connection of one or a few members of the plexus with the nucleolus was without physiological significance to the other members.

Another feature of the prophase that has attracted much attention is the relation of the satellites to the nucleolus. S. Navashin (17,18) was the first to call atten-

tion to these structures. In Galtonia candicans he was able
to show that the satellite-bearing chromosomes in somatic
prophase were in contact with the nucleolus by their satellites.
The work of Heitz (6) has already been referred to. McClintock
(12) described the satellites borne on the members of chromo-
some pair VI of Zea mays although most of the evidence here
is on meioticmchromosomes. These are definitely the nucleolus-
forming chromosomes. Sorokin (25) observed structures
("nucleolosomes"), probably satellites, constantly associated
with prophase nucleoli in Ranunculus abortivus, Anemonella
thalictroides and Isopyrum biternatum. She was able to de-
finitely establish the identity of "nucleolosomes" and true
chromosomal satellites in R.chius. Smith (23) has presented
a clear account of the satellites in relation to the nucleolus
in Galtonia candicans. In somatic prophases he found at least
one of the satellite chromosomes near the nucleolus and in
many cases the satellite was in contact with it. Dermen (1)
observed some relation between satellite chromosomes and
nucleoli,but he does not seem to be convinced of its constancy
or importance.

It is difficult to demonstrate the satellites in the
somatic prophase of Tulipa. While they have been definitely
observed at this stage it was very difficult to determine the
exact number of chromosomes bearing them in root-tip nuclei.
The large number and size of the chromosomes together with

the minuteness of the satellites (in most cases) make it
practically impossible to do this. At metaphase satellites
were more easily seen but, except for a few cases the nucleo-
lar matter had all disappeared at this stage. The metaphase
satellites are more distinct in T. sprengeri than in any
other tulip examined. A maximum of seven satellited chromo-
somes was found in one metaphase plate but usually the number
observed was much less. The highest observed number of
nucleoli in this tulip is eight.

In addition to the fact that the satellites may be
hidden beneath the chromosome arms the attachment thread is
often so short that the satellite and chromosome arm seem to
be fused. A similar situation has been reported by Holings-
head and Babcock (7) in Crepis. The work of McClintock (12)
and of M. Navashin (16) is undoubtedly of great importance in
this connection. This will be considered in connection with
a discussion of meiosis below. It is sufficient to say at
this point with respect to Tulipa, that if the same chromo-
somes function in nucleolus formation in both the root-tip
and in microsporogenesis Heitz's contention with respect to
these chromosomes can be almost certainly verified, at least
in the case of the single late tulip Adonis.

The interphase nucleoli of Tulipa have been studied
principally in respect to their number in the root-tip. De
Mol (13) was the first to call attention to the regular

numbers of nucleoli that characterize certain plants. For
example, in diploid, triploid, and tetraploid varieties of
Hyacinth he found maximum nucleolar numbers of two, three,
and four respectively.

In the majority of investigated organisms the nucleo-
lar number is relatively low. Thus, to mention a few, Sorokin
(25) found one or two nucleoli in the Ranunculaceae investi-
gated by her, Zirkle (33) and McClintock (12) report one and
two (one in about 90% of the cells) in somatic cells of Zea
mays, Dermen (1) found two and rarely three nucleoli in
Callisia, Heitz (6) in Vicia faba with two SAT-chromosomes
found two, and Frew and Bowen (3) a single nucleolus in
Cucurbita pepo and C.maxima. Of particular interest, however,
are those species in which the nucleolar number is relatively
high. Zirkle (34) found nuclei containing five, six, or seven
nucleoli of common occurrence in Pinus strobus. A limited
number contained four or eight but the average was six. De-
tailed counts of a number of nucleoli are not presented.
According to Zirkle (34, pg.93) plurality of nucleoli is of
common occurrence in some genera. He lists Abies, Cedrus,
Larix, Picea, Pseudotsuga, Sciadopitys and Tsuga as plants
having high nucleolar numbers. He also states that other
gymnosperms have regularly but two nucleoli in a nucleus, but
that even these may be subject to variability. In the low
number group he cites Agathis, Ginkgo, Juniperus, Sequoia,

Taxus and Thuja. Dermen (1) found as many as 14 nucleoli
in a cell of Pinus strobus. Schaede (20) found three to five
nucleoli in resting cells of Lilium martagon and, correspond-
ingly more in telophase. In all of the investigations referred
to the number of nucleolar bodies was more numerous in telo-
phase than in interphase. Fusion of nucleoli in telophase
probably accounts for this. In some cases at least it is
doubtful whether the masses of nucleolar matter observed at
this stage should be called nucleoli in the sense that they
represent the definite morphological units generally implied
by this term. In one way they represent nucleoli in the pro-
cess of formation.

De Mol (13) has reported that the "complex" nucleoli
present at the end of telophase may fragment to give several
"simple" nucleoli. Discussion of prophase so-called nucleolar
fragmentation will not be discussed here other than to say it
almost certainly does not occur in Tulipa. As Dermen (1) has
pointed out it is very doubtful if it is a real occurrence.

De Mol's (13) work on nucleolar number was very
thorough, but the forms worked with by him possessed relative-
ly low nucleolar numbers. So far as the writer is aware there
has been no thorough numerical analysis of a plant with a high
nucleolar number. A detailed statistical analysis of the data
obtained in Tulipa will not be given in this paper. The most
significant facts can be observed by inspection of the tables
and graphs. Each clon is obviously characterized by a defi-

nite and characteristic maximum nucleolar number. The
average number of nucleoli (see table 2) occurring in a
given clon is usually roughly proportional to the maximum.
Thus Adonis has a maximum of 6 and an average of 3.6.
Herman Waller is characterized by a maximum of 7 and an
average of 4.0. Louis XIV has maximum and average nucleolar
numbers of 9 and 5.2 respectively. Each of these varieties
has a somatic chromosome complement of 24 chromosomes. In
many of the tulips thousands of nuclei have been examined and
the nucleolar numbers have never gone above the maximum
characteristic for the different clons. The only logical con-
clusion that can be drawn is that a definite number of nucleo-
lus-forming chromosomes are functioning in each case. It
should be pointed out in this connection that while the exact
number of satellited chromosomes have not been determined the
number of these observed in some cases is quite high.- in T.
sprengeri only one less than the maximum nucleolar number.
The concept of a definite number of nucleolus-forming chromo-
somes is in keeping with the findings of Heitz (5), McClintock
(12), De Mol (13) etc. on other genera. In one tulip, Adonis,
it has been definitely shown that the maximum nucleolar number
in the root-tip corresponds exactly to the number of satellited
chromosomes (nucleolus chromosomes) occurring in the first
meiotic prophase. The exact number of nucleolus-forming
chromosomes at meiosis have not been determined for any other
tulip.

A further study of the numerical data shows that the average nucleolar number is subject to variation but, if adjustments are made for different levels in the root an average number fairly characteristic of the clon can be obtained.   As mentioned before, nucleolar numbers lower than the maximum are probably due to fusions between plastin masses that develop in the telophase. In this study it was noted that the average number of nucleoli sometimes showed a uniform variation between different levels of the same root (fig. 4). Spatial segregation of the nucleolus-forming chromosomes is obviously one of the important factors concerned here. As Heitz (5) has shown, when the nucleolus-organizing portions of two chromosomes lie close together in the telophase nucleus the chance of fusion between the two developing nucleoli is greatly enhanced.

Another point of interest in connection with nucleolar number is its relation to an increase in chromosome number through auto-polypleidy. De Mol (13) has claimed a direct correlation between the two. Dermen (1), however, was unable to demonstrate a consistent proportional relationship between the two in a polyploid series of Petunia. That auto-polyploidy in Tulipa may result in an increase in nucleolar number is clear from the situation in chimeral sectors of root-tips (fig. 11). It does not follow, however, that an

increase in chromosome number is always associated with an
increase in nucleolar number. For example, T.stellata,
T. chrysantha, and T. clusiana constitute a polyploid series
(diploid, tetraploid and pentaploid respectively) the members
of which are probably not specifically distinct although vary-
ing in certain minor characters. The 48-chromosome T.chrysantha
has an average nucleolar number (see table 2) considerably
lower than could be expected if a progressive increase in
chromosome number were always accompanied by an equivalent
increase in the number of nucleoli. Other similar cases can
be found in table 2. It should be stressed that the species
mentioned above are not auto-polyploids in the strictest
sense, i.e., they have not all been derived from the same
seed by purely vegetative propogation. There has been ample
opportunity for the occurrence of a number of chromosomal
irregularities due to segmental inter-change, fragmentation,
etc. Furthermore it seems likely that at least some of these
forms are rather heterozygous. The phenomenon of "amphiplasty"
discovered by M. Navashin (15,16) is of interest in connection
with the seemingly irregular inheritance of the nucleolus-form-
ing ability of certain chromosomes. This will be discussed
in more detail under meiosis.

Volumetric studies of nucleoli and nuclei have
shown that large nuclei generally have higher total nucleolar
volumes than do small nuclei. This is in keeping with Dermen's
(1) observations. Nuclei with a high number of nucleoli

usually have lower total nucleolar volumes than do nuclei
with only a few nucleoli.

An increase in chromosome number may be asso-
ciated with an increase in nucleolar volume (see heteroploid)
chimeras), but this relation does not necessarily hold be-
tween different diploid and triploid varieties, e.g.,
Carrara (2n) and Inglescombe Yellow (3n). Humphrey (9),
however, has observed a consistent relation between chromo-
some number and nucleolar volume in tomato. There is little
correlation, positive or negative, between nuclear size per
se and nucleolar number. In Carrara (fig. 12) a slight
positive correlation is indicated but the exceptions were of
such a nature as to indicate little significance to the seem-
ing relationship. A negative correllation is indicated in
T. sprengeri but exceptions also occur. It seems that the
relation between nuclear size and nucleolar number, if any,
is at best an indirect one.

Nuclear size might appear to be related to nucleo-
lar number because of the possible effect on spacing of the
chromosomes. It is obvious, however, that the size of an
interphase nucleus will not necessarily pre-determine the
spatial arrangement of the chromosomes in the ensuing telo-
phase. At the latter stage the chromosomes are always very
closely associated (fig. 1, B.C.). It is important to note
that Dermen (1) and Lutman (10) have both clearly shown that
nucleolar volume is closely related to the nutrition of the

cell. Regardless of all the factors involved it seems almost certain that the spatial arrangement of the nucleolus-forming chromosomes at telophase, and the relative amounts, of plastin generated at that stage, determine largely the number of nucleoli formed. Large amounts of plastin would encourage fusion of nucleoli and thus a reduction in their number.

The nucleolar situation in meiotic and pre-meiotic stages is rather different than in the root-tip. Differences occur in number, volume and structure of the nucleoli. There appears to be a reduction in nucleolar number between the last pre-meiotic interphase and some early stage of the first prophase. This apparently occurs previous to synapsis of the chromonemata. In Inglescombe Yellow, at least, there is apparently no reduction in nucleolar volume associated with this. Some sort of prophase fusion of nucleoli is therefore indicated. Dermen (1, pg.297) observed that "In pinus at early leptotene and during early diplotene, as many as nine nucleoli were found, the number decreasing as the development of the nucleolus advances but, the volume of total nucleoli not showing any decrease". The relation between the pre-meiotic and first prophase stages is therefore apparently similar in the two genera.

As the result of each meiotic division in Tulipa the nuclear and nucleolar volumes of each resulting nucleus

are progressively reduced but the total nucleolar volume for the whole tetrad of microspores is about the same as that of the pollen mother cell (table 9). Dermen (1,footnote pg.305), found that in Callisia the nucleolar volume varied in different parts of the plant system but was constant in all cells at all stages in microsporogenous tissue, measuring about 4 microns. He reports that "--- the size may be about 4 microns", in the root-tip. This is quite different from Tulipa where the average nucleolar volume in the mitotic region of the root-tip is much higher than in the sporogenous cells.

The first meiotic prophase nucleoli are usually characterized by a peculiar bud-like structure which is associated with the attachments of the satellited chromosomes. The latter may not all be attached to the nucleolus but at least one is. This attachment is very definite (fig. 17, A to E). Association of the satellited chromosomes seems to be of wide occurrence but, reports as to its constancy in any one organism vary. Newton and Darlington (19, 3c, pl.I; 4, pl. II, etc.) figure distinct bud-like structures on nucleoli of Tulipa in first meiotic prophase. The only satellite illustrated in this paper occurs in a somatic metaphase plate of Keizerskroon (19, text fig. 1). None of these structures are mentioned. The attachment of the chromosomes to the nucleoli are not designated although in fig. 3c, pl. 1, the trivalent is apparently definitely attached to the nucleolus.

Smith (23) has shown definite attachment of the satellited chromosomes to the nucleolus in Galtonia candicans. The heterotype nucleoli were characterized by one or two "buds" the satellites of the chromosomes being associated with these in the constricted region. The buds are somewhat similar to the ones seen in Tulipa but in Galtonia the distinction between "bud" and satellite is much more clearly defined. Dermen (1) frequently observed "a bud-like growth" on the side of the meiotic nucleolus but, was unable to establish its relationship with other nuclear structures. He could not demonstrate more than occassional association between the satellited chromosomes and the nucleolus. In Tulipa, at least, the nucleolar "buds" are apparently of considerable significance being associated intimately with the satellites and attachment of the chromosomes to the nucleolus as before mentioned. Horton (8) in Triticum observed attachment of the satellites to the diakinetic nucleoli in about 20 percent of 2305 nuclei examined. McClintock (12) has clearly demonstrated regular attachment of the nucleolus-organizing chromosomes in Zea mays. No explanation is offered for the different results obtained by these workers but, in Tulipa the association between satellited chromosomes and nucleoli in first prophase seems to be of constant occurrence in the varieties examined.

Of particular significance in Tulipa is the fact that the satellited chromosomes are not always morphologically alike in the heterotypic prophase. Thus in Adonis there is

one pair in which each homologue bears a satellite and four pairs in which only one of each bears one of these structures. The satellited chromosomes are the only ones that are attached to the nucleolus. The occurrence of satellite differences in otherwise homologous chromosomes was reported by S. Navashin in <u>Galtonia</u> <u>candicans</u>. He described races in which either all large, all small, or both large and small satellites were present. The first two he called "symmetrical" races, the latter "assymetrical" races. In all of the races the satellites were typically associated with the nucleolus. Smith (23), however, was unable to show distinct races or forms in <u>Galtonia</u> <u>candicans</u> on the basis of Navashin's criterea. Smith (23) in referring to some work of Emme (2) states that the latter found a number of varieties of barley to be "--- polymorphic with regard to the presence or absence of one or both satellites of a particular pair of chromosomes". Emme called these chromosomes "Nebenchromosomen". In 44 varieties of <u>Hordeum</u> he found two with 2 of these peculiar chromosomes, 17 with one each and the rest without any. His figures do not give the impression that these structures are true satellites. The kinetochores of the chromosomes are not definitely shown, the "neben" bodies appearing much like a portion of the chromosome arm. The attachment threads are very coarse in some instances. That Emme (2 pg.232) did not consider these portions of the chromosome arm proper is clearly shown by

his statement "Das Fädchen ist scheinbar ein echter Faden und keine 'constriction' nach Sakamura". The relation of these chromosomes to the nucleolus was not considered. If these chromosomes in barley are of the nucleolus-generating type the situation is very similar to that observed in Tulipa.

The differences in nucleolus-organizing power, observed in the haploid nuclei of some tulip varieties, is of particular interest. The nucleolus-forming chromosomes may only partially lose this ability in dyad and microspore nuclei or there may be no nucleolar organization whatever at these stages. In the latter case (Mrs. Moon) the plastin apparently originates from all the chromosomes forming an indefinite number of nucleolar granules which may be more numerous than the chromosomes. Every microspore nucleus shows the same lack of organization i.e., there is no segregation of the factors responsible for this condition. Hybrids formed from an "organized" type and the "unorganized" variety (the latter used as pollen parent) show ordinary nucleolar behavior in the root-tip. The nucleolus-forming power of the chromosomes is therefore present in the hybird. As the hybrids (see table 2, Louis XIV X Mrs. Moon) had nucleolar maxima of at least eight, some of the nucleolus-forming chromo- somes must have come from the pollen parent, Mrs. Moon. This is substantiated by microspore studies in Louis XIV which show that the nucleolus-organizing chromosomes in this tulip are about equally distributed in meiosis. Louis XIV has a

maximum of 9 nucleoli per nucleus in the root-tip.

Two papers by McClintock (12) and M. Navashin
(16) have already been referred to.   They have an important
bearing on the occurrence of asymmetry of satellite chromo-
somes in Tulipa and upon the peculiar types of haploid nucleo-
lar organization just described.   McClintock (12) found
chromosome VI in Zea mays to be characterized by a deeply
staining body just behind the satellite stalk that functions
as a nucleolus-forming organ. As a result of X-ray treatment
a chromosomal interchange resulted in which this body was split
in two, each inter-changed chromosome getting a section.
Whereas in ordinary haploid, diploid and triploid tissues
possessing normal chromosome VI there were respectively one,
two and three nucleoli, various peculiar nucleolar situations
resulted in plants having one or more inter-changed chromo-
somes. Plants homozygous for the inter-change developed four
nucleoli in their somatic telophases two being typically larger
than the others. The sizes of the nucleoli were proportional
to the sizes of the portions of the chromosome-forming bodies
present. Plants heterozygous for the interchange developed
three nucleoli in their somatic telophases, the microspores
showing segregation.   One of her very significant contribu-
tions was the demonstration of different "functional capacitys"
for the two types of inter-change chromosomes. When both
types were present in the same nucleus the chromosome with
the largest segment of the nucleolus-organizing body formed

a large nucleolus and the other chromosome a small one. When the latter was present alone in a nucleus it developed a typical large nucleolus. She found furthermore (12,pg.322) that "The activity of the nucleolar-organizing element is hindered by certain genomic deficiencies". When this happened many small nucleolus-like bodies developed but were never organized into definite nucleoli. According to her "These small nucleoli appear to develop from a swelling and later collection into droplets of the matrix substance of the chromosome". Creighton (see McClintock (12) pg. 321) observed a similar phenomenon in Zea when chromosome complements lacking the entire nucleolus-forming region of chromosome VI were formed in microsporogenesis. An indefinite number of nucleolar bodies were found in such microspores. Of interest in connection with the apparent disappearance of satellites, is McClintock's (12 pg.298) observation that the length of the attachment thread in chromosome VI varied greatly in different nuclei. This seemed to depend "---- upon the distance the satellite was removed from the nucleolar-organizing body in the previous prophase and also upon the stage at which the satellited chromosome is released from the nucleolus in the late prophase". This difference in satellite thread length carries over into metaphase.

McClintock's (12) results are very suggestive in connection with the work of M. Navashin (16) on Crepis.

Navashin (15) had, previously observed the apparent loss of a satellite in certain Crepis hybrids. This phenomenon he termed "amphiplasty". In his recent paper (16 pg.199) he reports that this change "--- is reversible and the satellited chromosomes recover their normal shape as soon as the pure complement is extracted from the hybrid by means of segregation". He found "-- that the presence of an incomplete haploid set of the one species is sufficient to cause changes in two satellited chromosomes of the other". This "loss" of the satellite he found was in reality due to its fusion with the arm of the chromosome. McClintock (12) suggests that this phenomenon in Crepis may be explained on the assumption of differential nucleolus-organizing powers of the satellited chromosomes of the hybrid. Thus, due to the failure of certain of these to function in nucleolus formation their satellite stalks never become elongated and the satellite cannot be differentiated from the chromosome arm. Navashin did not report nucleolar behavior in these plants.

It is possible that certain nucleolar phenomena observed in Tulipa can be explained on the basis of McClintock's contentions. The occurrence of different numbers of functioning nucleolus-forming chromosomes in the varieties and species-clons suggests that a situation similar to that occurring in Crepis may exist. The behavior in microsporogenesis shows conclusively that the ability of the nucleolus-forming chromosomes to function in some cases depends upon the combination

of chromosomes present. Thus, in Mrs. Moon both haploid sets
are unable to function alone. In this respect the situation
in _Zea_ differs. McClintock (12) observed segregation in the
abnormal behavior in microsporogenesis. As pointed out above,
this does not occur in the variety of _Tulipa_. In the latter
case it was therefore possible to definitely test the fertility
of the microspores showing lack of nucleolar organization.
There is little if any reduction in fertility due to this
condition. This shows furthermore that nucleolar divisions
can occur without the presence of a limited number of definite-
ly organized nucleoli because the pollen grains of Mrs. Moon
each contain several nuclei.

SUMMARY

(1)   The development of nucleoli in mitosis is described.   The nucleolar plastin appears to originate from the matrix in telophase.   This material is organized into nucleoli about certain loci.   If these loci, or collecting centers, lie close together the developing nucleoli fuse.

(2)   A detailed numerical study of interphase root-tip nucleoli has shown that each clon is characterized by a fixed maximum nucleolar number.   The average is subject to variation which seems to be in part due to the spatial arrangements of the nucleolus-organizing chromosomes and the relative volumes of plastin formed in telophase.   Some or all of the chromenemata apparently remain attached to the nucleoli in interphase.   Volumetric studies of interphase nuclei and nucleoli show that nuclear size and nucleolar volume tend to be directly proportional.   Nuclei with a high number of nucleoli generally have a lower total nucleolar volume than do nuclei with only a few of these bodies.   There appears to be little or no direct relationship between nuclear size and nucleolar number, however.

(3)   All, or nearly all, of the chromosomes are definitely attached to the nucleoli in mitotic prophase, the chromosomes being inter-connected by lateral anastomoses. Satellited chromosomes were definitely observed, but it was not possible to determine the exact number of these in somatic

prophase or metaphase. The satellites are associated with the nucleoli in prophase.

(4) Autoheteroploidy (as observed in chimeras) results in an increase in nucleolar number and volume. Varieties forming a polyploid series of related forms (not strictly autopolyploids), however, may not show a consistent variation in nucleolar number and volume.

(5) A study of first meiotic prophase nucleoli has established the identity between satellited and nucleolar chromosomes in the single late tulip, Adonis, 4 pairs of chromosomes are hetero-morphic, and one pair homo-morphic for satellites. Such lack of morphological homology in pairs of chromosomes apparently occurs in some other varieties. In Adonis the number of satellited chromosomes is equivalent to the maximum number of nucleoli occurring in the root-tip (six). The morphological relationships between nucleoli and satellited chromosomes are described for the first meiotic prophase.

(6) The results of numerical and volumetric studies at the last pre-meiotic interphase and the first meiotic prophase are presented. At both of these stages the nucleolar number and volume is much less than in root-tips. Very little, if any, change in total nucleolar volume occurs between the last pre-meiotic interphase and the second meiotic interphase (considering total nucleolar volume of all four microspores.)

(7)   The nucleolus-organizing ability of the
chromosomes may be partly or entirely suppressed in haploid
nuclei.   Thus the ability of given chromosomes to form
nucleoli is dependent in some cases upon the combination of
chromosomes present in the nucleus.   Complete lack of
nucleolar organization does not prevent nuclear division in,
or fertility of, the microspores.   The nucleolus-organizing
function of the chromosomes is present in root-tips of hybrids
formed from a cross in which the pollen came from a variety
showing no organization in haploid nuclei.   In this case
some of the nucleolus-forming chromosomes in the hybrid came
from the pollen parent.

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Explanation of plate 1.

(Figs. 1-11, diakinesis stages in single last tulip Adonis.
        Flemming's fixation)

Fig. 1.   Homomorphic pair of chromosomes attached to nucleo-
          lus (same shown in photomicrograph, fig.17, D).
Fig. 2.   Homomorphic pair of chromosomes, satellites closely
          paired.
Fig. 3.   Homomorphic pair of chromosomes attached to nucleolus,
          satellites cannot be distinguished from the "bud".
Fig. 4a. Nucleolus attached to homomorphic, and one pair of
          heteromorphic chromosomes.
Fig. 4b. Nucleolus attached to two of the heteromorphic pairs
          of chromosomes.
Fig. 5.   One of the heteromorphic pairs of chromosomes.
Figs. 6 & 7.   Nucleoli attached to heteromorphic pairs of
          chromosomes.
Fig. 8.   Heteromorphic pair of chromosomes.
Fig. 9.   Nucleolus attached to two pairs of heteromorphic
          chromosomes.
Figs. 10 & 11.   Two diakinetic nuclei each containing 12
          bivalents. Nucleoli labled (N), satellites (S), the
          nucleolus chromosomes are numbered the same in both
          nuclei, i.e., 4,5,7,11,12. Pair 7 is homomorphic
          for satellites. (See text for complete description).
Figs. 12.Two nucleoli with attached chromosomes from a nucleus
          in pachytene.
Figs. 13 to 18.   Satellited chromosomes from root-tip meta-
          phase plates of Adonis. Remnants of nucleolar matter
          are still attached in 15 and 16.

(Figs. 19 to 22 from root-tip cells of T.eichleri. Bouin's
          fixation.)

Fig. 19. Satellited chromosome at metaphase with associated
          nucleolar material.
Fig. 20. Satellited and non-satellited chromosomes attached
          to prophase nucleolus.
Fig. 21. Satellited chromosome at metaphase.
Fig. 22. Nucleolus with associated chromosomes at late pro-
          phase. Nucleolar matter (plastin) is marked (N).
          Chromosomes marked (c)-have been cut transversely in
          sectioning.
Figs. 23 and 24.   Tandem satellited chromosome of the Darwin,
          Allard Piérson. The satellites are divided in 24.

          Camera lucida drawings X 2000. Reduced abt. 1/8.

Plate 1
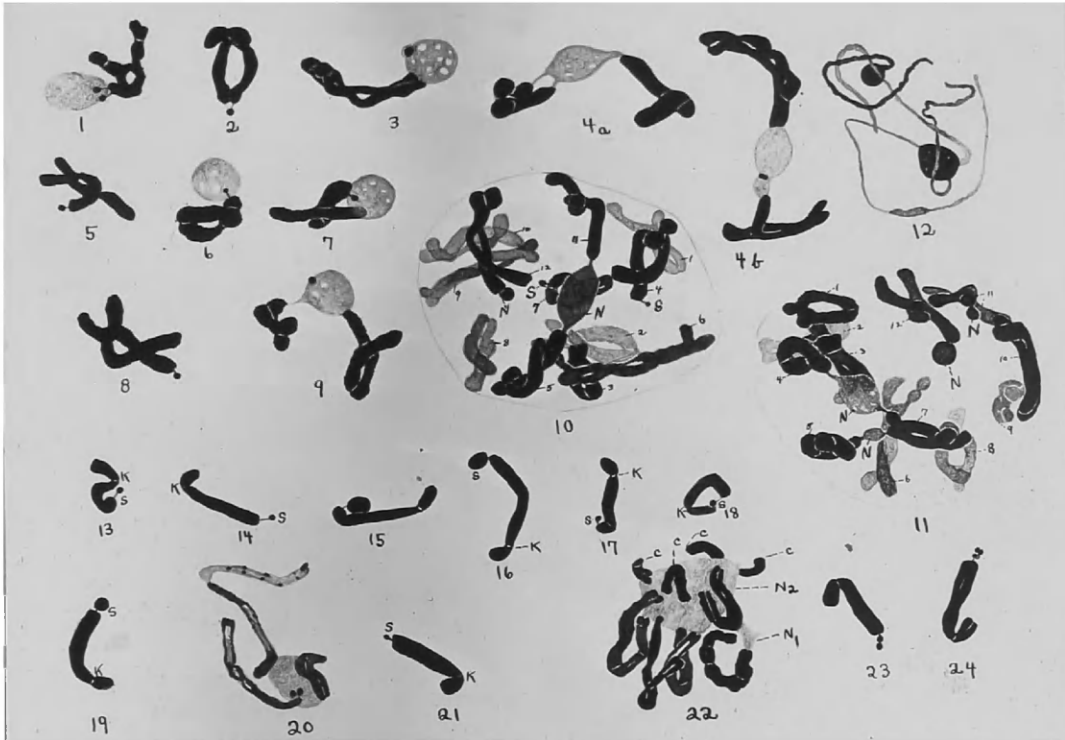
Nucleoli in Tulipa

APPROVED *Ronald Bamford*

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