CYTOLOGICAL FEATURES OF NICOTIANA GLUTINOSA HAPLONTS

By John Milton Webber

Associate cytologist, Division of Egyptian Cotton Breeding, Bureau of Plant Industry, United States Department of Agriculture

This paper represents the sixth in a series, the first five numbers of which appeared in the University of California Publications in Botany (1, 14, 22, 23, 24). The investigation on which this paper is based was begun while the writer was a research associate in the department of botany at the University of California, and was completed after he joined the staff of the Bureau of Plant Industry, United States Department of Agriculture.

INTRODUCTION

Nicotiana glutinosa L. has been grown in pure line for over 25 years in the botanical gardens of the University of California. It is one of the most distinct species of the genus and is strictly monomorphic. The leaves are small, cordate, long-petioled, and extremely glandular. The flowers, which are light yellow tinged with deep red, are short-cylindrical at the base, but above the base they expand abruptly into an irregular, obliquely one-sided funnel. The limb is somewhat bilabiate. The stigma and anthers are connivent just under the middle lobe of the upper lip. Although the species is included in the N. rustica L. section of the genus (7) its zygomorphic flowers show a close relationship to N. tomentosa Ruiz and Pav. of the N. tabacum L. section. It possesses 12 pairs of chromosomes.

Three Nicotiana glutinosa haplonts have been found among the plants in the botanical garden. Although the first of these was discovered among 24 seedlings that were subjected to X-radiation, the variation was apparently spontaneous and not the result of the treatment (11). The two other haplonts, 30.179-9 and 30.180-49, occurred in two F₃ populations of 50 plants each, grown from X-rayed seed during the spring of 1930. The parents of both of these populations were selected as having lower vigor and fertility. It is probable that these two haplonts were due to haploid parthenogenesis stimulated by an increased incompatibility of male and female gametes.

Except for considerable reduction in all the parts, the haplonts are normal in appearance. The flowers are slightly lighter in color, and the anthers and stigma, although noticeably smaller, are essentially normal. The plants are self-sterile and produce no seed when pollinated with diplont pollen. Photographs of diploid and haploid plants are shown in figures 1 and 2.

1 Received for publication Apr. 21, 1933; issued February 1934.
2 The writer is much indebted to Dr. T. H. Goodspeed, professor of botany, University of California, for the experimental material and for the opportunity to pursue the study, and to Dr. I. E. Webber, of the Division of Blister Rust Control, Bureau of Plant Industry, for helpful suggestions in regard to histology and for aid in the preparation of the manuscript.
3 Reference is made by number (italic) to Literature Cited, p. 866.
Haplonts in general exhibit many interesting cytological phenomena, some of which may serve as a basis for the interpretation of certain complex processes of meiosis in diplonts and hybrid plants. The early prophase stages of haplonts furnish evidence regarding the origin and nature of the double chromosomes seen in the later stages of the diplont. Since, in general, haplonts form univalent chromosomes in the first metaphase, many irregularities occur during the remaining stages of sporogenesis. Such irregularities as univalent division, fragmentation, and elimination are frequent and exceptionally clear. The formation and behavior of binucleate germ cells and of unreduced gametes and finally the steps of degeneration are likewise frequent and
Figure 2.—*Nicotiana glutinosa*: *A*, Typical flowers of diplont 30.180-48, shown against background ruled in centimeters; *B*, typical flowers of haplont 30.180-49; *C*, typical leaf of diplont 30.180-48; *D*, typical leaf of haplont 30.180-49.
clear. Similar products of such irregularities observed in other plants doubtless originated in many cases from phenomena closely related to those occurring in haplonts.

Because of the reduced number of chromosomes in the somatic cells, the root tips are obviously most favorable for the study of chromosome morphology. Moreover, such root tips frequently contain diploid cells and tissues. The formation of diploid areas in haplont roots and comparisons of such areas with tetraploid areas in roots of diplonts are extremely interesting. The cytology of root initials, callus tissues, and cuttings presents evidence as to the nature of diploid areas in haplonts and their relative frequency of occurrence in root and shoot.

Studies in chromosome number and morphology in *Nicotiana* have demonstrated that characteristic chromosome features distinguish certain species. The results for *N. tabacum* (23), *N. alata* Link and Otto (1, 22), *N. longiflora* Cav. (14), *N. sylvestris* Spegaz. (24), and *N. langsdorffii* Schrank, together with the data included herein, indicate that specific chromosome features furnish evidence as to the origin and relationship of species within the genus. It is encouraging that such evidence is accumulating and that it harmonizes closely with the evidence obtained from interspecific hybridization.

**MATERIAL AND METHODS**

The seeds giving rise to *Nicotiana glutinosa* haplonts 30.179-9 and 30.180-49 and to sister diplonts were germinated in pots. The seedlings were later transplanted into flats; when approximately 6 weeks old, they were set out in the field.

When approximately 16 weeks old, plants 30.179-9 and 30.180-49 were suspected of being haplonts and a few root tips were carefully collected from each plant. Soon after this 30.179-9 was injured and died. When 22 weeks old, 30.180-49 was known to be a haplont, and anther, ovary, and additional root-tip material were collected. At this time the plant was potted, removed to the greenhouse, and 20 stem cuttings were made.

Two weeks later root tips were gathered at random and from marked roots of the haplont. When the plant was 26 weeks old, root tips were similarly collected, and material for a study of root initials was obtained. By this time the roots had become so pot-bound that it was necessary to remove all exposed roots. Subsequent root-tip collections were made when the plant was 28, 30, 35, 37, and 41 weeks old. All exposed roots were removed after the root-tip collection made when the plant was 37 weeks old.

During this period and until the writer last saw the haplont, when it was 1½ years of age, it continued to grow vigorously and to produce numerous flowers. Frequent cytological examinations showed that its shoots remained haploid. As previously stated, the sexual cells of the plant were highly sterile.

Of the 20 cuttings, 13 produced callus tissues over their proximal ends and 7 died. Two of the surviving 13 were used for the study of callus tissue and root initials. In order to stimulate distal callus formation, a fresh cut was made across the apical internode of 5 of the

remaining cuttings. The cuts on 3 of these were immediately covered with petrolatum. All 5 were kept in a humid chamber and only 1 shoot bearing 2 leaves was allowed to develop on each. In no case did the cuttings form callus tissue at the distal end. The remaining 6 cuttings were allowed to develop, and their root tips and flower buds were subjected to cytological examination. All shoots produced were haploid and remained haploid until the plants died.

Diploid material of Nicotiana glutinosa was obtained from plants 27.028-6, 27.028-7, 28.028-53, and 30.005-1. The populations containing these four plants were grown from pure untreated seed.

The cytological observations were made on paraffin material, permanent smears, and acetocarmine smears. All cut material was killed and fixed in the following chromoacetic-formalin mixture:

1 part: 65 cc water, 10 cc glacial acetic acid, and 1 g chromic acid.
1 part: 40 cc commercial formalin and 35 cc water.

Before paraffin infiltration, callus tissues were softened in diaphenol. Paraffin sections were cut 10μ thick and stained in Heidenhain’s iron haematoxylin.

Somatic chromosome drawings and measurements were obtained from division figures located in the upper portion of the zone of cell division in the periblem. In this region the chromosomes vary only slightly in size and shape.

Camera-lucida drawings were corrected by direct observation. The drawings are reproduced at the following diameters: Somatic chromosomes and callus cells, at 4,750; pollen mother cells and sporads, at 2,000; and female gametophytes, at 950. No attempt has been made to show the relative levels at which the chromosomes lie.

CHROMOSOME MORPHOLOGY IN HAPLONT AND DIPLONT NICOTIANA GLUTINOSA

The haploid chromosome number of Nicotiana glutinosa, recorded by Goodspeed (9, 10) as 12, has been repeatedly confirmed by cytologists. Christoff (3) determined and figured the 24 somatic chromosomes of the species. His figures clearly show that the chromosomes of the complex vary but little in size. He found that the somatic chromosomes of N. glutinosa are shorter and slenderer than those of N. paniculata.

In the present study a large number of favorable somatic metaphase plates were examined in root tips from both haplonts and diplonts. A casual observation of diploid plates (pl. 1, G–I) gives the impression that the chromosomes are all of approximately the same size but readily divisible into a group of V-shaped and a group of rod-shaped chromosomes. However, a detailed study of these chromosomes shows slight variations in size and form within each of the two groups.

A close examination of the chromosomes composing the V-shaped group shows that they may be classified under six types (fig. 3). Type 1 comprises two pairs of chromosomes having median constrictions. Each segment of this group of four chromosomes averages 1.80μ in length. Type 2 likewise comprises two pairs of medially constricted chromosomes. However, the segments of this type average 2.00μ in length. Type 3 comprises one pair of medially constricted chromosomes, the segments of which average 2.26μ in length. Obviously the chromosomes of the foregoing five pairs are...
strictly V-shaped and are very nearly the same in size, which makes it difficult to identify them in diploid plates.

Type 4 comprises two pairs of submedianly constricted chromosomes, each with segments averaging 1.60μ and 2.15μ in length. Type 5 comprises a single pair of submedianly constricted chromosomes. The segments of this pair average 1.80μ and 2.26μ in length. The chromosomes of types 4 and 5, as in the case of types 1, 2, and 3, are very similar but are easily distinguishable from the chromosomes of the first three types because of their submedian constriction and consequent approach to an L-shape.

The pair of chromosomes composing type 6 is the most easily distinguished of the entire complex. The chromosomes of this type have submedian constrictions, are decidedly L-shaped, and have well-defined proximal satellites. The segments of the pair average 1.40μ and 2.40μ in length.
Cytological Features of Nicotiana glutinosa Haploids

PLATE 2

Nicotiana glutinosa haplont 30.180-49.  
A. Haploid root tip with a small diploid area in the dermatogen.  
B. Haploid root tip with a large diploid area in the periblen. This chimera nearly surrounds the ple- 
rome and greatly distorts the usual symmetrical cell arrangement of the tip.  
C. Embryo sac with a 
single large nucleus. Compare with D.  
D. At left, embryo sac with four characteristic nuclei; at 
right, characteristic disintegrated embryo sac.  
E. Pollen mother cell. Adnormal interkinesis. Daugh-
ter nuclei are connected by chromatin thread. Compare with F.  
F. Pollen mother cell. Interkinesis. Chromatin elements are well separated and clearly exhibit homotypic split. This condition is char-
acteristic for the stage in both haploids and diplonts. This mother cell is larger than that shown in 
E and contains more than 12 chromosome elements; it probably represents an advanced stage of a 
binucleate prophase (G).  
G. Pollen mother cell. Binucleate synizesis, showing characteristic chromatin 
granule in plasma, and well-separated nuclei.
Upon close examination it is found that the chromosomes of the rod-shaped group may likewise be classified as types (fig. 3). Type 7 comprises the smallest pair of chromosomes of the complex. The chromosomes of this pair average only 3.40μ in length and have practically terminal constrictions. Type 8 likewise comprises a single pair of chromosomes. The constrictions of this pair, however, are further removed from the proximal end than are those of the preceding pair, and the chromosomes are slightly longer, averaging 3.70μ. The chromosomes of the pair composing type 9 are similar to those of type 8 in that they have subterminal constrictions. These chromosomes, however, average 0.80μ longer than those of type 8. Although the chromosomes of the three latter types do not exhibit greater differences than those of types 1, 2, and 3 or types 4 and 5, they are more easily identified. The chromosomes of the rod-shaped group are less twisted and bent than are those of the V-shaped group. Hence, in the former group, differences in form and size are more striking and more easily measured.

A casual observation of haploid plates (pl. 1, A-F) clearly shows that there are 9 V-shaped and 3 rod-shaped chromosomes. A detailed study of such plates shows the presence of the 6 types of V-shaped and the 3 types of rod-shaped chromosomes described in connection with the diploid plates. Owing to the fewer number of chromosomes in such plates, the chromosome types are more easily identified than in the diploid plates.

The somatic chromosome types just described, in addition to being characteristic of the two haplonts 30.179-9 and 30.180-49 and all diplonts examined, are also distinguishable in the diploid cells in the root tips of haplonts (pl. 1 and fig. 3). It is likewise possible to distinguish 12 rod-shaped chromosomes and to identify 4 chromosomes of type 6 in one tetraploid metaphase in a diploid root tip from a haplont.

In root-tip metaphase plates of *Nicotiana sylvestris*, the writer (24) has noted that the chromosomes often appear to lie in pairs. A similar orientation is apparent in diploid metaphase plates of *N. glutinosa* (fig. 3, C). The writer (25) has suggested that such orientation is due to similarity of form or the location of spindle-fiber constrictions. This suggestion is supported by the fact that similar chromosomes in haploid *N. glutinosa* plates often appear to lie together or to occupy similar positions within the achromatic figure (pl. 1, F; fig. 3, B).

**OCCURRENCE OF DIPLOID TISSUES IN HAPLONT NICOTIANA GLUTINOSA**

Large portions of the root tips of haplont 30.180-49 were entirely or partly diploid (pl. 2, A and B). Diploid cells and the boundaries between diploid and haploid areas may be rather accurately determined on the basis of cell and nuclear size and chromosome number. Table 1 shows the number of root tips examined and the approximate age of the plant when they were collected. In the case of each root tip, the data are based on a careful examination from the root cap to the few scattered divisions in the upper zone of cell enlargement.
From the data in table 1 it appears that the haplont formed few or no diploid root tips until it was more than 22 weeks old. After this period the percentage of diploid tips gradually increased until the thirty-seventh week, when 55.56 percent of the root tips examined were wholly diploid. As noted in a preceding section, the plant was removed from the field to the greenhouse at the end of 22 weeks, and after 26 and 37 weeks the roots were severely cut back. These facts may account for the sudden appearance of diploid roots at 24 weeks and the slight decrease in the percentage of diploid roots following the twenty-sixth and the thirty-seventh week.

Of the 6 haploid root tips containing $2n$ areas, 2 had diploid areas in the dermatogen (pl. 2, A), 2 in the plerome, and 2 in the periblem. Four of the diploid areas were comparatively small and limited to either the zone of cell division or the zone of cell enlargement. One, occurring in the plerome, extended from the zone of cell enlargement to the root cap. The remaining diploid area, occurring in the periblem, extended from the last section examined in the zone of cell enlargement into the root cap, or approximately 820$\mu$. This diploid area (pl. 2, B) nearly surrounded the plerome and greatly distorted the usual symmetrical cell arrangement of the root tip. Each of the two last-mentioned diploid areas contained more cells and formed a greater proportion of the root tip in the zone of cell division than in the zone of cell enlargement.

The small tetraploid area in a diploid root tip was situated in the periblem of the upper portion of the zone of cell division. It was composed of from 8 to 10 cells, 1 being in the metaphase stage of mitosis.

Marked roots, which had produced either wholly haploid or wholly diploid tips, were found to continue to produce their respective type for a period of over 2 weeks. Fifteen root tips were examined from the marked haploid root and 19 from the marked diploid root.

Of 19 pieces of rootlets gathered at random from the haplont, 13 had wholly diploid root initials, 5 had wholly haploid initials, and 1 had 1 haploid and 2 diploid initials. There was a total of 32 diploid and 10 haploid initials. This material was collected at the end of 26
weeks. Hence, the high percentage of diploid initials indicates that if the roots had not been cut back at 26 weeks the percentage of 2n tips at 28 weeks (table 1) would have been considerably higher.

The callus covering the proximal or root end of the cuttings apparently originated from the vascular cambium. Unfortunately the cells of the forming callus were very irregular in shape and arrangement, so that the mitotic figures were difficult to study. The bulk of the callus, however, was composed of haploid cells. In the forming callus of 1 cutting 4 isolated diploid cells and 1 diploid area composed of approximately 5 cells were visible. In such callus tissues binucleate cells were about as common as diploid cells.

Although several root initials were visible in the callus, in no case did they arise within this tissue. Apparently the initials always originate from the vascular cambium located several sections above the callus. The young initials grow into the callus and finally through it. The callus thus forms a protective tissue for the initials. However, since the initials penetrate the callus more readily than the epidermis, callus tissue must afford less protection than epidermal tissue.

In 1 cutting 5 initials were visible in the callus and 1 in the cortex. In sectioning, the initials were cut at various angles and hence were difficult to study. However, all initials were apparently wholly haploid.

Of the 6 cuttings allowed to develop, only 1 formed diploid root tips in its early history. Two of the first 12 tips collected from this plant were diploid. The first diploid tips on the remaining 5 cuttings appeared approximately 3 weeks later than on the other plant. As in the case of the parent haplont, after the cuttings began to form diploid root tips, the percentage of such tips gradually increased. Of the 130 root tips examined from these cuttings, 22 were wholly diploid and 2 partly diploid.

The significance of the cytological evidence already submitted will be discussed later. It may be noted here that since the extent of diploidy is similar in root tips, marked roots, and root initials, the findings in each confirm those in the others. Moreover, the extent and nature of diploidy in haplont Nicotiana glutinosa is very similar to that found in haplonts of N. tabacum (23), Crepis capillaris (15), and Lycopersicum esculentum (19).

CHROMOSOME DOUBLING IN HAPLONT NICOTIANA GLUTINOSA

Several observations indicate that chromosome doubling in the root tips of haplont Nicotiana glutinosa occurs through failure of anaphase separation. In the majority of cases the stages of mitoses in haploid tips are as regular as those in diploid tips. Occasionally, however, in haploid figures, the nucleolus does not disappear during the prophase. Remnants or traces of the nucleolus are present during the metaphase and rarely during the anaphase. In the latter stages (fig. 4, A), the achromatic figure and the majority of chromosomes are apparently normal. However, some of the chromosomes always appear imperfectly formed and attached to the remnant of the nucleolus. In several of these abnormal figures every chromosome, except those attached to the remnant of the nucleolus, is divided and well separated. It is probable that in some of these abnormal figures,
anaphase separation fails and a single nuclear membrane is formed about the entire diploid chromosome group.

Diploid cells in haploid callus tissues probably originate from binucleate cells (fig. 4, B). As previously noted, several binucleate cells were visible in haploid callus, but no evidence was obtained as to their method of formation. The nuclei of these cells always lay in contact with each other or in close proximity. In some cases, probably, the nuclear membrane broke along the lines of contact and the sister nuclei united.

MEIOSES IN POLLEN MOTHER CELLS AND EGG MOTHER CELLS OF HAPLONT NICOTIANA GLUTINOSA

The prophase stages of the egg mother cells and pollen mother cells of haplont Nicotiana glutinosa are usually as regular as and very similar to those of diplont N. glutinosa or other species of Nicotiana (2).

![Figure 4](image)

The spireme of the haploid, however, is unpaired throughout the prophase (fig. 5, A). Hence the characteristic diploid ring and X-shaped diakinetic pairs are lacking in the haplont (fig. 6, B). During diakinesis in the haplont, 12 short, thick, rod-shaped univalents lie about the periphery of the nucleus.

Several twin ovules and binucleate egg mother cells and pollen mother cells are visible (pl. 2, G; fig. 5). The sister nuclei of these twin ovules and binucleate cells are always in the same stage of division and are haploid in nature. Binucleate cells usually have a single small chromatin granule in the cytoplasm. Although the origin of this granule is unknown, it is probably connected with that of the binucleate condition.

3 For convenience, the following symbols used in the University of California publications will be employed to designate the stages of meiosis: I and II, first and second meiotic divisions, respectively; I-M and II-M, first and second metaphase, respectively; I-A and II-A, first and second anaphase, respectively; and I-T and II-T, first and second telophase, respectively.
Since the binucleate cells occur in early meiotic prophase, they probably originate during an archesporial mitosis. Although direct evidence as to their mode of origin is lacking, several conditions indicate that they are not a product of extrusion of the nucleus from one germ cell to another (13, 23). These conditions are, briefly, as follows: (1) Binucleate germ cells are surrounded by an unbroken cell wall; (2) sister nuclei of binucleate pollen mother cells and egg mother cells lie far apart, thus apparently repelling each other; (3) it is extremely difficult, even in degenerate germ tissues, to separate the nucleus from the cytoplasm; and (4) egg mother cells are well separated by somatic tissue and air spaces, yet binucleate egg mother cells occur.

The remaining meiotic behavior is extremely irregular and variable. The 12 rod-shaped univalents contract until they are more or less spherical bodies. Immediately after the disappearance of the nuclear membrane a bipolar spindle is formed. The univalents are scattered over the spindle (pl. 3, A and B; fig. 6, D). They are approximately the same size as the II-M or univalent chromosomes of the diploid species.

One pollen mother cell is visible, with 2 achromatic figures and 12 univalents (fig. 5, B) scattered over each spindle. This pollen mother cell is probably an advanced stage of the binucleate prophase condition explained above.

Since the haplont does not form a I-M equatorial plate there is no foundation for orderly I-A distribution. The univalents are apparently distributed haphazard to the poles (pl. 3, C-E). All types of chance distribution from $0 < 12$ to $6 < 6$ occur. In many cases, however, univalents become laggards and go through an apparent division or fragmentation. This division usually fails and the univalent is finally distributed to a pole, or rarely left in the plasma. Now and then a laggard completes division, and the resulting parts

![Figure 5](image-url)
are likewise included in the daughter nuclei or rarely left in the plasma. Since the products of such division are of various sizes, the division is undoubtedly a fragmentation.

In general, interkinesis is very regular. The chromosomes at this stage are similar to those of the diploid state and exhibit the homo-

FIGURE 6.—Pollen mother cells of Nicotiana glutinosa diplont 30.005-1 (at left) and haplont 30.180-49 (at right): A and B, diakinesis; C and D, profile views of I-M; E and F, polar views of II-M, F showing 3 chromosome fragments; G and H, II-A. × 2,000.
Egg mother cells of *Nicotiana glutinosa* haplont 30,180–49.  
A. Profile view of I–M equatorial plate, showing an extremely rare condition.  
B. Profile view of I–M showing a typical figure.  
C–E, Typical I–A figure.  
F, Typical II–M figure.
Cytological Features of Nicotiana glutinosa Haplonts

PLATE 4

Nicotiana glutinosa. A and B, Diplont 30.005-1. A, Polar view I-M. B, Polar view II-M. C-I, Haplont 30.189-40; typical II-M figures, resulting from complete 0<12 to 0<6 I-A distribution; note relative size of pollen mother cells and chromosome of haplont and diplont.

856—2
typic split. The daughter nuclei of one pollen mother cell (pl. 2, E) are attached by a fine chromatin thread. This figure probably represents a suspended I-A or semiheterotypic division (21). As indicated in table 2, approximately 14.08 percent of the pollen mother cells show chromosomes or chromosome fragments in the plasma.

**Table 2.—Chromosomes or fragments in plasma of pollen mother cells at interkinesis**

<table>
<thead>
<tr>
<th>Number of chromosomes or fragments</th>
<th>Pollen mother cells showing chromosomes or fragments in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>0</td>
<td>177</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
</tr>
</tbody>
</table>

As shown in table 3, the II-M plates exhibit various numbers of chromosomes. The table, expanded from 0 to 12, shows a mode of 6, as would be expected from I-A random distribution, and a mean value of 5.56. Typical II-M plates depicting complete random distribution are shown in plate 4, C—I, and plate 3, F. Although the total number of chromatin units in the plates of each of these pollen mother cells is normally 12, it is occasionally less than this and rarely more. Out of 1,161 pollen mother cells (table 3) in which both plates were countable, 158, or 13.61 percent, showed chromosomes or chromosome fragments in the plasma. This percentage is in agreement with that of such chromatin units found in the plasma during interkinesis. The number of such elements in the plasma ranges from 1 to 4, as in the case of interkinesis. Fifteen, or 1.29 percent, of the 1,161 pollen mother cells (table 3) showed chromosome fragments within the II-M plates. Two of these pollen mother cells showed 3 + 1 < 8 + 1 fragments; 2 showed 4 + 1 < 7 + 1; 1 showed 4 + 1 < 7 + 2 (fig. 6, F); 9 showed 5 + 1 < 6 + 1; and 1 showed 5 < 6 + 1.

It is to be noted (pl. 4, C) that II-M plates, which contain all 12 univalents, cannot be mistaken for I-M plates. Such II-M plates are always at the side of the cell or in the approximate position of one of the poles of the I-M spindle. Furthermore, such II-M pollen mother cells are always surrounded by other cells in the same stage of division.

The II-A chromosomes, which are slightly smaller than the II-M chromosomes, are about the same size as those of the diploid at this stage (fig. 6, G and H). In general behavior II-A is fairly normal. Occasionally, however, laggards occur. These laggards do not show the apparent division exhibited by the laggards in the case of I-A and apparently are ultimately included in one of the granddaughter nuclei. The majority of chromatin units left in the plasma during I are supplied with miniature spindles and divide at II-A. However, occasionally such a unit lacks a II spindle and fails to divide. Since the dividing units are considerably larger than the others, they probably are whole univalents. The chromatin units that fail to
divide and probably the laggards within the major II-A spindles are chromosome fragments.

Table 3.—Number of pollen mother cells showing chromosomes variously distributed on II-M plates

<table>
<thead>
<tr>
<th>Type of distribution of chromosomes</th>
<th>Number of pollen mother cells showing chromosomes on—</th>
<th>Total number of pollen mother cells</th>
<th>Type of distribution of chromosomes</th>
<th>Number of pollen mother cells showing chromosomes on—</th>
<th>Total number of pollen mother cells</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Both plates a</td>
<td>Single plates b</td>
<td></td>
<td>Both plates a</td>
<td>Single plates b</td>
</tr>
<tr>
<td>0</td>
<td>39</td>
<td>37</td>
<td>115</td>
<td>5</td>
<td>343</td>
</tr>
<tr>
<td>12</td>
<td>39</td>
<td>37</td>
<td>156</td>
<td>6</td>
<td>237</td>
</tr>
<tr>
<td>11</td>
<td>48</td>
<td>58</td>
<td>156</td>
<td>6</td>
<td>237</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>71</td>
<td>196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>88</td>
<td>94</td>
<td>280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>188</td>
<td>187</td>
<td>641</td>
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</tbody>
</table>

* 158 pollen mother cells exhibiting chromosome fragments or chromosomes in the plasma are not included in the table.

Plates exhibiting fragments are not included.

The several counts of pollen mother cells at II-A were 5 of \( \bigtriangleup + \Bigtriangleup \); 6 \( \bigtriangleup + \bigtriangleup \); 1 each of \( \bigtriangleup + \bigtriangleup \) (fig. 6, \( H \)), \( \bigtriangleup + \bigtriangleup \), and \( \bigtriangleup + \bigtriangleup \), and \( \bigtriangleup + \bigtriangleup + \bigtriangleup + \bigtriangleup \). The last of these pollen mother cells during I-A probably involved the following conditions: A random distribution of \( 4 \bigtriangleup + 6 \); 1 univalent left in the plasma; and the fragmentation of 1 univalent, one part of which was included in the daughter nucleus with 4 univalents and the other part left in the plasma. Two exceptionally large pollen mother cells were visible. One of these (fig. 5, \( C \)) contained 2 groups of 17 chromosomes, 2 groups of 6 chromosomes, and 2 well-separated chromosomes in the plasma. This unusual pollen mother cell probably represents a more advanced stage of the binucleate condition, which, as previously explained, contains two I-M achromatic figures. The I-A distribution probably was \( 6 \bigtriangleup \rightarrow 6 \) and \( 11 \bigtriangleup \rightarrow 1 \). However, at one side of the pollen mother cell the adjacent spindle poles fused, so that the distribution was \( 17 \bigtriangleup \rightarrow 6 \). Normal homotypic division \( \rightarrow 1 \) followed, and the exceptional cell resulted.
As is indicated in table 4, the sporads were highly irregular. Since the spores were equivalent in size in only a few sporads, the segregation in the table is based entirely on the number of spores.

**Table 4.—Frequency of occurrence of microspores of various types**

<table>
<thead>
<tr>
<th>Microspores</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diads</td>
<td>48</td>
<td>7.52</td>
</tr>
<tr>
<td>Triads</td>
<td>18</td>
<td>2.82</td>
</tr>
<tr>
<td>Tetrads</td>
<td>456</td>
<td>71.47</td>
</tr>
<tr>
<td>Above tetrads</td>
<td>116</td>
<td>18.18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>638</strong></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 4 show that the majority of microsporads were tetrads. Although the spores of these tetrads were in the main unequal in size, a few were equal (fig. 7, B). The spores of the latter tetrads were considerably smaller than those of the diads (fig. 7, D) or of the tetrads of the diploid species (fig. 7, A). Obviously such spores contain less than the haploid chromosome complex and probably are the products of a uniform $6<>6$ or similar I-A distribution.

Tetrads, as well as the other types of sporads, frequently contain micronuclei. Figure 7, D and C, show, respectively, a normal diad and a diad containing two small micronuclei. Approximately 30 to 40 percent of the diads contained such micronuclei. Possibly these diads resulted from a very irregular I-A distribution, such as $1<>11$ or $2<>10$. If this was the case, then the adjacent products of daughter nuclei lay in such close proximity that they were included in one spore. A few of these micronuclei and the majority of micronuclei of other types of sporads undoubtedly resulted from chromosomes or fragments left in the plasma at I and II.

The spores of normal diads are approximately the same size as those of the tetrads of the diploid state. They probably arise from a $0<>12$ I-A distribution and consequently contain a normal haploid chromosome complex.

Triads are possibly produced by a process similar to that by which diads containing micronuclei are produced. The sporads containing more than four spores undoubtedly result from highly irregular meiotic divisions or from binucleate pollen mother cells. Typical sporad types are shown in figure 7.

Up to II-T the behavior of the egg mother cells and pollen mother cells is practically identical. Possibly, however, the behavior of the egg mother cells is slightly more uniform and the mean I-A distribution nearer the mode. However, following II-T, the majority of egg mother cells rapidly disintegrate to a deep-staining mass. As degeneration proceeds, the surrounding somatic cells elongate and gradually supplant the egg mother cells (pl. 2, D). After such replacement the somatic cells fail to develop further and finally the entire ovule disintegrates.

Although in the majority of plants the meiotic divisions produce a group of 4 spores, in numerous cases the divisions are highly irregular and from 1 to 12 or more spores are produced. Cases are known where 2 spores are characteristically formed. In literature dealing with these conditions such a statement as "the majority of tetrads contain only four spores" is rather common. It therefore appears that the term "tetrad" (tetra (Greek), four; and di (Latin), toward) or "tetrad stage" is not expressive of the condition that obtains. The term "sporad" (sporo (Greek), seed, spore) is proposed for this stage or group of cells. Alternative terms, such as "sporocorymbus" (corymbus (Greek), cluster, group) are not in keeping with the terms "tetrad", "diad", etc., and are more cumbersome.
Although an occasional egg mother cell develops beyond II-T, such development is never normal and is rarely duplicated in another egg mother cell. The megasporads which develop always have at least two spores, the nuclei of which are approximately the same size as those of the microdiad. In addition to the large spores, there are occasionally one or two small ones. No definite evidence was obtained as to which spore develops into the megaspore and finally produces the female gametophyte. However, judging from the size of gametophytic nuclei and from a few questionable chromosome counts of these nuclei, only spores containing approximately 12 chromosomes develop.

Further development is highly irregular and no definite stages occur. In the majority of cases, apparently, rapid division follows and from 4 to 7 nuclei are formed (pl. 2, D). Although in a few cases the nuclei are scattered throughout the gametophyte, no definite arrangement occurs. In the majority of cases the nuclei are in a single
compact group. Whether these nuclei remain in this condition until degeneration or whether they fuse is undetermined. However, later in development, the appearance of extremely large nuclei (pl. 2, C) indicates that the latter condition occurs. Such nuclei are visible in ovary material collected during anthesis. Although several of these large nuclei are in prophase, no gametophyte containing more than one such nucleus is visible. Counts of the latter nuclei indicate that they contain 35 or more chromosomes. Approximately at the time when fecundation should take place, degeneration starts, and all ovules, including those in which somatic cells have replaced the embryo sac, rapidly disintegrate.

As stated previously, no definite arrangement of stages occurs, hence no mature or normal embryo sac is formed. The most nearly normal condition is shown in figure 8, A. The three nuclei of this figure may represent the two synergids and the egg. In this case, the egg is extremely large in proportion to the synergids and may represent the union of several nuclei. Figure 8, B and C, shows two of the many irregularities. Although no explanation is offered for these unusual figures, apparently in the one shown in figure 8, B and A, a polyploid somatic cell is replacing the embryo sac. Figure 8, C, represents a condition among well-advanced ovules and hence should be beyond the meiotic divisions. The nuclei at the bottom of the embryo sac likewise indicate an advanced stage. However, the 12 chromosomes...
in the upper part of the embryo sac are the size of univalents and have a decidedly meiotic form.

Since preparations of the male gametophyte are extremely difficult to obtain, no study of its development was made. However, the young pollen grains are extremely variable in size and shape, and only a few appear equivalent to those of the diploid species. During anthesis a few grains of the latter species were found in a whitish powder obtained from the anthers.

DISCUSSION

Inasmuch as the meiotic behavior of the *Nicotiana glutinosa* haplont is similar to that of other haplonts, especially in *Nicotiana*, this discussion will be confined mainly to somatic behavior and phenomena concerning such behavior. Moreover, since the literature dealing with haplonts has recently been reviewed by Gates and Goodwin (8) and by Kuhn (18), it will not be summarized here.

CHROMOSOME NUMBER AND MORPHOLOGY

That the normal somatic chromosome number is 24 (3) is confirmed by the present study of the chromosomes in diplo and haplont *Nicotiana glutinosa*. Since no pairing occurs during meiosis in haplont *N. glutinosa*, it is reasonably certain that 12 is the basic haploid number of chromosomes for the species.

Somatic chromosomes of *Nicotiana glutinosa* are similar in shape to those of *N. tabacum*, in which \( n = 24 \) (23); to those of *N. alata*, in which \( n = 9 \) (1, 22); and to those of *N. sylvestris*, in which \( n = 12 \) (24). They are, however, considerably smaller in diameter than the chromosomes of these species and are not so variable in size. The chromosomes of *N. glutinosa* differ from those of *N. longiflora*, in which \( n = 10 \) (14), in that the chromosomes of the latter species are uniformly rod-shaped. To some extent, therefore, specific chromosome distinctions separate the complex of *N. glutinosa* from the complexes of the other species mentioned.

According to the data and figures presented by the authors just cited, the chromosomes of *Nicotiana sylvestris* and *N. tabacum* are more nearly alike than those of any other of the species mentioned. In interspecific hybrids it has been shown that chromosome affinity is much greater in the *N. tabacum-N. sylvestris* combination than in any other combination of these five species (3; 4, p. 181; 7, p. 273–274; 9; 12).

COMPARISON OF DIPLOIDY IN HAPLONT ROOTS AND TETRAPLOIDY IN DIPLOID ROOTS

As stated previously, the extent of diploidy is similar in root tips, marked roots, and root initials of haplont *Nicotiana glutinosa*. Only 2.21 percent of the haploid root tips gathered at random were partly diploid. This percentage indicates that the occurrence of diploidy in haplont roots is less frequent than is commonly believed (14, 17, 19, 23). In fact, diploidy in haplont roots does not appear to originate more frequently than does tetraploidy in diploid roots. Of the wholly diploid root tips of haplont *N. glutinosa*, 1.02 percent showed

7 The degree of affinity between *Nicotiana alata* and *N. longiflora* seems to be questionable (7).
tetraploid chimeras. In a previous paper the writer (24) has shown that in diploid *N. sylvestris*, 2.50 percent of the root tips contain tetraploid chimeras, and he has reported a tetraploid chimera in *N. sanderae* Sander. Since 1930 the writer has observed such chimeras in *N. rusbyi* Britt. and *N. trigonophylla* Dun. Although in the last three cases no records were kept, it is certain that in each case tetraploidy originated as frequently as diploidy in haplont *N. glutinosa* root tips.

On the other hand, unless some phenomenon aside from frequent origin occurs, the high percentage (55.56) of wholly diploid root tips cannot be explained. The writer (24, p. 360) has stated:

The diploid cells of the haplonts are by far the most vigorous cells of the entire root tip and multiplication is more rapid in the diploid cells of such a root. The descendants of a diploid root initial in a haploid plant will in all probability persist in that meristem, and possibly in time dominate the tissues of the organ.

Such a change is precisely what happened in the *Nicotiana glutinosa* haplont 30.180-49. It has already been shown that the two large diploid chimeras in haploid root tips contain more 2n cells in the zone of cell division than in the zone of cell enlargement. It is also shown that the percentage of wholly diploid root tips increased from 0 to 55.56.

Such development and domination of diploidy in roots of haplont *Nicotiana glutinosa* is in direct contrast to that found in the case of tetraploidy in roots of diplont *N. sylvestris*. In the latter case the writer (24, p. 360) has stated:

That the larger sector * * * is gradually reaching its maximum size is indicated by the comparatively uniform decrease in the number of tetraploid cells from the region of elongation to the growing point. * * * If the root tip had been allowed to grow, this sector, and probably the majority of other sectors, would have become "islands" of tetraploid cells.

Hence, the size of 2n and 4n areas which develop from such initial cells in the roots of haplonts and diplonts is apparently determined by growth-rate relations of these tissues. Consequently such growth-rate distinctions must also determine the frequency of wholly 2n and 4n roots, since these roots must arise from such areas in the pericycle.

**COMPARISON OF ROOT AND SHOOT IN RELATION TO POLYPLOIDY**

It is well known that diploidy in haplonts occurs more frequently in roots than in shoots (23, 15, 19). A similar condition exists in the case of tetraploidy in diplonts. Although the reason for the greater prevalence of these chimeral tissues in the root is apparently unknown, several clues are afforded by the nature of the root and shoot, especially by the relative amount of meristematic tissue in these organs and the relative degree of protection such tissues receive.

Undoubtedly the number of apical meristems in the root system is greater than in the shoot. Meristematic tissue in the shoot is covered with a more effective protective tissue than is that in the root. Moreover, the apical meristem of the shoot receives additional protection from young leaves and is therefore better shielded from environmental changes that may initiate polyploidy. Hence, it seems probable that polyploidy originates more frequently in the root than in the shoot.

Although the environmental variations to which the shoot is subjected are ordinarily greater than those that affect the root, as great
or greater variations are usually encountered by roots grown for
cytological investigations. Such roots, generally from potted plants
that soon become more or less pot-bound, are subjected to various
and sudden changes in temperature and moisture. Since the root
is not so effectively constructed as the shoot to endure such variations,
the greater frequency of polyploidy in roots grown under such con-
ditions is not surprising.

The behavior of haplont *Nicotiana glutinosa* is in harmony with the
foregoing facts. It was shown in a preceding section that all roots
gathered from field plants or from plants (cuttings) grown in an
ample volume of soil were wholly haploid. On the other hand, as
soon as the plants become more or less pot-bound, diploidy suddenly
occurred.

It is probable, however, that tetraploid and also diploid chimeras
are of more frequent occurrence in shoots than has been observed,
since apical meristems of shoots are very seldom examined.

**HAPLONT CALLUS TISSUE IN RELATION TO DIPLOIDY**

As previously shown, the callus at the proximal ends of shoot
cuttings of haplont *Nicotiana glutinosa* originates from the vascular
cambium and is composed chiefly of haploid cells, although a few
diploid and binucleate cells occur. The mode of origin is in agree-
ment with that reported by Priestley and Swingle (20). The occur-
rence of diploid and binucleate cells in the proximal callus is probably
due to the effect of environmental changes on the unprotected dividing
cells. Such diploid cells have no effect on the composition of roots
produced by cuttings, since new roots originate from the vascular
cambium several sections above the callus.

No distal callus is formed in haplont cuttings of *Nicotiana glutinosa*. However, distal callus similar in composition to the proximal callus
of haplont cuttings of *N. glutinosa* has been reported by Jørgensen (16)
and by Lindstrom and Koos (19). The occurrence of diploid cells
in the callus of decapitated haplonts may be attributed to causes
similar to those resulting in diploid cells in proximal callus. How-
ever, unlike the diploid cells of the proximal callus, those of the distal
callus may have a profound effect on the composition of new tissues
Since Priestley and Swingle (20) found that buds are exogenous in
origin, whereas roots are endogenous, the occasional occurrence of
diploid and tetraploid shoots from decapitated haplonts may be
accounted for by the chimeral composition of the distal callus.

**MEIOTIC FEATURES OF HAPLONT NICOTIANA GLUTINOSA**

As previously stated, the meiotic behavior of haplont *Nicotiana
 glutinosa* is very similar to that of other *Nicotiana* haplonts (2, 5, 6,
11, 17, 23). Because of this similarity and since Hollingshead (14)
has recently discussed the meiotic resemblances and differences of
haplonts, it appears unnecessary to present any further compilations
here. Hence, the new, unusual, and important meiotic and gene-
trophytic features of the *N. glutinosa* haplont are merely enumerated.
These are as follows:

The occurrence of binucleate egg mother cells and pollen mother cells and of
twin ovules during the meiotic prophase.

The complete lack of any observable conjugation during meiosis.
The lack of the homotypic split or apparent equational division of univalents during I.

The apparent fragmentation of univalents during I-A.

The formation of two I-M achromatic figures in binucleate germ cells.

The success of I-A random distribution, the 12 univalents being distributed, in nearly all cases, in all types of distribution from 0<>12 to 6<>6.

The very rare occurrence of semiheterotypic divisions.

The II-M mode of 6 and mean value of 5.56.

The occurrence of chromosome fragments at II-M.

The fairly normal II-A distribution.

The occurrence of II-A pollen mother cells containing 48 chromosomes, which probably represent advanced stages of the binucleate condition. The possible fusion of the I-M achromatic figures in binucleate cells, giving rise to II-M and II-A plates containing more than 12 chromosomes.

The occurrence of highly abnormal sporads and occasional diads, the spores of which contain the complete haploid chromosome complex.

The disintegration of the majority of egg mother cells following II-T, and their gradual replacement by somatic cells.

The formation of an occasional female gametophyte.

The great irregularity and lack of definite stages in the female gametophyte.

The apparent fusion of nuclei in the female gametophyte and the occurrence of polyploid nuclei.

The degeneration of all ovules at the approximate time when fecundation should occur.

The occurrence of a few apparently normal pollen grains.

**SUMMARY**

Two additional haplonts of *Nicotiana glutinosa* were found during the spring of 1930 in the botanical gardens of the University of California. One of these plants is described and figured.

The somatic chromosome complex of *N. glutinosa* consists of 24 chromosomes. The basic haploid number is apparently 12.

The chromosomes of the somatic garniture exhibit morphological differences permitting the recognition of nine distinct types.

Diploidy frequently occurs in the root system of haplont *N. glutinosa*. The percentage of wholly diploid root tips observed increased from 0 at 22 weeks to 55.56 at 37 weeks. Diploid areas were present in 2.21 percent of the haploid root tips, and 1.02 percent of the diploid root tips contained tetraploid areas.

Diploid areas extending from the zone of cell division into the zone of cell enlargement contain more cells in the former zone and hence are larger than in the latter zone.

Marked diploid and haploid roots produced wholly diploid and wholly haploid tips, respectively.

Of 19 pieces of rootlets, 13 had wholly diploid root initials; 5 had wholly haploid initials; and 1 had 1 haploid and 2 diploid initials.

The basal callus tissue of cuttings is composed mainly of haploid cells, although a few diploid and binucleate cells occur.

Root initials in the callus originate several sections above the callus. All initials are wholly haploid.

Out of 6 cuttings, only 1 had diploid root tips in its early history.

Chromosome doubling in root tips is possibly due to the failure of anaphase separation, following the retention of the nucleolus to this stage. Chromosome doubling in callus tissue is possibly due to the fusion of the nuclei of binucleate cells.

Meiotic and gametophytic behavior is exceptionally irregular, and several new and unusual features occur. The term "sporad" is suggested for the spore or group of spores (tetrads, triads, microcytes,
etc.) resulting from meiotic divisions. A summary of meiosis is given in the final section of the discussion.

Evidence is presented that the high percentage of diploidy in haplont roots of *N. glutinosa* is due to the relative growth relations of 2n and n tissues rather than to the frequent origin of 2n initials.

Growth rates of 4n and 2n tissues in relation to the occurrence of tetraploid chimeras in diplonts are discussed.

Evidence is presented that diploidy in haplont roots is probably due to the effect of frequent and sudden environmental changes on poorly protected meristematic tissue.

Evidence is presented that the basal-callus tissue of cuttings forms a protective coat for adventitious root initials. Comparisons are drawn between the basal callus and root initials, and the conditions presumably existing in the apical callus and bud initials.

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