

INHERITANCE IN NICOTIANA TABACUM. XVI. STRUCTURAL DIFFERENCES AMONG THE CHROMOSOMES OF A SELECTED GROUP OF VARIETIES

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THE studies reported herewith deal with the structural relations among the chromosomes of fifteen horticultural varieties of *Nicotiana Tabacum*. They were initiated as a consequence of the discovery that hybrids of the standard variety, *Purpurea*, with the variety Cuba exhibit $19\text{II} + \text{O}6 + \text{O}4$ instead of 24II chromosomes in meiosis. For the purposes of the study 14 varieties of *N. Tabacum* were first crossed with *Purpurea*; then those varieties which produced hybrids exhibiting evidence of structural difference were intercrossed in order to determine their structural affinities.

THE VARIETIES STUDIED

The 15 varieties employed in the study include a number of commercial types, a few possessing unusual or freak characters, and some local varieties collected by DR. T. H. GOODSPEED in South America. The names, aside from those of established commercial varieties, are mainly locality or other designations employed for convenience. For purposes of precise identification, the UCBG (UNIVERSITY OF CALIFORNIA BOTANICAL GARDEN) number is also given. These varieties have all been continued by selfing, and they exhibit 24II chromosomes in meiotic metaphases, as revealed by acetocarmine smears of microsporocytes. The list follows:

1. *Purpurea* (UCBG 06-25), originally from the MISSOURI BOTANICAL GARDEN. Described by SETCHELL (1912) and since employed as the standard variety of reference for our investigations of *N. Tabacum*.
2. *Consolation* (UCBG 40-22), a variety with yellowish-green leaf color from Puerto Rico (NOLLA 1934).
3. *Trelease Turkish* (UCBG 40-24), a Turkish-type variety from PROFESSOR SAM F. TRELEASE, COLUMBIA UNIVERSITY.
4. *Belge* (UCBG 40-25), an old Canadian variety received from DR. N. A. MACRAE.
5. *Station Standup* (UCBG 40-26), a modern Canadian burley variety (MACRAE and HASLAM 1935).
6. *Holmes Samsoun* (UCBG 40-28), a Turkish variety incorporating the mosaic resistance of *N. glutinosa* (HOLMES 1938).
7. *Cuba* (UCBG 14-200), a white-flowering parthenocarpic variety (GOODSPEED 1915) originally received from MRS. ROSE HAIG THOMAS.
8. *Ambalema* (UCBG 37-51), a mosaic-resistant variety collected by NOLLA in Colombia (NOLLA and ROQUE 1933).
9. *Huadquina* (UCBG 36-170), a distinctive local type collected by DR. T. H. GOODSPEED in the vicinity of Machu Picchu, Peru.

10. Serrate (UCBG 40-23), a variety with somewhat incised leaves distributed by the U. S. DEPARTMENT OF AGRICULTURE.

11. Ceniza (UCBG 40-27), a variety with hairy filaments and glaucous leaves from Puerto Rico (NOLLA 1934).

12. Bergerac Catacorolla (UCBG 40-29), a freak variety, flowers with supernumerary reversed corolla attached externally to the tube at the base of the infundibulum (ARISZ 1927), collected by DR. H. P. OLMO at Bergerac, France.

13. Maryland Mammoth (UCBG 31-1), a commercial mammoth variety supplied by E. G. BEINHART.

14. Apolo-I (UCBG 39-245), a very distinctive weed-like local type collected by T. H. GOODSPEED in Bolivia.

15. Apolo-II (UCBG 39-246), a commercial-type variety collected by T. H. GOODSPEED at the same locality as the preceding variety.

STRUCTURAL DIFFERENCES AMONG THE VARIETIES

The hybrids of the 14 varieties with the standard type, *Purpurea*, may be classified in three groups with respect to MI associations:

1. Those with 24 bivalents (fig. 1), indicating structural identity with *Purpurea*. Nine varieties appear to be structurally identical with *Purpurea*—namely, Consolation, Trelease Turkish, Belge, Station Standup, Ambalema, Serrate, Bergerac Catacorolla, Maryland Mammoth, and Apolo-II; but a few, for example Belge and Apolo-II, gave some evidence of intrachromosomal dislocations relative to *Purpurea*.

2. A hybrid with 23 bivalents and two univalents (fig. 3). The presence of a non-conjunctional pair of chromosomes was a unique feature of the *Purpurea*-Holmes Samsoun hybrid.

3. Those with multivalent (amphibivalent) associations. The hybrids of this class arise from the varieties: Apolo-I, Ceniza, Huadquina, and Cuba. The number and character of the associations is recorded in the first line of table 1.

In order to explore the problem further, the five varieties which gave atypical results with *Purpurea* were intercrossed. These results are also recorded in table 1, where the number and character of associations only are indicated, the remaining chromosomes regularly forming bivalents.

CHARACTERISTIC FEATURES OF THE COMPLEXES

Not only does the structural analysis indicate the distinctness of the complexes, but their variation in association testifies to the same conclusion. The parental varieties, as selfed lines, consistently exhibit 24 bivalents in meiosis. Most of the bivalents are attached at both ends, but rods are sometimes seen as a consequence of chiasma formation in one arm only. The chiasma frequency is comparatively low; instances of two chiasmata in one arm are rather infrequent. The behavior of the different complexes is noted briefly below:

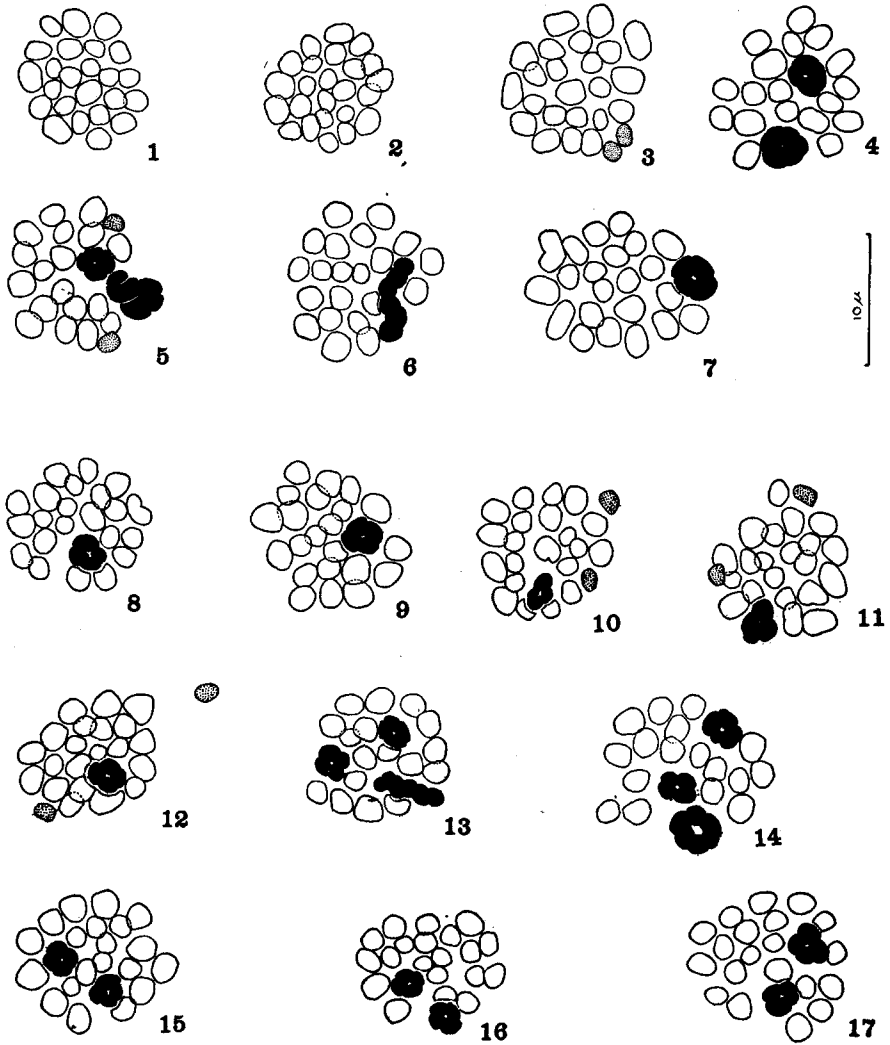


Fig. 1.—Purpurea-Serrate, 24II.—Fig. 2. Holmes Samsoun, 24II.—Fig. 3. Purpurea-Holmes Samsoun, 23II+2I.—Fig. 4. Purpurea-Cuba, 19II+1VI+1IV.—Fig. 5. Holmes Samsoun-Cuba, 18II+1VI+1IV+2I.—Fig. 6. Apolo-I-Ceniza, 21II+1VI.—Fig. 7. Purpurea-Huadquina, 22II+1IV.—Fig. 8. Purpurea-Ceniza, 22II+1IV.—Fig. 9. Purpurea-Apolo-I, 22II+1IV.—Fig. 10. Holmes Samsoun-Huadquina, 21II+1IV+2I.—Fig. 11. Holmes Samsoun-Ceniza, 21II+1IV+2I.—Fig. 12. Holmes Samsoun-Apolo-I, 21II+1IV+2I.—Fig. 13. Cuba-Ceniza, 17II+1VI+2IV.—Fig. 14. Cuba-Apolo-I, 17II+1VI+2IV.—Fig. 15. Apolo-I-Huadquina, 20II+2IV.—Fig. 16. Ceniza-Huadquina, 20II+2IV.—Fig. 17. Cuba-Huadquina, 20II+2IV.

The figures are from camera-lucida drawings of microsporocytes at MI. Bivalents, outlined; univalents, stippled; multivalent associations, solid black.

TABLE I

Maximum associations observed in various Tabacum hybrids.

	APOLO-I	CENIZA	HUADQUINA	CUBA	HOLMES SAMSOUN
Purpurea	4	4	4	6+4	1+1
Holmes Samsoun	4+1+1	4+1+1	4+1+1	6+4+1+1	
Cuba	6+4+4	6+4+4	4+4		
Huadquina	4+4	4+4			
Ceniza	6				

The non-conjunctional complex

This complex was observed in every cross of Holmes Samsoun with the other varieties (fig. 2, 3, 5, 10-12). The two univalents appear to be approximately the same size, and they are always located on the periphery of the plate, as is normally the case for unassociated chromosomes (OLMO 1936). They appear to be entirely independent. Sometimes they are located on opposite sides of the plate; sometimes close together, in which case, however, they usually lie in different planes. No case of association has ever been observed between them, nor have they ever been seen to conjugate with other chromosomes. A single chromatin bridge was observed in a cell of the Cuba-Holmes Samsoun hybrid, but it was impossible to connect it with the non-conjunctional pair.

Six-chromosome complexes

There are two different six-chromosome complexes; (a) the one appearing in hybrids of Cuba with Purpurea, Holmes Samsoun, Ceniza, and Apolo-I (fig. 4, 5, 13, 14) and (b) that of the Ceniza-Apolo-I hybrid (fig. 6). The former rarely forms a complete circle; often it appears as a chain of six, but more frequently it is broken into smaller units. Presumably these breaks are a consequence of failure of chiasma formation, dependent upon the length of the associated segments (DARLINGTON 1929), and do not occur at random. The number of breaks observed varied from one to five. By no means, however, were all the possible configurations observed; a very large number of plates would have been necessary to have made such a demonstration possible. In one instance (fig. 5) a complete ring appeared to be half twisted, resembling a figure-of-eight, but the occurrence of this configuration must remain doubtful in view of the high degree of contraction of the chromosomes. The latter complex very rarely produces a complete ring or chain of six chromosomes; but in the majority of instances a chain of four chromosomes and a bivalent are seen instead. Both complexes take up a peripheral position in the plate.

Four-chromosome complexes

The four-chromosome complexes (fig. 7-12) are best observed in the hybrids of Purpurea and Holmes Samsoun with Cuba, Huadquina, Ceniza and

Apolo-I, where only a single such complex is present. When these complexes form complete circles, they seem to have no preference for any definite position in the metaphase plate. As the structural analysis shows, the complexes of the four hybrids of Purpurea involve different chromosomes; and since Holmes Samsoun is structurally identical with Purpurea, except for the chromosome of the non-conjunctional pair, its hybrids with these four varieties form a parallel series. Correspondingly the frequencies of specific types of associations differ in the different hybrids, but they appear to be identical in the equivalent pairs of hybrids involving Purpurea and Holmes Samsoun. The configurations observed and their approximate frequencies for a group of selected hybrids are recorded in table 2. For convenience we may call these complexes the

TABLE 2
Types and frequencies of associations of the single four-chromosome complexes in Tabacum hybrids.

TABACUM HYBRIDS	CIRCLE OF FOUR	CHAIN OF FOUR	CHAIN OF THREE ONE SINGLE	TWO PAIRS	ONE PAIR TWO SINGLES	TOTALS
Huadquina-Purpurea	33	8	—	9	—	50
Ceniza-Purpurea	1	2	2	30	1?	36
Ceniza-Holmes Samsoun	2	1	1	30	—	34
Apolo-I-Purpurea	37	1	—	2	—	40
Apolo-I-Holmes Samsoun	18	1	—	1	—	20
Cuba-Purpurea	21	1	—	3	—	25

Apolo-I, the Ceniza, the Huadquina, and the Cuba complexes. The Apolo-I and Cuba complexes form a complete circle in the majority of the cells, indicating that failure of chiasma formation in one or more of the four arms of the association is very rare. The Huadquina complex likewise frequently forms a complete circle, but in about twenty percent of cases it forms an open chain and in an additional twenty percent a pair of bivalents. The Ceniza complex, on the other hand, rarely forms either a complete circle or a chain quadrivalent, but usually forms two bivalents. Presumably these differences depend upon the lengths of the translocated segments.

In the majority of cells, as seen in polar view, when the four-chromosome complexes form a complete circle they appear as flat rings in the plate; only rarely, in about five percent of the cells, are they arranged vertically—that is, non-disjunctionally. Whether the four chromosomes are really in one plane or in a zigzag arrangement usually cannot be determined; but occasionally it is possible by careful focussing to observe that adjacent chromosomes lie at different levels.

Two four-chromosome complexes are present in the Cuba hybrids with Ceniza, Apolo-I, and Huadquina (fig. 13, 14, 17) and in the Huadquina hybrids with Apolo-I and Ceniza (fig. 15, 16). As shown by the structural analysis, these complexes are the same as those described above, except for one of the two in the Cuba-Huadquina hybrid; one is the Cuba complex, which, as

described above, usually forms a complete circle; the other is from the Cuba-Purpurea six-chromosome complex by reason of the fact that Cuba and Huadquina have one relatively translocated chromosome (8-10) in common. It frequently breaks down and appears to consist of large chromosomes. When two four-chromosome complexes are present in a cell, they appear to behave independently and to exhibit the same types and frequencies of configurations as have been described individually above.

DISCUSSION

Considering the observations recorded in table 1, the chromosomal structure of the varieties may be formally analyzed relative to that of Purpurea as depicted in table 3. The results show that in a relatively small sample of *Tabacum*

TABLE 3
Structural analysis of the Tabacum varieties.

Purpurea	1-2	3-4	5-6	7-8	9-10	11-T-12	13-14	15-16	17-18	19-20 . . .
Holmes Samsoun	1-2	3-4	5-6	7-8	9-10	11-G-12	13-14	15-16	17-18	19-20 . . .
Cuba	1-3	2-4	5-7	6-9	8-10	11-T-12	13-14	15-16	17-18	19-20 . . .
Huadquina	1-2	3-4	5-6	7-9	8-10	11-T-12	13-14	15-16	17-18	19-20 . . .
Ceniza	1-2	3-4	5-6	7-8	9-10	11-T-12	13-15	14-16	17-18	19-20 . . .
Apolo-I	1-2	3-4	5-6	7-8	9-10	11-T-12	13-14	15-17	16-18	19-20 . . .

varieties structural changes arising from reciprocal translocation have occurred in no less than eight chromosomes. These structural alterations in the chromosomes appear to be confined mainly to local varieties collected in South America; the standard commercial varieties, and derivatives from them, all appear to be identical structurally with Purpurea, the variety chosen arbitrarily as the point of reference for our *Tabacum* studies. Two of the varieties, Cuba and Huadquina, have a relatively translocated chromosome in common, which may bespeak a common source of origin. Huadquina is a recent accession collected near Machu Picchu, Peru, but no record of the New World source of origin of the other appears to be available, except that it may have come from Cuba or Tobago. In view of the large number of translocations revealed by this preliminary study, further investigations are in progress involving a number of other varieties collected by T. H. GOODSPEED in various South American localities.

Of special interest is the situation in the mosaic-resistant variety, Holmes Samsoun, one chromosome of which fails to conjugate with the corresponding member of the standard *Tabacum* set. This variety was deliberately established in order to incorporate in *Tabacum* the well-known necrotic type of response of *N. glutinosa* to infection with the mosaic virus. The normal F₁ *glutinosa-Tabacum* hybrid exhibits the necrotic type of response, but it is completely sterile. To overcome the sterility, HOLMES (1938) resorted to the use of the amphidiploid *glutinosa-Tabacum* (CLAUSEN and GOODSPEED 1925), which gives a fertile sesquidiploid hybrid with *Tabacum*. In the origin of Holmes Samsoun, the Turkish variety, Samsoun, was first crossed with amphidiploid

glutinosa-Tabacum, then the sesquidiploid hybrid thus produced was back-crossed to Samsoun; and thereafter successive selfed generations, selected for mosaic resistance, were raised until eventually a constant mosaic-resistant type was established, the Holmes Samsoun of this paper. Our supply of seed of the variety was kindly provided by DR. F. O. HOLMES himself. It is not clear just now how the mosaic resistance became incorporated in the variety, since no parallel cytological studies were conducted, but the results reported herewith seem to indicate that an entire *Tabacum* chromosome has been displaced by a corresponding *glutinosa* chromosome, which although apparently functionally a satisfactory substitute is nevertheless unable to conjugate with its *Tabacum* analogue. In the succeeding paper in this series, GERSTEL (1943) reports a demonstration of the connection between this non-conjunctional pair of chromosomes and the mosaic-resistant feature.

SUMMARY

The structural relations of the chromosomes of 15 horticultural varieties of *N. Tabacum* were studied.

Ten of them including the standard variety, Purpurea, appear to be identical structurally. All of the strictly commercial-type varieties of the sample were included in this group.

One variety, Holmes Samsoun, gives hybrids which exhibit a non-conjunctional pair of chromosomes, apparently on account of replacement of a *Tabacum* by an entire *glutinosa* chromosome in the genesis of this mosaic-resistant variety.

Three varieties—Apolo-I, Ceniza, and Huadquina—contain single reciprocal translocations relative to the standard type.

One variety, Cuba, has three reciprocal translocations; one between two and two among three chromosomes.

Structural analysis of the translocation types demonstrate that they involve eight different chromosomes.

The varieties Cuba and Huadquina have a relatively translocated chromosome in common.

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