

INTERSPECIFIC HYBRIDIZATION IN NICOTIANA. II.
A TETRAPLOID *GLUTINOSA-TABACUM* HYBRID,
AN EXPERIMENTAL VERIFICATION OF
WINGE'S HYPOTHESIS

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Numerous investigations have shown that in plants the chromosome numbers of the species of a genus are often in arithmetical progression. Excellent examples are afforded by *Triticum*, $n=7, 14$ and 21 ; by *Rosa*, $n=7, 14, 21, 28$; and by *Chrysanthemum*, $n=9, 18, 27, 36$ and 45 . Appeal has often been made to tetraploidy as a method of increase in chromosome number, but WINGE (1917) has pointed out that successive doubling of chromosome number would give rise to geometrical rather than arithmetical series, and has suggested as an alternative hypothesis interspecific hybridization followed by doubling of chromosome number. The process suggested by WINGE would establish tetraploid interspecific hybrids having $2(n_1+n_2)$ chromosomes, where n_1 and n_2 represent the haploid numbers of the parent species; and since such forms are essentially homozygous diploids, they may reasonably be expected to be fertile and constant. WINGE was unable, however, to present any experimental evidence in support of his hypothesis. In this article a preliminary account is given of a tetraploid hybrid of *Nicotiana glutinosa* and *N. tabacum*, and attention is invited to *Primula kewensis*, apparently a tetraploid *P. floribunda-verticillata* hybrid, which together offer the necessary experimental verification of the hypothesis.

The two species of *Nicotiana* employed in the present investigations are very distinctly different. *N. glutinosa* is particularly characterized by its villose pubescence; its distinctly petioled, cordate leaves; its bilabiate flowers; and its sparsely branched, racemous inflorescence. It is one of the most distinct species of the genus. *N. tabacum* is familiar to almost everyone as the tobacco of commerce. Descriptions and more complete illustrations of the forms employed in the experiments described below may be found in SETCHELL'S (1912) account of the genus. The chromosome number of *N. glutinosa* is $n=12$ (GOODSPEED 1923), of *N. tabacum*, $n=24$ (WHITE 1913, GOODSPEED 1923).

The two species have frequently been crossed. Reciprocal hybrids may be obtained, although hybridization is attended with some difficulty. Usually only a few viable seeds are produced in a capsule; in our experience an average of about ten or twelve. The F_1 hybrids are weak in germination and development, but they grow on to maturity. This, in brief, is the behavior which has been noted for all varieties of *N. tabacum* which have been tested, except one, the variety "Cuba" (cf. GOODSPEED 1915). From "Cuba" ♀ × *glutinosa* ♂ full capsules of seed are obtained. These seeds are of the same order of viability as pure seed of the species; the seedlings are vigorous; the hybrid plants develop to a height approximately equal to that of "Cuba"; and they branch profusely. GÄRTNER (1849) apparently also observed marked differences in the vigor of F_1 *glutinosa-tabacum* hybrids, when different *tabacum* varieties were employed; but as FOCKE (1881) points out, it is difficult to know how to judge these results because of the numerous discrepancies in GÄRTNER'S account of his observations. Despite these differences in vigor, the F_1 hybrids are always intermediate in appearance and they are apparently completely sterile. Numerous attempts to secure seed by backcrossing to the parental species under a variety of conditions have failed, and no seed has been found in open-pollinated capsules.

In 1922 from a single capsule of *glutinosa* ♀ × *tabacum* var. *purpurea* ♂, three plants were secured, which were grown under the garden number, 22062. Two of these plants were obvious hybrids. They were both small plants, about two feet in height, with few, slender branches and small leaves. The flowers exhibited a strong tendency towards the bilabiate shape of *N. glutinosa*, but the color was carmine, like that of *purpurea*. The leaves showed distinct evidences of *glutinosa* in their cordate shape. One of these plants was partially fertile, the other completely sterile. No other differences were noticed at that time. The third plant was very strikingly different from the other two; in fact, if our notes and memory may be depended upon, it was identical with the *purpurea* haploid which was obtained later (cf. CLAUSEN and MANN 1923). Unfortunately, we lost it during the winter of 1922-1923 because of unfavorable greenhouse conditions.

A number of flowers on the single partially fertile plant were hand-pollinated and gave selfed seed without difficulty. The capsules were harvested separately. In the season of 1923, 155 plants were obtained from one of these capsules. These, however, were set out in the field late in the season, and they did not mature. In 1924 a culture of 65 plants was grown under the garden number, 24123. For purposes of comparison

there was available at the same time a culture, 24192, of 15 F_1 plants of *purpurea* ♀ × *glutinosa* ♂. Much to our surprise, with one exception, the 65 F_2 plants of 24123 were uniform and almost identical with the F_1 plants of 24192. There were, however, important minor differences, which were found constantly to characterize the two populations. The F_1 plants were completely sterile. They set no seed on open-pollination, and twenty-five attempts at back-pollination with each of the parental species failed to give seed. Under these circumstances, capsules were retained for as long as three weeks, but in no case did they reach maturity. The plants of the F_2 population were reasonably fertile, and uniform in this respect. Large plump capsules were obtained from open-pollinated and hand-pollinated flowers and also from crosses with the parent species. These capsules contained a fair quantity of seed, but not so much as capsules of normal species.

The plants of the two populations exhibited a close correspondence in morphological characters. Both populations were very uniform. The F_2 plants had slightly, but constantly, larger flowers than F_1 plants, and the anthers were conspicuously larger. F_2 plants produced abundant pollen, most of the grains of which were normal in appearance: F_1 plants produced scanty pollen, consisting entirely of shrivelled empty grains. F_1 plants averaged about two feet in height, F_2 plants about a foot and a half. Despite the difference in height, which was probably due to their unfavorable start in the flats, the general impression given by F_2 plants was that of a slight enlargement to scale of characters of F_1 , aside from those features obviously connected with the difference in fertility.

One F_2 plant stood out from the rest by reason of its remarkable robustness. This plant eventually attained a height of six feet, and produced numerous stout branches. Despite the difference in size, however, the general morphological characters were those of the other plants of the population on an enlarged scale. Vegetative characters were proportionately enlarged, flower size only slightly. The plants of this population were all very weak as seedlings, and they grew very feebly during the time they were in flats. It is believed that the general small size of the plants in the population was due to this stunting during their early growth and that 24123P55, the robust individual, merely by some fortunate chance overcame this difficulty.

The uniformity of F_2 and its close resemblance to F_1 immediately suggested the need for cytological examination. Excellent aceto-carmines smears of pollen mother cells were easily secured; the stage of development of anthers containing them in proper condition being rather later

than is usually the case. Examinations were made of material from several plants, including the robust plant described above, which gave exactly the same results as the others. The general impression of the cytological figures was one of regularity of meiotic division rather different from the irregular distribution seen in normal F_1 *glutinosa-tabacum* hybrids. Numerous counts of first-metaphase figures showed 36 bivalents. In a few instances it was possible to count both metaphase plates in the second division, and to determine that each contained 36 chromosomes. There was of course some doubt as to the exact count in a number of figures, but only to the extent of one or two chromosomes. There were minor irregularities in distribution, evidenced by precocious splitting, lagging, and microcyte formation; but these features, while noticed, were not studied in detail. There is no doubt that the chromosome number of the plants of this population was uniformly $n = 36$, $2n = 72$.



Fig. 1



Fig. 2

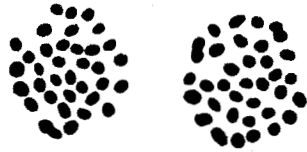


Fig. 3

FIGURE 1.—Portion of an anaphase of the normal F_1 *glutinosa-tabacum* hybrid.

FIGURE 2.—The same of the tetraploid hybrid.

FIGURE 3.—Homotypic metaphase, polar view, of the tetraploid hybrid.

Pollen-mother-cell heterotypic anaphase conditions in the sterile F_1 *glutinosa-tabacum* hybrid and in the tetraploid hybrid are illustrated in figures 1 and 2, while figure 3 shows two homotypic metaphase plates of the tetraploid, each polar view containing 36 chromosomes. Figure 1 was drawn from fixed material, the other two figures from aceto-carmin preparations. As will be noted (figure 1), the behavior of the bivalent and univalent chromosomes closely parallels that found in the F_1 *tabacum-sylvestris* hybrid elsewhere described (GOODSPEED 1923). The bivalent partners are approaching the poles while the univalents are in the equatorial zone, either dividing or preparing to divide. In the tetraploid, on the other hand, there appear to be no univalent chromosomes and the bivalent partners move in regular fashion to the poles. No attempt is made in either figure 1 or 2 to represent the full chromosome complement.

Pollen conditions are illustrated in figures 4 and 6 for the tetraploid hybrid and in figure 5 for the normal sterile F_1 . These figures are repro-

duced from photomicrographs of pollen preparations stained with aceto-carmine. As will be noted, the pollen of the normal hybrid consists exclusively of shrivelled grains devoid of contents. The pollen of the tetraploid hybrid consists mostly of large grains apparently normal in protoplasmic contents. Measurements were made of pollen grains of the tetraploid hybrid and of its *tabacum* parent, but unfortunately no pollen of *glutinosa* was available at the time measurements were made. The average diameter of pollen grains in the tetraploid hybrid was found to be 46.3 microns, of *tabacum*, 36.7. The volumes are therefore, in the ratio of approximately 2 : 1 (106 : 49).

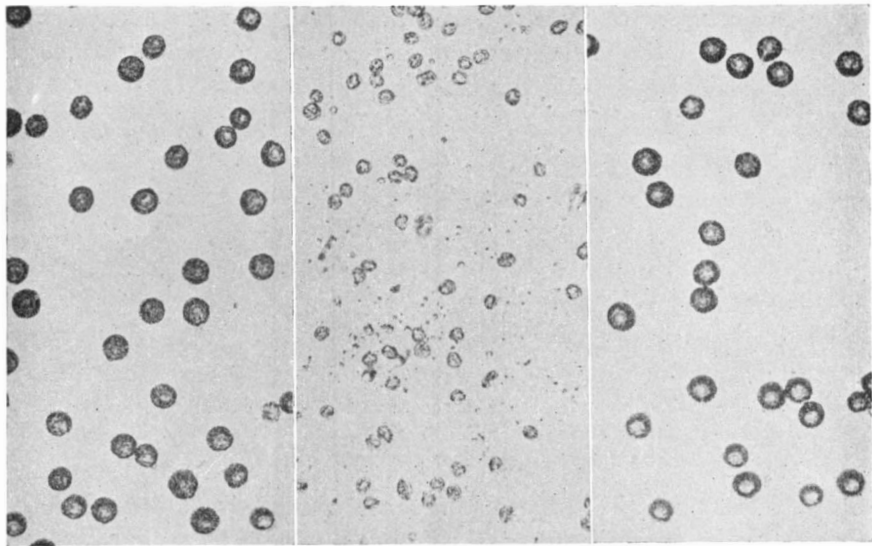


Fig. 4

Fig. 5

Fig. 6

FIGURE 4.—Portion of a photomicrograph of pollen of the tetraploid *glutinosa-tabacum* hybrid, 24123P55.

FIGURE 5.—Of the normal F_1 , 24192P4.

FIGURE 6.—Of another plant of the tetraploid hybrid, 24123P58. The preparations were stained in aceto-carmine and the photomicrographs were taken at the same magnification.

The cytological findings supply an obvious explanation for the uniformity and constancy of this hybrid. Since in *glutinosa*, $n = 12$, and in *tabacum*, $n = 24$, the F_1 hybrid normally has 36 chromosomes. This was undoubtedly the case in the sterile F_1 described above. The original fertile F_1 plant, 22066P2, must have arisen from a doubling of the chromosome number immediately or soon after fertilization, by which a tetraploid hybrid with 36 pairs of chromosomes was produced. Such a plant may be represented by the chromosomal formula, 12 GG + 24 TT;

and, if *glutinosa* and *tabacum* homologues pair regularly, fertility and constancy follow as a matter of course, for every gamete would then contain 12 *glutinosa* and 24 *tabacum* chromosomes. If this explanation is correct, an interspecific hybrid may be expected to become fertile and constant by simple doubling of its chromosome number.

These observations naturally recall the case of *Primula kewensis*, the much discussed hybrid of *P. floribunda* with *P. verticillata*. According to accounts of its origin as described by Miss DIGBY (1912) and by the Misses PELLEW and DURHAM (1916), the original hybrid was sterile; but it eventually produced a fertile bud-sport which gave rise immediately to the fertile, comparatively constant form now known as *P. kewensis*. Miss DIGBY found that the chromosome numbers of *P. floribunda* and *P. verticillata* were both $n=9$ and $2n=18$, that the sterile hybrid had 18 chromosomes and the fertile *P. kewensis*, 36. *P. kewensis*, therefore, is evidently a tetraploid hybrid; and as WINKLER (1920) and RENNER (1924) suggest, this fact probably accounts for its genetic behavior. If it contains 9 pairs each of chromosomes of *P. floribunda* and *P. verticillata*, and homologues of each species pair regularly, the situation is exactly the same as that described in the tetraploid Nicotiana. It seems more reasonable to adopt this explanation of its chromosome number, since it accounts so well for the genetic results thus far obtained with it, rather than that of transverse fission suggested by FARMER and DIGBY (1914) on the basis of chromosome measurements, which has been accepted by GATES (1924) in his recent discussion of polyploidy.

The confirmation of WINGE's hypothesis afforded by the instances described above extends only to establishment of the tetraploid chromosome condition, and not to the method of origin described by him. The establishment of the condition is evidently a mutational event, analogous to that which occurs in the establishment of the tetraploid condition in pure species. The tetraploid hybrid condition may, however, arise in a variety of ways: (1) by doubling of chromosome number immediately subsequent to fertilization; (2) by bud-variation in an F_1 interspecific hybrid; (3) by crossing together tetraploid representatives of two different species; and (4) by irregular distribution of chromosomes in an interspecific hybrid in which the chromosomes do not pair in meiosis, as suggested by COLLINS and MANN (1923). It may be possible, therefore, that tetraploid hybrids have the significance in the origin of new chromosome numbers ascribed to them by WINGE.

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