THE BOTANICAL REVIEW

VOL. XI DECEMBER, 1945 No. 10

CYTOTAXONOMY OF NICOTIANA

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INTRODUCTION

During the past fifteen years there has accumulated a body of new morphological, distributional and cytological information concerning the some 60 valid species of the genus *Nicotiana* sufficiently large and sufficiently significant to become subject to review along with cytotaxonomic conclusions which have been derived from it. In the University of California Botanical Garden studies of the genus *Nicotiana* were first undertaken by the late Prof. W. A. Setchell over 35 years ago (134) and have been carried on continuously since that time. Originally they had to do with genetic analysis of numerous varieties of *Nicotiana tabacum* (82, 136) and with the character of *Nicotiana* species used or cultivated in the pre- and post-Columbian periods (135). Later the scope of the *Nicotiana* investigations was broadened to include cytogenetic and other studies of the origins and relationships of all the species of the genus. To this latter end an almost complete living collection of species and varieties of *Nicotiana* has been grown for many years. In addition to the *Nicotiana* investigations just mentioned the only other comprehensive ones of like nature are those which have been carried on in Russia and Bulgaria by Dr. D. Kostoff and his assistants.

As will be indicated below, the majority of *Nicotiana* species are today found in temperate South America. In order to determine their distribution, the extent of their natural variation and the possible occurrence of previously unknown species, three expeditions have been sent to South America from the University of California Botanical Garden during the past ten years. Members of these expeditions made numerous collections of herbarium specimens and seeds of species of *Nicotiana* in Peru, Bolivia, Chile, Argentina and Uruguay. Additional material has been received from collaborators in South America and in Australia and the South Pacific. These accessions have included a number of previously unknown or little known species grown and investigated for the first time in the University of California Botanical Garden; among them are *N. benavidesii* (53, 56), *N. cordifolia* (48, 54, 56), *N. knightiana* (53, 56), *N. thyrsiflora* (53, 56), *N. setchellii* (55, 56), *N. otophora* (56, 66), *N. wigandioides* (48, 49, 56), *N. arentsii* (57), *N. corymbosa* (48, 54, 56), *N. spegazzinii* (56), *N. linearis* (54, 56), *N. suaveolens* (48, 68, 153, 154), *N. exigua* (153, 154), *N. maritima* (50, 152, 153, 154), *N. velutina* (152, 153, 154), *N. gossei* (154), *N. excelsior* (153, 154), *N. megalosiphon* (48, 152, 153, 154), *N. goodspeedii* (50, 152, 153, 154), *N. occidentalis* (154), *N. rotundifolia* (48, 50, 152, 153, 154), *N. debneyi* (48, 50, 152, 153, 154) and *N. fragrans* (154).

Following brief summaries of the taxonomy and cytology of the genus *Nicotiana*, the cytotaxonomy of the three subgenera will be reviewed and commented upon. Where no citation of literature accompanies factual information or conclusions drawn therefrom reference is made to material not as yet published by the author and his associates. To avoid repetition of citations and otherwise to conserve space, references to the literature concerning the cytology of the numerous interspecific hybrids of *Nicotiana* are largely confined to the list of such hybrids on p. 579.

TAXONOMY

The genus *Nicotiana* has been referred to Tribe Cestrineae by Bentham and Hooker and to Tribe Cestreae, Subtribe Nicotianinae, by Wettstein. Unlike some other members of the Solanaceae, no specialized feature distinguishes it from other genera. In the Bentham and Hooker (7) classification *Nicotiana* stands closest to *Fabiana, Vestia* and *"Dittostigma"* (probably *Nicotiana longiflora* var. *acutiflora,* according to Millán 113); in Wettstein's (151), *Petunia, Bouchetia, Nierembergia* and *Parabouchetia* are added to these. Morphological resemblance between Solanaceae and Scrophulariaceae, an acknowledged feature of the tribes Cestreae and Salpiglossideae (126), is not consistent nor pronounced in *Nicotiana,* although it occurs.

Taxonomically the Linnean genus *Nicotiana* today remains intact, although the many species added in the interim have considerably broadened its definition. Between 1781 and 1874, 13 attempts were made to erect genera for particular *Nicotiana* species, and one of these, *Lehmannia* of Sprengel (142), survived as late as 1899 (28). The suggestion that the genus *Petunia* should be included under *Nicotiana* (151) has not been accepted, although Fries (42) recognized in *Petunia* the subgenus *Pseudonicotiana* comprising the most *Nicotiana*-like *Petunia* species.

The treatment of *Nicotiana* by Bentham and Hooker, while not entirely complete for inflorescence, is a useful short diagnosis of the genus. Rather comprehensive descriptions of the classical species are given by Don (30), Dunal (32), Comes (28) and Grabovetzkaya (72). The first taxonomic organization to embrace many species of the genus was that of Don (30), in which four sections—*Tabacum, Rustica, Petunioides* and *Polydiclia*—were recognized. Wettstein's (151) reduction to three sections by elimination of *Polidiclia* has been generally accepted. Only Gray (74) has abandoned emphasis on corolla shape and color in favor of capsule dehiscence.

On purely morphological grounds Splendore (141) in 1906 and Anastasia (1) in 1914 postulated the hybrid derivation of certain species of *Nicotiana* and advanced hypotheses concerning the phylogenetic structure of the genus. Between 1912 and 1930 a number of investigators (*cf.* 16, 34, 70, 71, 83) supplied information on the cytology and genetics of many species and thereby implemented a mode of attack upon phylogenetic problems in *Nicotiana* which Splendore and Anastasia had anticipated. East (34) referred to "a number of genetic centers which may possibly be made the basis for various genetic subdivisions when our information is more complete". Goodspeed (50) crystallized and expanded this "genetic group" concept, applied it to the entire genus and contrasted clusters of related species with amphidiploids.

Recently Kostoff (97, 98) has set aside the Donian organization of the genus in favor of eight "sections", to seven of which he attaches the designations given by Goodspeed (48, 50, 56) to the "genetic groups" that the latter described, although the composition of certain of Kostoff's "sections" is not the same as that of the "genetic groups" carrying the same designation. By contrast, the following classification proposed by the author (58) retains, as did Wettstein's three of Don's major subdivisions, referring to them as subgenera and recognizing within each of them a series of sections, some of which, in general, correspond to the author's "genetic groups", others to amphidiploid species, while still others are of a more inclusive nature. This classification reflects to some extent the cytological evidence reviewed in this article.

Of a considerably greater number of species of *Nicotiana* that have been described, only the following 58 are here recognized as valid. As will be noted below, chromosome number and morphology are known in 55 of these species, and 53 of them have been involved as parents in F_1 interspecific hybrids, the meiotic chromosome behavior of which has been analyzed (*cf.* p. 579). Three of the species listed, *N. ameghinoi* Speg., *N. ingulba* Black and *N. stenocarpa* Wheeler, are presumably known only from the wild state, and their cytology has not been investigated. The validity of *N. frigida* Phil. (54), closely allied to members of the Acuminatae, is not entirely clear, and this species is not therefore being retained here.

SUBGENUS *Rustica*

Section Paniculatae: *N. paniculata* L., *N. knightiana* Goodsp., *N.*

solanifolia Walp., *N. benavidesii* Goodsp., *N. raimondii* Macbr., *N. cordifolia* Phil., *N. glauca* Grah.

Section Thyrsiflorae: *N. thyrsiflora* Goodsp.

Section Rusticae: *N. rustica* L.

SUBGENUS *Tabacum*

Section Tomentosae: *N. tomentosa* R. & P., *N. tomentosiformis* Goodsp., *N. otophora* Griseb., *N. setchellii* Goodsp., *N. glutinosa* L.

Section Genuinae: *N. tabacum* L.

SUBGENUS *Petunioides*

Section Undulatae: *N. undulata* R. & P., *N. wigandioides* Koch & Fint., *N. arentsii* Goodsp.

Section Trigonophyllae: *N. trigonophylla* Dun., *N. palmeri* Gray.

Section Alatae: *N. alata* Link & Otto, *N. langsdorffii* Weinm. apud Roem. & Schult., *N. bonariensis* Lehm., *N. longiflora* Cav., *N. plumbaginifolia* Viv., *N. sylvestris* Speg. & Comes, *N. repanda* Willd., *N. stocktonii* Brandeg., *N. nesophila* Johnst.

Section Noctiflorae: *N. noctiflora* Hook. (syn. *N. cavanillesii* Dun.), *N. petunioides* (Griseb.) Millán (syn. *N. pampasana* O. Kuntze), *N. ameghinoi* Speg., *N. acaulis* Speg.

Section Acuminatae: *N. attenuata* Torr., *N. acuminata* (Grah.) Hook., *N. pauciflora* Remy (syn. *N. caudigera* Phil.), *N. corymbosa* Remy, *N. miersii* Remy, *N. linearis* Phil., *N. spegazzinii* Millán, *N. clevelandii* Gray, *N. bigelovii* (Torr.) Wats., *N. nudicaulis* Wats.

Section Suaveolentes: *N. suaveolens* Lehm., *N. maritima* Wheeler, *N. velutina* Wheeler, *N. exigua* Wheeler, *N. gossei* Domin, *N. excelsior* Black, *N. benthamiana* Domin, *N. megalosiphon* Heurck & Müll. Arg., *N. goodspeedii* Wheeler, *N. occidentalis* Wheeler, *N. rotundifolia* Lindl., *N. debneyi* Domin, *N. fragrans* Hook., *N. ingulba* Black, *N. stenocarpa* Wheeler.

Of the species listed above, three have previously been dealt with cytologically and otherwise under different designations by a number of investigators. Priority of publication necessitates the replacement of *N. pampasana* O. Kuntze by *N. petunioides* (Griseb.) Millán, and *N. caudigera* Phil. by *N. pauciflora* Remy. In addition, it appears necessary with the evidence at present available to replace *N. cavanillesii* Dun. by *N. noctiflora* Hook. (*cf.* 73).

CYTOLOGY

Taxonomy has some reflection in the chromosome number constitution of the genus *Nicotiana.* For the Solanaceae (exclusive of *Nicotiana*), 12 pairs of chromosomes or multiples of 12 represent the mode, while 7, *ca.* 8, 9, 10, 11, 14, 17, 22, 30 and 34 pairs have also been reported (*cf.* 146). The chromosome numbers of *Nicotiana* species (18, 45, 48, 50) show a mode at 12 pairs with 9, 10, 16, (32 *cf.* p. 570), 18, 19, (154), 20, 21 (154), 22 and 24 pairs also occurring. This sequence of chromosome numbers is wide in range for a genus of the Solanaceae and includes certain numbers characteristic of Scrophulariaceae. Of the 55 species of *Nicotiana* for which chromosome numbers have been reported, 28 are 12-paired, eleven are 24-paired, four are 16-paired, three are 9-paired, two are 10-, 19- or 20-paired and the remaining numbers correspond to a single species in each case. Presumably all 24-paired American species are of amphidiploid origin. It has been suggested (16, 29, 87, 98) that 6 represents the basic chromosome number for the genus.

Chromosome morphology in most of the 55 species above referred to has recently been determined from comparative studies of pollen grain and root tip mitoses (50, 59, 154). In the case of a few species pollen grain mitoses alone have been investigated. The chromosomes of the genus are relatively small to medium in size (96). Although size distinctions within a genom or between members of different genoms are appreciable, the size range is not great. An average of 2.2 μ for the genom of shortest and 5.5 μ for the genom of largest chromosomes was previously reported (50), but it is now known (59) that the average chromosome size in *N. acaulis* somewhat exceeds 5.5 μ (*cf.* p. 567).

In *Nicotiana,* chromosome morphology, apart from differences in size, is sufficiently distinctive in many species to possess significance for interpretation of their origins and relationships. The chromosomes have been referred to (59) as median, submedian or subterminal where the arm ratio (length of long arm vs. that of short arm) is respectively 1 : 1, greater than 1 : 1 but less than 3 : 1, and 3 : 1 or greater. Simplifying the situation as much as possible for the moment by combining median and submedian positions of the centromere, it appears that for the genus as a whole these chromosomes predominate over subterminal ones in the ratio of approximately 5 to 3 (59). However, for each subgenus a distinctive ratio obtains. Thus, in subgenus Rustica the ratio is 9 to 1; in subgenus Tabacum, 5 to 2; and in subgenus Petunioides, 4 to 3. Employing the term "karyotype" in accordance with Lewitsky's definition (106, *cf.* 4) to designate the physical properties of the chromosomes of a complement visible at somatic metaphase, it is impossible, therefore, to establish a common karyotype pattern for *Nicotiana* (59). The suggestion (107, 131) that the possession by a species of *Nicotiana* of a genom consisting largely of median chromosomes connotes a more primitive rather than a derived condition, is largely borne out by other evidence.

It is to be noted that in *Nicotiana* the region of spindle attachment is localized in a chromosomal organ, the centromere. The genoms of all species show at least one pair of chromosomes bearing proximal satellites. The satellite thread may be long or short, and the segment may vary in size from just within the limits of visibility to approximately one-fifth the total length of the chromosome, distinctions in length of thread and size of segment being relatively constant specific characters. Frequently there are two satellited pairs (rarely one of these pairs with distal rather than proximal satellites) and, in the species of higher chromosome number, sometimes three satellited pairs. When only one pair is involved the satellites are characteristic of subterminal chromosomes, or, if no such chromosomes occur, of the short arms of a submedian pair. In species where two or more pairs are satellited the satellites involve a subterminal pair if present (*cf.* p. 571), and usually one or more submedian pairs, although in some instances both satellited pairs are subterminal. Strictly median chromosomes are rarely satellited.

Evidence of the relation of the nucleolus to the chromosomes in *Nicotiana* has been observed in only a few species. Kater (81) in *N. longiflora* traced the origin of the nucleolus to portions of telophase chromosomes. Correlation of the number of satellited chromosomes and the number of nucleoli at comparable stages (before fusion) and the presence of pycnotic regions associated with the nucleoli in resting nuclei have been observed in several species of section Alatae (3, 64)—*N. alata, N. langsdorffii, N. longiflora, N. plumbaginifolia* and *N. sylvestris*—and in the last named species the nucleoli have been seen at telophase to have their origin in matrical material surrounding definite nucleolus-organizing regions of three chromosomes, two satellited and a third with pycnotic terminal region but no satellite. Gates (43) refers to the situation in *N. sylvestris* and interprets all three chromosomes as nucleolus-organizing. Additional constrictions for which a nucleolus-organizing function has not yet been demonstrated in *Nicotiana* occur in certain chromosomes (59, 154). Their presence is reflected by flexures distinct from flexures referable to coiling.

Studies of chiasmatype in species of *Nicotiana* are somewhat difficult because of the relatively small size of the majority of the chromosomes involved and their extreme contraction at the first meiotic metaphase (MI). Nevertheless, detailed knowledge of its karyotype permits both analysis of some to many of the bivalents of a given species and determination of the relation between somatic chromosome size or morphology, or both, and chromosome behavior at MI (50). Thus, a pair of either large or small median or nearly median chromosomes forms one chiasma in each arm to produce a ring bivalent. In such case, where small chromosomes are involved, terminalization is usually complete by MI, while it may not be entirely complete in bivalents of large chromosomes. In the case of submedian chromosomes a chiasma often appears only in the long arms and often is unterminalized at MI to give a characteristic cross-shaped bivalent. However, long chromosomes of this type frequently form two chiasmata, at times one in each arm. In the latter instance the chiasma in the short arms is usually terminalized at MI. Pairs of subterminal chromosomes, if short, commonly show a single, terminalized chiasma, but, if unusually long, may form two or, rarely, three chiasmata, in the last case including one in the exceedingly short arm or "head" (107).

The relation between these various chiasmatypes and distinctions in chromosome morphology is shown by comparison of chiasma frequencies with the ratios of median and submedian to subterminal chromosomes present. Thus, studies of four species of subgenus Rustica, five species of subgenus Tabacum and ten species of subgenus Petunioides show that the chiasma frequency per pair in the three subgenera is 1.87, 1.57 and 1.40 respectively (59). In other words, in the first subgenus where the genoms consist of chromosomes almost all of which are median or submedian, 87% of the bivalents show two chiasmata; in the second subgenus, where the ratio of median or submedian to subterminal is 5 to 2, two chiasmata appear on 57% of the bivalents; and in the third subgenus, where approximately equal numbers of median or submedian and subterminal chromosomes occur, 40% of the bivalents have two chiasmata. The consistent relation between karyotype pattern, chiasma frequency and terminalization coefficient of the series of representative species studied has made it possible to predict with accuracy the character of pairing at MI in all other species for which the karyotype pattern is known.

Meiotic chromosome behavior of 131 F_1 interspecific hybrids of *Nicotiana* has been determined by the author and his associates. Other investigators (*cf.* 16, 17, 31, 40, 114, 116, 117, 132, 133), and in particular Kostoff (98), have reported upon certain of the same hybrids and upon 78 additional ones. On page 579 is a list of F_1 interspecific hybrids in *Nicotiana* with literature citations. In general, our findings agree with those of others who have studied the same hybrids, while the majority of such discrepancies as occur are susceptible of interpretation. In almost every instance our conclusions are based upon determinations of the extent and character of chromosome pairing at MI in from 50 to 100 pollen mother cells (PMC), all cells in a given field being independently analyzed by two investigators. In computing number of pairs where multivalents occur, a trivalent is taken to have the value of one pair, a quadrivalent of two pairs, *etc.* Meiotic behavior has been examined in plants at different stages of maturity and under different seasonal and cultural conditions. Our evidence indicates that, although certain hybrids are peculiarly sensitive to distinctions in environment, the majority do not reflect by variation of meiotic behavior such distinctions, unless they are extreme (*cf.* however, 11, 96, 102, 132). It has been reported (98) that the extent or character of pairing is different for certain reciprocal hybrids, but in our experience this is not the case.

Where reference is made to the range of chromosome pairing of a group of hybrids it will be understood that not all of the individual hybrids involved show the total inclusive range reported. Likewise, the mode may differ slightly from one hybrid to another. For example, on page 555 hybrids between members of section Tomentosae and members of sections of the other two subgenera are discussed. Chromosome behavior at MI of 18 hybrids is involved, and a combined pairing range of zero to 8 with mode at 2 and at 3 is reported. These inclusive statements refer to a series of pairing ranges for individual hybrids of zero to 4, zero to 5, *etc.*, up to zero to 8, the corresponding modes being either 2 or 3 in each instance.

Twenty-eight of the 131 F_1 interspecific hybrids we have studied (50, 60, 154) and five additional ones reported by Kostoff (98) show at MI as complete or almost as complete pairing as would occur in the parental species. By contrast, another considerable group of hybrids is characterized by almost complete lack of pairing, 33 such hybrids having a range of zero to 3 (rarely 4) pairs with the mode at zero (rarely 1 or 2) pairs (50, 60). Kostoff (83, 97, 98) reports a similar range of pairing for 12 of these same hybrids and for 37 additional ones. For the 33 hybrids the mode of zero includes well over 50% of the PMC analyzed, and such lack of conjugation is taken to be a reflection of lack of genic equivalence or similarity so far as the species involved are concerned (50, p. 388). Such conjugation as occurs has been assigned to non-homologous association or autosyndesis (50, 88, 103) In 22 hybrids which we have studied and seven additional ones reported by Kostoff (83, 97, 98), there is a low but variable degree of pairing, the range in number of pairs being at times considerable (0–8). In this category the mode is consistently low with approximately 25% of the PMC showing only two or three pairs at MI. This type of pairing indicates the presence of a number of small homologous segments. In another group of hybrids (a total of 31 reported) the range in number of pairs is also wide (from one or two pairs to almost complete pairing), but the mode is higher than in the last instance and is usually at least one-half the total number of pairs possible in the particular interspecific hybrid involved, with 25% of the PMC in the modal class. The final category of pairing, where 46 hybrids have been reported, is one in which the number of pairs is the same as the haploid number of chromosomes of the parental species with the lower chromosome number, *i.e.*, "Drosera scheme" pairing (128). Obviously, it occurs only in interspecific hybrids the parents of which differ in number of chromosomes. It has been found particularly characteristic of hybrids between amphidiploid species and each of the two species the progenitors of which entered into the origin of the particular amphidiploid. Thus, the extent of pairing exhibited by F_1 interspecific hybrids of *Nicotiana* can, in general terms, be referred to as either complete, lacking, low variable, high variable, or of the "Drosera scheme" type (*cf. N. "eastii"* p. 577).

Chromosome behavior at diakinesis is consistent with that at MI (50, 60), but knowledge of earlier prophase conditions in species as well as in hybrids is fragmentary (39, 64, 98, 102, 119). In numerous instances complete pairing at MI is followed by at least partial fertility (*cf.* 98, 114). Hybrids exhibiting other classes of pairing—low variable, high variable and "Drosera scheme" where one parent is of amphidiploid origin—are almost completely sterile, non-conjunction, non-disjunction, lagging chromosomes, *etc.*, resulting in failure of production of viable gametes (12, 98 p. 738, 103).

The quality of the pairing, as far as consistent with the karyotype patterns involved, shows correlation with its amount (50, 60). Thus, as the amount of pairing increases progressively through the categories of pairing above referred to, the chiasma frequency increases and the terminalization coefficient decreases. When there are few bivalents they are rod shaped, united by a single and frequently terminal chiasma. As the mode in number of pairs increases an increasing number of ring bivalents is seen, along with other bivalent configurations which are products of two or more chiasmata and decreased terminalization. For example, in F_1 *N. paniculata* × *N. miersii*, both species having predominantly median or submedian chromosomes and the hybrid exhibiting practically no pairing (0–2), the chiasma frequency is 1.00 per pair and the terminalization coefficient 0.85; whereas in F_1 *N. paniculata* × *N. solanifolia*, only median or submedian chromosomes and complete pairing being involved, the corresponding figures are 1.75 and 0.50, and in F_1 *N. noctiflora* × *N. petunioides*, involving all subterminal chromosomes and complete pairing, they are 1.05 and 0.60. In the example just given amount of pairing is contrasted in the first two hybrids and karyotype pattern in the last two.

In addition to bivalents, the configurations of which are reflections of chiasmatype, F_1 interspecific hybrids, particularly those of the lower pairing categories, and haploids of *Nicotiana* exhibit union of univalent chromosomes by "offspindle attachments". Two or more univalents lying at random off the MI plate region may become attached, at times forming a chain when from three to six chromosomes are involved (125, *cf.* 105). Kostoff (98) uses the term "tandem attachment" for an apparently equivalent condition. This phenomenon has been interpreted as fixation artifact (39), persistence of the threads sometimes seen to connect univalents at diakinesis (102), as related to non-homologous pachytene association (3, 157), or residual homology (125).

Of numerous species of *Nicotiana* in which meiotic behavior has been studied, only two, "*N. eastii*" (95, 154, *cf.* p. 570) and *N. arentsii* (57), have shown multivalents at MI, and both are known to be of polyploid origin. On the other hand, Kostoff reports multivalents at MI in *N. alata* and in *N. sylvestris* (98). It is difficult to evaluate this report because the two species in question have long been under critical cytological observation by us (3, 64) and have given no evidence of valencies higher than two. Of the 131 F_1 interspecific hybrids analyzed cytologically by us (3, 50, 60, 154), approximately one-half are characterized at MI by the presence of one or more trivalents and, less frequently, higher valencies. In the hybrids he has studied Kostoff (98) refers to trivalent formation in about the same proportion of instances but rarely mentions the occurrence of higher valencies or the percentage of PMC in which multivalents are seen. Our results in this latter connection indicate that the F_1 interspecific hybrids can be classified as follows: those in which multivalents occur in from 1% to 6% of the PMC, in from 6% to 25% (most frequently 15% to 25%), in approximately 50%, and in approximately 100% of the PMC, and that these subdivisions based upon amount of multivalent formation are in general correlated with categories of extent of pairing and with degree of relationship of the species involved in the hybrids in question.

Spontaneous and induced alterations in the chromosomal content of a number of species of *Nicotiana* have been reported. Thus, for example, Mallah (109), investigating structural differences among the chromosomes of 15 varieties of *N. tabacum,* found that while the majority of them appear to be identical structurally, four varieties show structural interrelationships indicating reciprocal translocations, relative to the standard type, affecting eight chromosomes. The monosomic and trisomic condition has been shown to occur in various species (*cf.* 2, 19, 21, 24, 26, 34, 61, 64, 120, 121, 139).

Haploidy (*cf.* 86, 91, 111, 150) and chromosome doubling leading to tetraploidy, octoploidy, amphidiploidy and sesquidiploidy have frequently been reported (*cf.* 36, 37, 66, 98, 144, 145, 148, 149). Most of these categories of chromosomal alterations involving an entire chromosome complement or individual members of chromosome complements, and other conditions such as asynapsis, have been induced by treatment with high frequency radiation of reproductive and vegetative tissues of a number of species of *Nicotiana* (38, 46, 47, 51, 52, 62, 63, 64, 143). Effects of colchicine, acenaphthene, sanguinarine, centrifuging, temperature extremes, grafting and aging of seed have been shown to induce chromosome doubling, aneuploidy and structural chromosome alterations in *Nicotiana* (8, 84, 90, 92, 93, 94, 101, 138, 140, 147).

CYTOTAXONOMY

In what follows notes on the morphology and distribution of the constituent sections of the three subgenera will be supplemented by the cytological information pertaining to each of the sections with a comment, in the case of each subgenus, on the significance of the combined evidence in terms of the taxonomic arrangement (*cf.* p. 536) of the genus adopted here.

As already noted, analyses of somatic chromosome morphology in the 55 species referred to below are based upon studies of mitoses in pollen grains and root tips (50, 59, 131, 154). Meiotic chromosome behavior at MI in some 210 F_1 interspecific hybrids is also discussed. Reference has already been made (p. 540) to the character and quality of pairing in these hybrids and their parental species, to the correlation between chiasmatype and karyotype and to the chiasma frequencies and terminalization coefficients of the three subgenera. Although species the close relationship of which is otherwise variously indicated commonly show a high degree of compatibility, the possibility of securing a mature F_1 hybrid between *Nicotiana* species is not a reliable index of relationship. It has been reported that compatibility shows "greater relationship to chromosome number than to taxonomic status" (16, 114), that its extent indicates "the trend of relationship in a general way" (34), and that "crossibility decreases with decrease in relationship" (98). However, many F_1 hybrids which earlier investigators failed to obtain have since been successfully made. There have been efforts to set up categories of compatibility (16, 34, 98, 110, 112). It has been shown that in *Nicotiana,* as elsewhere, crossibility is related to the use of the species possessing the higher chromosome number as female parent, although there are notable exceptions (34, 50, 83, 98). In certain cases seasonal effects, mechanical conditions and stage of maturity are said to influence crossibility (35, 83, 98). Undoubtedly of significance is the possibility of genic blocks to successful hybridization. In this general connection attention is called to the fact that the appended list of F_1 interspecific hybrids in *Nicotiana* includes only those for which cytological evidence is available. It is, therefore, in no sense an index of compatibility, since numerous additional hybrids have been obtained (*cf.* 110, 112). References to the literature concerning many of the hybrids discussed here are given in the list only. The morphology of the majority of the hybrids referred to has been described in considerable detail (67, 98, 124).

Weight is assigned to the extent and quality of pairing at MI of interspecific hybrids in interpretation of relationships between species and larger taxonomic subdivisions (50 p. 388). Meiotic behavior and categories of pairing in hybrids have already been discussed (*cf.* p. 542). The fact that the phylogenetic conclusions reflected in the present taxonomic arrangement are supported by evidence from morphology, distribution and karyotype pattern as well as by extent of pairing in F_1 interspecific hybrids should be borne in mind in connection with a statement by Darlington (29 p. 172) which gives the impression that pairing relations alone have been used as a criterion in establishing species origins and relationships in the genus *Nicotiana.*

Subgenus Rustica

Section Paniculatae: *N. paniculata, N. knightiana, N. solanifolia, N. benavidesii, N. raimondii, N. cordifolia, N. glauca.*
Section Thyrsiflorae: *N. thyrsiflora.*
Section Rusticae: *N. rustica.*

Stout herbs or short-lived shrubs with yellow or green, tubular or tubular-salverform corolla.

This subgenus represents, in general, a morphological unit. Vegetatively, it presents two patterns: (a) a relatively thick stemmed, erect, bushy herb or shrub type provided with well spaced, petioled leaves (Paniculatae and Rusticae); (b) a spicate plant type clothed with crowded, narrow, sessile leaves (Thyrsiflorae).

Three flower types that correspond to the three sections are distinguishable on the basis of corolla shape and stamen characters—those of the first and last sections less distinct from each other, and that of the Thyrsiflorae more distinct from those of the other two. In the Paniculatae all six species are clearly inter-related; *N. paniculata* and *N. knightiana* show close morphological affinity in the majority of characters, while resemblance in flower relates *N. raimondii* to *N. cordifolia* and *N. solanifolia* to *N. benavidesii.* In leaf shape and in habit (exclusive of inflorescence) *N. benavidesii* approaches *N. glutinosa* of subgenus Tabacum, section Tomentosae. The structure of the inflorescence together with longer life span and greater woodiness sets *N. glauca* somewhat apart from the other members of the Paniculatae.

Five of the nine species of this subgenus occur only in Peru, and for a sixth, *N. rustica,* which is also known from Ecuador, the Peruvian Andes represent the center of distribution. All of these six species occupy arid or semi-arid habitats, but only *N. paniculata* is widespread in latitudinal distribution (near the coast), while *N. thyrsiflora* is restricted to the northern highlands, *N. benavidesii* and *N. raimondii* to the southern Andes, and *N. knightiana* to the southern coast. Of the remaining three species in the subgenus, *N. cordifolia* is endemic on Mas-a-Tierra Island in the Juan Fernandez group west of central Chile, *N. solanifolia* has a limited distribution in the fog belt on the coast of northern Chile and *N. glauca,* although introduced and now widely distributed elsewhere, is found in the wild state only in northwestern Argentina. The first two of these geographically outlying species possess considerable morphological resemblance to all the remaining species except *N. thyrsiflora* and *N. glauca.* The last named reflects something of a morphological hiatus so far as the subgenus as a whole is concerned.

Section Paniculatae. a. Karotypes. Each of the seven species of this section—*N. glauca, N. raimondii, N. solanifolia, N. cordifolia, N. benavidesii, N. knightiana, N. paniculata* (in order of decreasing average chromosome size)—possesses 12 pairs (48, 50, 59). Except in *N. glauca* and *N. benavidesii,* all the chromosomes of these species are median or submedian, and in each species one submedian pair is satellited. *N. benavidesii* conforms to this basic pattern except that its karyotype contains two subterminal pairs, one satellited (59). The detailed formulae for six of the species of this section are: *N. raimondii, N. solanifolia* and *N. cordifolia*—$4\ m+8^1\ sm$; *N. knightiana* and *N. paniculata*—$6\ m+6^1\ sm$; *N. benavidesii*—$6\ m+4^1\ sm+2^1\ st$ (the figure referring to the number of pairs, the superscript designation to the number of satellited pairs, and *m* and *sm* to median or submedian centromere position). By contrast with this general uniformity of karyotype pattern, most of the chromosomes of *N. glauca* are subterminal with the formula $1\ m+1\ sm+10^1\ st$ (50, 59).

One other study of *N. glauca* (131) made of root tip mitoses only, provides the formula $1\ m+11^1\ st$. The chromosomes of *N. glauca* have frequently been reported to be the longest in the genus (16, 50, 98, 131). The rod-like form of most of the *N. glauca* genom creates such an impression, whereas actual measurement shows that the total chromosome length in *N. raimondii* or *N. solanifolia* approximates, and in *N. acaulis* (subgenus Petunioides, section Noctiflorae) considerably exceeds, the total length in *N. glauca.*

b. Meiotic chromosome behavior in F_1 interspecific hybrids. As shown in the subjoined list (p. 579) which, as already noted, refers only to F_1 interspecific hybrids which have been studied cytologically, both *N. raimondii* and *N. solanifolia* have been crossed with all other species of the Paniculatae and *N. benavidesii* and *N. paniculata* with all except *N. cordifolia.* With the last named *N. knightiana* has been crossed, and if, as appears certain, *N. paniculata* and *N. knightiana* are practically equivalent genically, the series of F_1 intrasectional hybrids includes all possible combinations of the species concerned, except those involving *N. glauca.* With the exception of F_1 *N. benavidesii* × *N. solanifolia* which, however, shows high variable pairing with a range of 2 to 11 pairs and a mode at 6 pairs, chromosome conjugation is complete or nearly so at MI of these nine hybrids. In all except those involving *N. cordifolia* multivalents occur in from 12% to 25% of the PMC.

With the exception of *N. cordifolia, N. glauca* has been crossed with every other species of the section. In each case pairing is lacking or of the low variable type. For example, in F_1 *N. panicu-*

lata x *N. glauca* the pairing range is zero to 3 with the mode at zero pairs. In this case Kostoff (83) reported a range of 9 to 12 pairs and of 6 to 10 pairs for the reciprocal and, later (98), a range of 4 (rarely 3) to 10 pairs and some trivalents for the original hybrid.

Between members of the Paniculatae and members of the other two sections of subgenus Rustica and also members of the other two subgenera, F_1 hybrids have been made. Within the subgenus, *N. benavidesii, N. paniculata* and *N. solanifolia,* when crossed with *N. rustica,* show "Drosera scheme" pairing (*cf.* p. 551) with considerable multivalent formation. For F_1 *N. rustica* x *N. glauca* an approximation of "Drosera scheme" behavior has been reported (98 p. 675) in the sense that 12 pairs are "very rarely formed" (98 p. 677). Low variable pairing occurs in F_1 *N. thyrsiflora* x *N. glauca* (*cf.* p. 550). As a genically representative species of all the members of its section (*N. glauca* excepted), *N. paniculata* has been crossed with at least one member of each of the eight sections of the other two subgenera, except the Trigonophyllae and the Suaveolentes (*N. tabacum, N. glutinosa, N. undulata, N. noctiflora, N. miersii, N. langsdorffii, N. bigelovii, N. alata, N. sanderae*). We have studied all but the last three of these hybrids, and meiotic behavior in every instance is irregular and pairing is either lacking or of the low variable category. According to this evidence the reports by Kostoff (98) of pairing ranges from 2 to 7 for F_1 *N. paniculata* x *N. alata* and x *N. sanderae,* from 3 to 8 for F_1 *N. paniculata* x *N. langsdorffii,* for which he previously (83) reported zero to 7 pairs (*cf.* also 16, 31), and from 2 to 12 for F_1 *N. paniculata* x *N. tabacum* are difficult to understand. Four F_1 hybrids between species of the Paniculatae and members of subgenus Tabacum (*N. benavidesii, N. raimondii* and *N. solanifolia* with *N. tabacum* and *N. benavidesii* x *N. glutinosa*) show low variable pairing, while two hybrids involving members of subgenus Petunioides, F_1 *N. solanifolia* x *N. suaveolens* (section Suaveolentes) and F_1 *N. solanifolia* x *N. bigelovii* (section Acuminatae) show lack of pairing.

In the F_1 hybrids of *N. glauca* with *N. noctiflora* of section Noctiflorae, with *N. nudicaulis,* with *N. bigelovii* and with *N. attenuata,* three species of section Acuminatae, subgenus Petunioides, the author has observed a negligible amount of pairing. Kostoff (98) has studied the first three of these hybrids and his results are in agreement with ours. He reports similar pairing for F_1 *N. glauca* × *N. suaveolens* and × *N. megalosiphon* of section Suaveolentes. Present evidence indicates (60) a pairing range of zero to 8 pairs (most frequently 4 or 5) for F_1 *N. tabacum* × *N. glauca,* whereas Sarana (133) finds "up to 12 pairs" and Kostoff (98) reports 9 to 12 pairs. Similarly, the latter investigator (98) for F_1 hybrids between *N. glauca* and three other species of subgenus Tabacum (*N. tomentosa, N. glutinosa* and *N. tomentosiformis*) reports high pairing, and an approximation of "Drosera scheme" pairing for hybrids between *N. glauca* and five members of section Alatae, subgenus Petunioides (*N. alata, N. langsdorffii, N. sanderae, N. longiflora* and *N. plumbaginifolia*). These results are difficult to reconcile and are subject to review, especially since others find a negligible amount of pairing in F_1 *N. glauca* × *N. plumbaginifolia* (125), and the author has found little pairing in *N. glauca* × *N. tomentosa.*

Section Thyrsiflorae. This section consists of one 12-paired species, *N. thyrsiflora.* A difficult plant to grow to maturity, it has proved impossible thoroughly to analyze its karyotype or investigate its relationships with other species. Nevertheless, it is clear that like all species of the Paniculatae except *N. glauca,* all its chromosomes are median or submedian, one pair being satellited (59). The one interspecific hybrid made, F_1 *N. thyrsiflora* × *N. glauca,* shows at MI a range of zero to 5 pairs, with the mode at 1 pair (60).

Section Rusticae. Here also the section consists of a single species, *N. rustica.* The only species of the subgenus possessing 24 pairs (122), it has been shown to be an amphidiploid, progenitors of members of the Paniculatae (presumably *N. paniculata*) and of *N. undulata* (subgenus Petunioides, section Undulatae), having entered into its origin by hybridization followed by chromosome doubling (18, 50, 66, 89, 98, 100, 103, 104).

a. Karyotype. From study of root tip mitoses the karyotype of *N. rustica* has been reported to consist of chromosomes without morphological distinctions (130) and small and sharply flexed (15), uniform except for the presence of two subterminal pairs with one pair bearing proximal satellites (131), and very similar to *N. paniculata* chromosomes (89). Detailed analysis of chromosome morphology in pollen grain mitoses (59) shows 12 *m* and 12^2 *sm* with only slight range in size, the average length being somewhat less than that of the *N. paniculata* complement which contains the smallest chromosomes of all the members of the Paniculatae (50, 59).

b. Meiotic chromosome behavior in F_1 interspecific hybrids. As already noted (*cf.* p. 549), F_1 hybrids between *N. rustica* and three members of the Paniculatae (*N. paniculata, N. benavidesii* and *N. solanifolia*) exhibit "Drosera scheme" pairing, most complete in the hybrid involving *N. paniculata.* Equivalent pairing with considerable multivalency obtains in F_1 *N. rustica* × *N. undulata* of subgenus Petunioides. In hybrids of *N. rustica* with *N. alata, N. palmeri, N. sanderae, N. langsdorffii* and *N. glauca* (*cf.* pp. 549, 566), Kostoff (98) reports high variable pairing with some multivalency. By contrast, in hybrids of *N. rustica* with one or more members of the Undulatae, Acuminatae, Noctiflorae and Suaveolentes of subgenus Petunioides (*N. wigandioides, N. noctiflora, N. pauciflora, N. miersii, N. attenuata, N. bigelovii, N. nudicaulis, N. suaveolens*), and in F_1 *N. rustica* × *N. tabacum* (subgenus Tabacum) there is lack of pairing or low variable pairing in every case (*cf.* pp. 556, 562, 567, 569, 573). In other words, only four of 18 F_1 interspecific hybrids involving *N. rustica* consistently show appreciable pairing at MI, and in those four "Drosera scheme" pairing occurs. Therefore it appears that the gametic complement of 24 *N. rustica* chromosomes, which in the haploid individual shows no pairing (80), consists of two subgenoms of 12 each, one derived originally from a progenitor of *N. paniculata* or of its close modern relatives and the other from a progenitor of *N. undulata.* Confirmation of this hypothesis was obtained by the production of fertile, 24-paired *N. rustica*-like plants from unions of unreduced male gametes of F_1 *N. undulata* × *N. paniculata* with normal female gametes of *N. rustica* (50).

Comment. The Paniculatae contains seven species the intimate relationship of six of which, indicated by their morphology and distribution, is confirmed by their common karyotype pattern, their extensive compatibility and the complete or nearly complete pairing that their F_1 hybrids exhibit. The karyotype of *N. glauca* and pairing in F_1 hybrids involving this species represent an exception to the uniformity in cytological detail characteristic of the section in which it is placed and of the subgenus as a whole. Since, however, its morphological expression is essentially in accord with that of other members of the Paniculatae, it has provisionally been retained there. Because of certain morphological deviations and the distributional and cytological evidence, there may, on the other hand, be sufficient justification for placing *N. glauca* in a section by itself. In the Thrysiflorae, although confirming cytological evidence is meager, the morphological distinctions between *N. thyrsiflora* and members of the Paniculatae justify the separation of this species under a sectional designation, at least until chromosome behavior in interspecific hybrids involving it has been determined. On certain morphological and distributional grounds *N. rustica* might be considered a member of the Paniculatae, one species of which has apparently entered into its amphidiploid origin. However, it is placed in a distinct section in part because the other parent involved in such origin is a member of a different subgenus.

Subgenus Tabacum

Section Tomentosae: *N. tomentosa, N. tomentosiformis, N. otophora, N. setchellii, N. glutinosa.*

Section Genuinae: *N. tabacum.*

Stout herbs or limited perennial shrubs with showy, white, reddish or yellowish flowers, the gaping corolla throat expanded into a cup, the stamens more or less exserted.

This subgenus consists of a nucleus of three closely related species (*N. tomentosa, N. tomentosiformis, N. otophora*) from which, and from one another, the three remaining species are somewhat separated morphologically. The first three are few- to many-stemmed perennials (or limited perennials), subarborescent in height, habit and deciduous character of the lower leaves, with a distinct inflorescence axis, little or no branching of the lateral elements of the inflorescence, and the throat cup of the corolla distinct. *N. tomentosa* is highly polymorphic, *N. otophora* less so; *N. tomentosiformis* has closest affinity with *N. tomentosa* but approaches *N. otophora*. *N. setchellii* is practically identical with the preceding three species in leaf character, rather similar in habit and inflorescence, least similar in its subcampanulate flower. *N. glutinosa* exhibits the flower type of the first three species but differs from them in inflorescence, leaf and life span. The remaining species of the subgenus, *N. tabacum,* is exceedingly polymorphic. It is morphologically reminiscent of *N. tomentosa, N. tomentosiformis* and *N. otophora* but rarely is characterized by either their woodiness, longevity, subarborescence, relatively simple inflorescence pattern or highly specialized flower. In shape the corolla represents a compromise between the cup-throated and salverform types, varying at times in one direction or the other.

The distribution of the subgenus is essentially continuous and restricted, largely Andean. *N. tomentosa* is found from a little north of central Peru to western Bolivia. *N. glutinosa* shows a range from the coast of the northern half of Peru along the western flank of the outer Cordillera into southern Peru, while *N. setchellii* is apparently restricted to north Andean Peru. Although often giving the appearance of a naturally occurring species in the lower Andes from northern Peru to northern Argentina, *N. tabacum* is there probably only an escape from cultivation and, otherwise, has no known present day distribution except as a crop plant or relic of cultivation. *N. otophora* is found in the Yungas of central Bolivia and the subtropical zone of northwestern Argentina; *N. tomentosiformis* in western Andean Bolivia. Of the species of the subgenus Tabacum, *N. glutinosa* alone is characteristic of warm, arid areas, and in the Andes tends to occupy the region in which occurs *N. benavidesii,* the species of the subgenus Rustica with which it has characters in common. All other species are usually restricted to deep valleys in which they seek sheltered and well lighted situations. Their habitat is, therefore, temperate to semi-tropical and even at times marginal in the moisture-laden *ceja de la montaña.*

Section Tomentosae. a. Karyotypes. Of the five 12-paired species composing this section, the three which on morphological grounds are most closely related (*N. tomentosa, N. tomentosiformis, N. otophora*) possess the common karyotype pattern 7^1 *m* or *sm* + 5^1 *st,* while for *N. setchellii* and *N. glutinosa* it is 12^1 *m* or *sm* (50, 59). Of the first three species, *N. tomentosiformis* and *N. otophora* are practically identical in chromosome morphology. Both complements consist of seven small median chromosomes and five subterminal ones, the latter three to four times the length of the former. Such a strikingly dimorphic genom has no parallel elsewhere in the genus. The genom of *N. tomentosa* is also somewhat dimorphic with four submedian, three median and five subterminal pairs, but the entire complement is smaller and size distinctions between the subterminal and the median or submedian pairs are not striking. Like *N. tomentosiformis* and *N. otophora, N. tomentosa* has one median and one subterminal pair satellited. In *N. tomentosa* one pair of each of the three types of chromosomes shows an additional constriction (*cf.* p. 540) not apparent in the two other species. An earlier analysis (50) of the genom of *N. tomentosiformis,* made of root tip mitoses only, does not entirely agree with the above characterization of its karyotype which is based upon studies of pollen grain mitoses also (59).

As noted above, in *N. glutinosa* and *N. setchellii* a different karyotype pattern occurs—12^1 *m* or *sm* (59); in *N. glutinosa* 5 *m* and 7^1 *sm,* in *N. setchellii* 7^1 *m* and 5 *sm.* The chromosomes of these two species show no marked distinctions in length. The *N. glutinosa* genom has been reported to consist of chromosomes uniform in size and shape (16), of median and submedian ones of seven types in terms of relative size, length of arms and presence of a satellite (131), and Webber (150), in the case of haploid plants, found nine median or submedian and three subterminal chromosomes. Additional evidence supports the karyotype formula first noted above, which is the one earlier reported by the author (50). In three submedian pairs the short arm is reduced but never to one-fourth the total length of the chromosome involved.

b. Meiotic chromosome behavior in F_1 *interspecific hybrids.* Each of the first four members of the Tomentosae has been crossed with all the others, and meiotic chromosome behavior studied. In F_1 *N. tomentosiformis* x *N. otophora* it could not be determined because of early degeneration of anther tissue. In each of the other five F_1 hybrids (*cf.* appended list of hybrids, p. 579) chromosome conjugation at MI is complete or practically so. The fifth member, *N. glutinosa,* has been crossed with all other species of the Tomentosae except *N. setchellii,* and according to my evidence pairing in these three F_1 hybrids is of the low variable type with a range of zero to 9 pairs, mode at 3 or at 5, with some multivalent formation (50, 60). For two of these hybrids—F_1 *N. glutinosa* x *N. tomentosa* and x *N. tomentosiformis*—Kostoff (98) reports a pairing range of 2 to 11 (most frequently 6 to 8) and 3 to 10 (most frequently 5 to 8), respectively, and some multivalency in both.

Hybrids between all members of the two sections of the subgenus have been studied cytologically. In F_1 *N. tabacum* × *N. glutinosa* considerable variation in amount of pairing occurs (*cf.* p. 556), whereas all the other F_1 hybrids involving *N. tabacum* and members of the Tomentosae show "Drosera scheme" pairing, with considerable multivalent formation.

One or more species of section Tomentosae has been crossed with members of the following sections of the other two subgenera: Paniculatae (*N. glauca, N. benavidesii, N. paniculata*); Alatae (*N. sylvestris*); Undulatae (*N. undulata, N. wigandiodes*); Acuminatae (*N. bigelovii, N. nudicaulis*); Trigonophyllae (*N. trigonophylla, N. palmeri*); Suaveolentes (*N. suaveolens*). Most of these 25 F_1 hybrids exhibit low variable pairing with a range of zero to 8 and mode at 2 or at 3 pairs (50, 60, 98). In F_1 *N. glutinosa* × *N. wigandioides* (the Undulatae), the author has found a range of 1 to 9 with mode at 6 pairs, and Elvers (originally reporting the hybrid as *N. glutinosa* × *N. tomentosa*) found 2 to 9, most frequently 4 to 6, pairs (39). In the case of two hybrids involving section Trigonophyllae the amount of pairing is consistently greater —F_1 *N. trigonophylla* × *N. tomentosa* showing 2 to 10 or 11 pairs with the mode at 5 or 7 pairs (50, 98), and F_1 *N. trigonophylla* × *N. setchellii* 2 to 10, mode 5 (60). Considerable variability in amount of pairing in different PMC under varying environmental conditions was seen in F_1 *N. palmeri* × *N. setchellii*, from low variable (0–7) to high variable (5–11) and frequent trivalency (60). In three F_1 hybrids, *N. glauca* × *N. glutinosa*, × *N. tomentosa* and × *N. tomentosiformis*, Kostoff (98) has found high pairing (3–10, 8–11 and 8–12 respectively) with some trivalency. In the only one of these hybrids that the author also has studied almost no pairing was seen (*cf.* p. 550).

Section Genuinae. a. Karyotype. In the amphidiploid origin of the single species of this section, *N. tabacum*, two species, each with 12 pairs of chromosomes, were concerned, and thus its genom shows 24 pairs. Ten of them are median or very nearly median, five are submedian and nine subterminal (59), one pair of each of these three morphological categories bearing proximal satellites ($10^1\ m+5^1\ sm+9^1\ st$). Of the ten median pairs, three are conspicuously long and more or less sharply flexed at the centromere as contrasted with the remaining seven which are exceedingly short and generally only slightly flexed (59). The longest pair with arms of almost equal length, as well as two additional pairs of submedian chromosomes, have conspicuous constrictions (*cf.* p. 540) near the extremities of the longer arms. The subterminal chromosomes show little variation in length, the longest being approximately as long as the longer median ones. In root tips Ruttle (129*a*) notes conspicuous distinctions in chromosome size and morphology in the *N. tabacum* genom and reports two satellited pairs. Sarana (131) refers to ten chromosome types on the basis of differences in size, position of centromere and presence or absence of satellites. By other investigators the *N. tabacum* karyotype has been described as consisting of chromosomes shorter and thicker than those of *N. rustica* (118), larger and not so sharply flexed as those of *N. rustica* (15), and showing considerable individual size distinctions (130).

b. Meiotic chromosome behavior in F_1 interspecific hybrids. As already noted, F_1 hybrids made between *N. tabacum* and every other member of its subgenus except *N. glutinosa,* show "Drosera scheme" pairing, with frequent multivalency. The F_1 hybrid *N. tabacum* x *N. glutinosa* has been studied by a number of investigators who have variously reported: an approximation of "Drosera scheme" behavior (25), lack of pairing (17), low variable pairing with the mode at 2 (50, 124) or at 4 (117) pairs. More recently, Kostoff (98) refers to a highly variable number of pairs with the most frequent range between 2 and 6, while the author's analyses (60) show the extremely wide range of zero to 11 pairs with the mode at 5 and some multivalency.

An extensive series of F_1 hybrids between *N. tabacum* and six members of subgenus Rustica (*N. raimondii, N. paniculata, N. benavidesii, N. solanifolia, N. glauca, N. rustica*), and eleven members of subgenus Petunioides (*N. sylvestris, N. suaveolens, N. debneyi, N. palmeri, N. bigelovii, N. nudicaulis, N. acuminata, N. pauciflora, N. alata, N. sanderae, N. longiflora*) have been analyzed cytologically. We have studied most of these F_1 hybrids, and our evidence indicates that in every case, with the exception of F_1 *N. tabacum* x *N. sylvestris* where "Drosera scheme" behavior obtains, they fall into the two categories of least pairing (*cf.* also p. 557).

Comment. Like subgenus Rustica and in terms of morphology, distribution, karyotype pattern and extent of pairing at MI and F_1

intra- and intersubgeneric hybrids, subgenus Tabacum has been shown to have distinct identity and to consist of (*a*) a core or nucleus of closely related species, (*b*) related but somewhat outlying species, and (*c*) an amphidiploid species in the origin of which, as will be shown below, progenitors of members of the subgenus in question and of a member of another subgenus were concerned.

Comparable to the position of *N. glauca* in the Paniculatae, is that of *N. glutinosa* in the Tomentosae. Less of the cytological but more of the morphological evidence than in the case of *N. glauca* might justify the separation of *N. glutinosa* under a sectional designation.

In the case of *N. tabacum* and its numerous F_1 interspecific hybrids much cytological evidence is available, the majority of which bears upon the nature of the amphidiploid origin of this, the most important, tobacco plant of commerce (33, 70, 123). As indicated above, the *N. tabacum* genom of 24 chromosomes consists of two subgenoms of 12 chromosomes each which differ from each other in karyotype pattern. The chromosome morphology of one subgenom conforms to that of the three species forming the nucleus of section Tomentosae, that of the other subgenom to the chromosome morphology of *N. sylvestris* (subgenus Petunioides, section Alatae). Pairing at MI in F_1 hybrids involving *N. tabacum* is, according to the author's evidence, of one of two types: (*a*) "Drosera scheme", but only in the case of hybrids made either with *N. sylvestris* or with the nucleus species of section Tomentosae, or (*b*) low variable (0–8, with mode at 2, 3 or 4 pairs). Analyses of the same hybrids by others are confirmatory in the first but in certain cases contradictory in the second instance. Thus, Kostoff (98) reports high variable, and rarely "Drosera scheme", pairing in F_1 *N. tabacum* x *N. paniculata,* x *N. glauca,* x *N. sanderae,* x *N. alata* and x *N. longiflora.* For the hybrid between the two amphidiploid species *N. tabacum* and *N. rustica,* "only a few loose bivalents" (16, 144) or zero to 7 pairs with a mode at 3 pairs (50, 60) have been found, while Kostoff reports 5 to 24 (98). Reexamination of our evidence fails to indicate the cause of the discrepancies between our results and those of Kostoff.

The significance of the foregoing cytological information for interpretation of the origin of *N. tabacum* is as follows. Winge's hypothesis (155, 156) that certain species have had their origin through interspecific hybridization followed by chromosome doubling was verified in the case of *N. "digluta"* (25) which originated by such doubling in F_1 *N. tabacum* x *N. glutinosa*. The proposal was then advanced (70) that *N. tabacum* had a similar origin, progenitors of *N. sylvestris* and of *N. tomentosa* (or *N. tomentosiformis*) being initially involved (*cf.* also 77). The cytological evidence that F_1 *N. tabacum* x *N. sylvestris*, x *N. tomentosa* and x *N. tomentosiformis* (*cf.* 9, 10, 20, 69, 70, 83) show "Drosera scheme" pairing and that pairing is largely lacking in F_1 *N. sylvestris* x *N. tomentosa* and also in haploid individuals of *N. tabacum* (*cf.* 14, 27, 70, 78, 102, 111), furnishes a background for this proposal. It is now known (60, 66) that *N. setchellii* and *N. otophora* crossed with *N. tabacum* also exhibit "Drosera scheme" pairing, and for this reason and because of present day overlapping in the ranges of distribution of *N. sylvestris* and *N. otophora* (56), and also on morphological grounds, it has been suggested that a progenitor of *N. otophora* rather than of *N. tomentosa* or *N. tomentosiformis* was concerned in the origin of *N. tabacam* (56, 66). Confirming evidence of the nature of its amphidiploid origin is derived from its dual karyotype constitution and from efforts to synthesize *N. tabacum* either from trigenomatic hybrids involving *N. sylvestris* and members of the Tomentosae (5, 6, 9, 10, 85, 108) or by artificially induced chromosome doubling in appropriate F_1 hybrids (75, 76, 77, 94). The individuals so produced resemble *N. tabacum* morphologically and show 24 pairs at MI but are largely sterile (22). Especially significant are the analyses of monosomic types in *N. tabacum* when crossed with *N. sylvestris*. Clausen and Cameron obtained all of the possible 24 monosomics (23) and have succeeded in classifying each of them as monosomic either for a member of the *N. sylvestris* or of the *N. tomentosa* subgenom of *N. tabacum*. In the 35-chromosome F_1 hybrids of monosomic *N. tabacum* crossed with *N. sylvestris* the MI pairing is 12 bivalents + 11 univalents if the *N. tabacum* parent is monosomic for a member of the *N. tomentosa* subgenom, or 11 bivalents + 13 univalents if it is monosomic for a member of the *N. sylvestris* subgenom. Twelve of the monosomic *N. tabacum* types were found to fall into one and twelve into the other of the two pairing categories when crossed with *N. sylvestris*.

Subgenus Petunioides

Section Undulatae: *N. undulata, N. wigandioides, N. arentsii.*

Section Trigonophyllae: *N. trigonophylla, N. palmeri.*

Section Alatae: *N. alata, N. langsdorffii, N. bonariensis, N. longiflora, N. plumbaginifolia, N. sylvestris, N. repanda, N. stocktonii, N. nesophila.*

Section Noctiflorae: *N. noctiflora, N. petunioides, N. ameghinoi, N. acaulis.*

Section Acuminatae: *N. attenuata, N. acuminata, N. pauciflora, N. corymbosa, N. miersii, N. linearis, N. spegazzinii, N. clevelandii, N. bigelovii, N. nudicaulis.*

Section Suaveolentes: *N. suaveolens, N. maritima, N. velutina, N. fragrans, N. megalosiphon, N. gossei, N. excelsior, N. ingulba, N. goodspeedii, N. exigua, N. rotundifolia, N. occidentalis, N. stenocarpa, N. debneyi, N. benthamiana.*

Annual, rarely perennial, herbs with the corolla almost always white and salverform.

This subgenus is not only larger but considerably more heterogeneous morphologically than the preceding two subgenera. Most typical are the four sections Alatae, Noctiflorae, Acuminatae and Suaveolentes. In practically all instances their species are vespertine and are characterized by a rosette, often of considerable size and duration, as contrasted with the remainder of the genus in which the flowers remain expanded in sunlight and which develop little or no rosette. The Alatae, Noctiflorae and Acuminatae have distinct identity, and the essential members of any one of these three sections would not readily be confused with those of either of the other two. The species of the Suaveolentes, a geographically isolated group, are composites of the morphologically distinguishing characters of the three other sections to which reference has just been made.

In the Undulatae certain subgenus Rustica characters are recognizable in *N. undulata,* and certain subgenus Tabacum characters in *N. wigandioides,* while *N. arentsii* combines major morphological elements of these two species. Thus, section Undulatae represents a bridge or transition between subgenera and is therefore placed first in the sequence of sections. Section Trigonophyllae is clearly a member of subgenus Petunioides, although the flower of *N. trigonophylla* is reminiscent of that of species of the Paniculatae in subgenus Rustica. Rarely, there is occurrence of characters of the latter subgenus elsewhere in subgenus Petunioides as, for example, in *N. langsdorffii* of the Alatae. Species especially closely related morphologically in subgenus Petunioides are: *N. longiflora* and *N. plumbaginifolia; N. acuminata* and *N. pauciflora; N. trigonophylla* and *N. palmeri; N. corymbosa* and *N. linearis; N. repanda, N. stocktonii* and *N. nesophila; N. maritima* and *N. velutina; N. rotundifolia* and *N. occidentalis; N. gossei* and *N. excelsior.* Species most divergent, either florally or vegetatively, from the other species in their respective sections are: *N. langsdorffii, N. sylvestris,* the *N. repanda-N. stocktonii-N. nesophila* group, *N. bigelovii,* and *N. benthamiana.*

By contrast with the strictly South American range of natural distribution of species of the subgenera Rustica and Tabacum, that of members of subgenus Petunioides includes North America, Australia and the South Pacific as well as South America. Two sections (Noctiflorae and Undulatae) are exclusively South American, another (Trigonophyllae) exclusively North American, a fourth (Suaveolentes) is confined to Australia and certain of the South Pacific islands, while the two remaining sections (Alatae and Acuminatae), although largely South American, have representatives in North America also. Apart from the Undulatae (Andes of Peru and Bolivia), the South American species of subgenus Petunioides occur south of the Tropic of Capricorn and therefore below the distributional range of the species of the two other subgenera. Thus, the Noctiflorae occur in western central Argentina, the center of distribution of the South American species of the Alatae and the Acuminatae is the Brazilian-Uruguayan border and central Chile, respectively, with the ranges of all three sections overlapping in northern Argentina and those of the Noctiflorae and the Acuminatae in Patagonia. In American distribution *N. longiflora* and *N. plumbaginifolia* of the Alatae and *N. acuminata* of the Acuminatae range most widely. The presence of *N. longiflora* at several coastal stations in eastern and southeastern United States and of *N. acuminata* in California and Washington is presumably due to man's intervention. It is doubtful that the same agency is responsible for the distribution of *N. plumbaginifolia* from southeastern Brazil to northwestern Peru and its reappearance in southern Mexico and northward along both Mexican coasts and in the West Indies. Three other species of the Alatae are found in North America and exclusively there: *N. repanda,* in northeastern Mexico, *N. stocktonii* and *N. nesophila,* endemics of the Revillagigedo Islands, southwest of Lower California. Similarly, in the Acuminatae there is a species (*N. acuminata*) found in both Americas, while three species are exclusively North American: *N. bigelovii* chiefly in California, *N. clevelandii* in southern California and Lower California, *N. nudicaulis* in northeastern Mexico.

A considerable morphological diversity within subgenus Petunioides is paralleled by diversity in the habitats of its constituent species. With the exception of *N. undulata,* a subalpine species, the members of the Undulatae and the Alatae tend to occupy subtropical situations, some more moist and others somewhat drier. The Trigonophyllae, Noctiflorae and Acuminatae are peculiar to dry habitats (deserts, high Andean plateaus, the Patagonian steppe). Many species of the Suaveolentes are characteristic of arid or semi-arid areas, interior and coastal; a few in the Australian deserts only. Rainfall is greater at certain of the coastal and at the insular stations.

Section Undulatae. a. Karyotypes. The two 12-paired species of the Undulatae differ in karyotype pattern: *N. undulata* has $6\,m + 6^1\,sm$, *N. wigandioides* $5\,m + 3\,sm + 4^1\,st$, with no marked size difference within each genom nor between the two genoms (57, 59). Sarana's report (131) of all subterminal chromosomes in *N. undulata* was undoubtedly due to error in identification of the species (98, p. 85). The karyotype of 24-paired *N. arentsii* ($11\,m + 9^1\,sm + 4^1\,st$) reflects completely the karotypes of *N. undulata* and *N. wigandioides,* the two species which entered into its amphidiploid origin (57, 59). In this connection it is of interest that at MI of *N. arentsii* certain particular bivalent configurations characteristic of MI in the parent species can be recognized (57).

b. Meiotic chromosome behavior in F_1 interspecific hybrids. The cytology of the three F_1 hybrids between the members of the Undulatae has been determined (57). F_1 *N. undulata* × *N. wigandioides* exhibits high variable pairing (2–9, mode 5). In F_1 *N. arentsii* × *N. undulata* and × *N. wigandioides* "Drosera scheme" pairing occurs with extensive multivalency, the modal class in each hybrid including a trivalent.

By contrast, among F_1 hybrids of *N. undulata* with three members of other sections of subgenus Petunioides (*N. longiflora* and *N. langsdorffii* of the Alatae and *N. attenuata* of the Acuminatae), with five members of subgenus Rustica (*N. rustica, N. paniculata, N. raimondii, N. benavidesii* and *N. cordifolia*), and with one member of subgenus Tabacum (*N. glutinosa*), all except one exhibit almost complete lack of pairing or pairing of the low variable type. This exception is F_1 *N. rustica* × *undulata* where "Drosera scheme" pairing with a high degree of multivalency occurs (*cf.* p. 551). *N. wigandioides* crossed with *N. rustica* exhibits low variable pairing and with *N. glutinosa,* subgenus Tabacum (*cf.* p. 555), only a slightly higher degree of pairing.

Section Trigonophyllae. a. Karyotypes. The karyotype formula of 12-paired *N. trigonophylla* is $4\ m+5\ sm+3^1\ st$ (50, 59). Composed of uniformly small chromosomes, the genom of this species ranks with those of *N. linearis* and *N. spegazzinii* (the Acuminatae) in being the smallest in the genus, with an average chromosome length of 2.2 μ (50, 59). Since *N. palmeri* is morphologically so closely related to *N. trigonophylla* as doubtfully to deserve specific recognition, the karyotype of the latter is assumed to be that of the former.

b. Meiotic chromosome behavior in F_1 interspecific hybrids. Complete pairing occurs at MI in F_1 *N. trigonophylla* × *N. palmeri,* whereas in the hybrids of both of these species with *N. debneyi* of the Suaveolentes there is lack of pairing. Other intersectional hybrids studied cytologically are F_1 *N. trigonophylla* and *N. palmeri* × *N. nudicaulis,* a 24-paired species of the Acuminatae, and F_1 *N. palmeri* × *N. repanda,* a 24-paired member of the Alatae. In the former two there is an approximation of "Drosera scheme" pairing and in the last high variable pairing (4–10) and considerable multivalency. Chromosome behavior at MI in intersubgeneric hybrids involving members of subgenus Rustica (*cf.* p. 551) and subgenus Tabacum (*cf.* pp. 555, 556) has already been discussed.

Section Alatae. a. Karyotypes. Both chromosome number and chromosome morphology divide this section into a 9-paired, a 10-paired, a 12-paired and a 24-paired group of species (59). In the 9-paired group *N. sanderae* Hort., although not included among valid members of the subgenus Petunioides, must be referred to because this designation has been used in the parentage of numerous hybrids. Actually, it deserves to rank only as a horticultural variety of *N. alata,* and the karyotypes of the two are identical. Otherwise the members of the 9-paired group differ more or less distinctly in chromosome morphology, although they give evidence of having been derived from a common karyotype (3, 50, 59). Thus it is possible in *N. alata, N. bonariensis* and *N. langsdorffii* to homologize individual chromosomes on the basis of size and other morphological distinctions, but the position of the centromere in corresponding chromosomes may vary from species to species to affect the karyotype pattern as a result of chromosomal alterations in the evolution of the species (3, 50, 59).

For these three species a basic karyotype of 5*m* or *sm* + 4^2 *st* was reported (50, 61, 129): in *N. alata* 2 *m* (large) + 3 *m* or *sm* (small) + 4^2 *st,* in *N. bonariensis* and *N. langsdorffii* 1 *m* (large) + 1 *sm* (large) + 3 *m* or *sm* (small) + 4^2 *st.* Sarana (131) refers to *N. alata* as possessing six morphological types of chromosomes. Avery (2, 3) gives for *N. alata* and *N. langsdorffii* 2 *m* (large) + 3 *m* or *sm* (small) + 4^2 *st* of varying length, for *N. bonariensis* 1 *m* (large) + 1 *sm* (large) + 3 *m* or *sm* (small) + 4^2 *st* of varying length. Results of recent analyses of pollen divisions (59) differ somewhat from the above reports on the karyotypes of the three species in question and indicate for *N. alata* 4 *m* (two large) + 2 *sm* (small) + 3^2 *st;* for *N. bonariensis* 2 *m* (one large) + 3 *sm* (one large) + 4^2 *st;* and for *N. langsdorffii* 2 *m* (one large) + 4^1 *sm* (one large) + 3^1 *st.*

Avery (3) describes in detail the morphology of the chromosomes in these species. To be noted are her references to apparently non-nucleolar constrictions (*cf.* p. 540), also observed by Sarana (131), near the ends of the arms of the two large median pairs in *N. alata,* and to the satellited pairs in each species, particularly the large satellite which characterizes the genom of *N. langsdorffii.* Analyses of pollen grain mitoses (59) confirm these features of chromosome morphology. The large proximal satellite in *N. langsdorffii* is striking, being practically as large as the smallest chromosome in the complement and approximately one-fourth the length of the chromosome to which it is attached by a thread nearly as long as the satellite itself. This pair of satellited chromosomes is classified in the most recent karyotype formula as submedian, although without the satellite it would be subterminal and was so considered in the karyotype reports earlier given (3, 50).

In addition to necessitating alteration in previously reported formulae of the 9-paired species, pollen division studies (59) have revealed certain other distinctions in chromosome morphology not observed in some previous investigations made of root tip mitoses. Avery (3) refers to two satellited pairs in *N. alata* as indistinguishable from each other and both provided with "small satellites", while in pollen grain mitoses (59) it is apparent that one satellite is distinctly larger than the other (*cf.*, also, 129). The satellite on the small pair of chromosomes in *N. langsdorffii,* the smallest pair in the 9-paired species, appears most frequently, both in pollen and in root tip mitoses, at the periphery of the plate, but it has not yet been determined whether or not it is distal. *N. langsdorffii* is reported to have a third pair of satellited chromosomes (3) which is said to have "partly lost its nucleolus-producing function, since this pair shows a satellite-like body which is close to the head of the chromosome but seldom separated from it by a distinct thread". In pollen divisions this pair has a similar appearance. At least the fact that six nucleoli have been observed (3) would indicate that this pair in addition to the two satellited pairs is nucleolus-organizing.

Chromosome morphology in *N. longiflora* and closely related *N. plumbaginifolia* has been variously described. Resende (127) interprets two pairs of chromosomes in *N. longiflora* as nucleolus-producing, one having proximal and the other distal satellites. Hollingshead (79) refers to chromosomes of *N. longiflora* as straight without "hook" ("heads") at the proximal end and with none showing satellites, although the two shorter pairs are characterized by more or less distinct distal constrictions. Sarana (131) classifies the chromosomes of *N. longiflora* into five morphological types, all rod-like with small "heads", varying only in size and in the possession by one pair of "additions on the distal end which may be satellites". Avery (3) for this species describes ten pairs of "headed" chromosomes (the "head" in some instances not well marked), with continuous slight variation in length, and for *N. plumbaginifolia* a similar karyotype but of shorter and narrower chromosomes. Pollen division analyses of these two 10-paired species (59) confirm Resende's interpretation (127) of two pairs of nucleolus-organizing chromosomes in *N. longiflora,* one with distal and one with proximal satellite, but contradict Avery's comment (3), based on observation of two nucleoli in

N. plumbaginifolia and two to four in *N. longiflora,* that the former has only one satellited pair in contrast to two such pairs in the latter species. In addition, the karyotypes of the two 10-paired species, $10^2\ st$, are practically indistinguishable in size as well as in morphology. Both species have been observed to have two satellited pairs, one of the shorter pairs in each genom bearing a distinct, relatively large distal satellite and one of the longer pairs a minute proximal satellite (59). As already defined (p. 538), subterminal chromosomes (*st*) are those in which the ratio of the long arm to the short arm is 3 : 1 or greater. In some instances, particularly where the ratio is greater than 3 : 1, the short arm may appear as a spherical "head" (*cf.* also "knob chromosomes", 4). In certain chromosomes of *N. longiflora, N. plumbaginifolia* and *N. acaulis* (p. 567) the short arm is so reduced that the position of the centromere is not distinguishable.

Studies of pollen grain mitoses (59) confirm the earlier report of the karotype of *N. sylvestris* as consisting of $3\ m + 5^1\ sm + 4^1\ st$ (64). Webber (149) and Brooks (13) classify one of the submedian pairs as subterminal, and both Webber and Kostoff (98) classify one of the submedian pairs as median. Sarana (131) reports eight types of chromosomes on the basis of length, position of centromere and presence of satellites. In pollen divisions we have recently observed (59) on the satellited submedian pair a characteristic feature referred to by Webber as a "subspherical knob at the end of the short arm", and by Goodspeed and Avery (64) as a "large terminal pycnotic chromomere". In *N. sylvestris* only a slight range in chromosome size occurs. In this species the twelve possible trisomic ($2n + 1$) types have been obtained (64, 65), and the studies of chromosome morphology just referred to made possible the identification of the chromosomes present in triplicate in a number of these types.

The three 24-paired species of the Alatae possess a similar karyotype pattern of predominantly submedian chromosomes (41, 59). The karyotype of *N. repanda* is $10\ m + 8\ sm + 6^3\ st$; that of *N. stocktonii* and *N. nesophila,* $10\ m + 11^1\ sm + 3^1\ st$ (59). The genom of *N. nesophila* is somewhat smaller than the genoms of the two other species.

b. Meiotic chromosome behavior in F_1 interspecific hybrids. Within the Alatae ten F_1 hybrids representing all combinations of the 9- and 10-paired species have been studied cytologically (3, 60, 98). In all of these F_1 hybrids except the two between *N. bonariensis* and the two 10-paired species, complete or "Drosera scheme" pairing obtains. An extremely high degree of multivalency occurs in the three F_1 hybrids between the 9-paired species with from one to three trivalents, quadrivalents or quinquevalents in almost every PMC—the mode including in the case of one hybrid three trivalents; in another, one trivalent and one quadrivalent; in the third, one quinquevalent. This multivalency is interpreted (3, 50) as confirming the karyotype evidence that chromosomal alterations have occurred in the evolution of these species. F_1 *N. bonariensis* × *N. longiflora* and × *N. plumbaginifolia* show almost complete lack of pairing, *i.e.*, zero to 3 pairs (Kostoff, 0–5). In view of the complete or "Drosera scheme" pairing in other intrasectional hybrids involving these species it has been suggested (3) that this absence of significant pairing is due to environmental and other factors and is not, therefore, a reflection of equivalent lack of genic homology.

The 12-paired species, *N. sylvestris,* has been crossed within the section only with 10-paired *N. longiflora* and with 24-paired *N. repanda.* Both F_1 hybrids show lack of pairing. Complete pairing has been found to occur in the three F_1 hybrids between the 24-paired species of the Alatae, *N. repanda, N. stocktonii* and *N. nesophila* (41).

Numerous F_1 hybrids between members of the Alatae and species of other sections of subgenus Petunioides have been studied cytologically. Of 14 such hybrids involving members of the Suaveolentes (*cf.* list p. 579) all have been consistently reported to exhibit no pairing (0–4, mode 0). Similar lack of pairing is shown by F_1 *N. bigelovii* × *N. sylvestris* and F_1 *N. undulata* × *N. longiflora* and × *N. langsdorffii.* By contrast Kostoff reports (98) high variable pairing, in certain instances approaching "Drosera scheme" pairing, in four F_1 hybrids: *N. miersii* × *N. langsdorffii, N. noctiflora* × *N. alata,* × *N. sanderae,* and × *N. langsdorffii.* Already noted is Kostoff's report of similar pairing in F_1 hybrids between *N. tabacum* (subgenus Tabacum) and three of the 9- and 10-paired members of the Alatae (*cf.* p. 557), and between three species of subgenus Rustica (*N. glauca, N. paniculata* and *N. rustica*) and various of the 9- and 10-paired members of the Alatae (*cf.* pp. 549, 550, 551). Evidence contradicting Kostoff's results has been reported in the case of two hybrids, F_1 *N. paniculata* x *N. langsdorffii* (*cf.* p. 549) and F_1 *N. glauca* x *N. plumbaginifolia* (*cf.* p. 550).

Reference has also been made to the high variable pairing occurring in F_1 *N. repanda* x *N. palmeri* (*cf.* p. 562) and to the significance of meiotic chromosome behavior in F_1 hybrids between *N. sylvestris* and all members of subgenus Tabacum for interpretation of the amphidiploid origin of *N. tabacum* (*cf.* p. 557).

Section Noctiflorae. a. Karyotypes. The three 12-paired species that constitute this section (*N. noctiflora, N. acaulis, N. petunioides*) are practically equivalent in karyotype pattern (59). With the exception of one submedian pair in *N. petunioides,* only subterminal chromosomes occur. In *N. acaulis* and in *N. petunioides* satellites were seen on two pairs of subterminal chromosomes, one of them on the long arm (distal) in *N. acaulis* (59). *N. noctiflora,* however, appears to have three satellited pairs of chromosomes, two with proximal and one with distal satellites (50, 59). In *N. acaulis* the chromosomes are considerably larger than in the two other species—indeed, in size they are outstanding in the genus—and the position of the centromere is usually so nearly terminal in the majority of the chromosomes that, as in *N. longiflora* (*cf.* p. 565), the short arms do not appear even as "heads".

b. Meiotic chromosome behavior in F_1 interspecific hybrids. No information is available for hybrids involving *N. acaulis,* but F_1 *N. noctiflora* x *N. petunioides* exhibits complete pairing at MI. Of intersectional hybrids F_1 *N. corymbosa* (Acuminatae) x *N. petunioides* shows high variable pairing (3–10, mode 7), and Kostoff (98) reports "Drosera scheme" pairing or an approximation thereof for F_1 *N. noctiflora* x *N. alata,* x *N. sanderae* and x *N. langsdorffii* (Alatae). By contrast is the almost complete lack of pairing that obtains in intersubgeneric hybrids of *N. noctiflora* with *N. paniculata,* with *N. rustica* and with *N. glauca* of subgenus Rustica (*cf.* 549, 550, 551).

Section Acuminatae. a. Karyotypes. This section consists of seven 12-paired species and three 24-paired species. Chromosome size and morphology divide the 12-paired species into two groups: for *N. acuminata, N. pauciflora, N. attenuata, N. corymbosa* and *N. miersii* the karyotype is $2\ m + 6^1\ sm + 4^1\ st$; for *N. spegazzinii* and *N. linearis,* with much smaller chromosomes, it is $1\ m + 3^1$

sm + 8^1 *st* (59). In contrast to the uniformly small chromosomes in the species of the latter group, in the former, and more particularly in *N. acuminata* and *N. attenuata,* there are marked size differences within the genoms, the two median and two of the submedian chromosomes being considerably longer than the other submedian and subterminal ones. Additional constrictions (*cf.* p. 540) are present near the distal end of one of the longer submedian pairs of chromosomes in some of the species of this group. In the chromosomes of the Acuminatae the position of the centromere is often unusually conspicuous with the result that the "heads" which represent the exceedingly short arms of the subterminal chromosomes are clearly distinguishable (*cf. N. longiflora,* p. 565).

Of the three 24-paired species, *N. bigelovii* and *N. clevelandii* are similar in karyotype (8 *m* + 10^1 *sm* + 6^1 *st*) but differ somewhat in the smaller average size of the chromosomes of the latter species (59). Within these genoms, as in the case of most of the 12-paired species of this section, there are marked differences in chromosome size, two median and two submedian pairs being among the longer chromosomes of the complements. Here, too, additional apparently non-nucleolar constrictions occur near the distal ends of one of the longer submedian pairs, and the short arms of some of the subterminal pairs appear as conspicuous "heads". In *N. bigelovii,* though not as yet in *N. clevelandii,* two satellited pairs (one submedian and one subterminal) have been observed. The karyotype of the third 24-paired species of the Acuminatae, *N. nudicaulis,* (5 *m* + 12 *sm* + 7^1 *st*) consists of chromosomes even smaller than those of *N. clevelandii,* but lacks the size differences that mark the genom of that species. Only one satellited pair has been identified (59).

b. Meiotic chromosome behavior in F_1 *interspecific hybrids.* Of the 12-paired species, each (with the exception of *N. spegazzinii*) has been crossed with at least one other, *N. acuminata* and *N. corymbosa* with two others. In F_1 *N. corymbosa* × *N. acuminata* and F_1 *N. pauciflora* × *N. acuminata* complete pairing occurs; in F_1 *N. corymbosa* × *N. linearis* and F_1 *N. attenuata* × *N. miersii* the author finds high variable to complete pairing, five to 11 pairs with mode at 9, and three to 12 pairs with mode at 9, respectively. In the last named hybrid Kostoff reports (98) only one to seven pairs. The three F_1 hybrids between the 24-paired species have been studied. In F_1 *N. bigelovii* x *N. clevelandii* practically complete pairing occurs. The author's analyses of F_1 *N. bigelovii* x *N. nudicaulis* show a pairing range of 2 to 12 with mode at seven pairs (60), while others have reported no pairing (16), and zero to 3, rarely 4 (98). In F_1 *N. clevelandii* x *N. nudicaulis* the pairing ranges from zero to nine pairs (mode at 6), with one or two trivalents occurring in approximately 25% of the PMC. Four F_1 hybrids between the 24-paired species and the 12-paired species of the Acuminatae (*N. bigelovii* and *N. clevelandii* with both *N. attenuata* and *N. miersii*) all show "Drosera scheme" pairing, or a close approximation of it, with some multivalency. This multivalency is very extensive in the case of F_1 *N. bigelovii* x *N. attenuata,* 50% of the PMC analyzed showing one or more trivalents, or frequently quadrivalents, and occasionally even higher valencies (50, 60).

Already noted is the occurrence of no pairing in F_1 *N. undulata* x *N. attenuata* (*cf.* p. 562), of high variable pairing in F_1 *N. corymbosa* x *N. petunioides* (*cf.* p. 567) and in F_1 *N. miersii* x *N. langsdorffii* (*cf.* p. 566), and of "Drosera scheme" pairing in F_1 *N. nudicaulis* x *N. palmeri* and x *N. trigonophylla* (*cf.* p. 562). Other intersectional hybrids studied are ten made between members of the Acuminatae and species of the Suaveolentes. All of these F_1 hybrids (*cf.* list p. 579), several of them studied by both Goodspeed (50, 60) and Kostoff (98) and a few by other investigators also, consistently show lack of pairing.

Lack of pairing in F_1 hybrids between three 12-paired species of the Acuminatae and *N. rustica* (*cf.* p. 551), between one of them and *N. paniculata* (*cf.* p. 549), and between two of them and *N. tabacum* (*cf.* p. 556), has already been referred to. Similar lack of pairing has been noted in F_1 hybrids of *N. bigelovii* with *N. sylvestris,* with four species of subgenus Rustica (*cf.* pp. 549, 551), and with three species of subgenus Tabacum (*cf.* p. 555); and of *N. nudicaulis* with four species of subgenus Tabacum (*cf.* pp. 555, 556), and with two species of subgenus Rustica (*cf.* pp. 549, 551). A somewhat higher degree of pairing occurs in F_1 *N. bigelovii* x *N. tabacum,* with a range of zero to 10 and mode at 4 pairs.

Section Suaveolentes. a. Karyotypes. Of the 15 species of this section the chromosome numbers and morphology of 13 have been investigated. These 13 species show a chromosome number range of from 16 to 24 pairs and many similarities in their karyotypes. The various complements are composed of median, submedian and subterminal chromosomes in ratios varying from species to species. There are four 16-paired species (*N. suaveolens, N. maritima, N. velutina, N. exigua*), one 18-paired (*N. gossei*), two 19-paired (*N. excelsior, N. benthamiana*), two 20-paired (*N. megalosiphon, N. goodspeedii*), one 21-paired (*N. occidentalis*), one 22-paired (*N. rotundifolia*), and two 24-paired (*N. debneyi, N. fragrans*). A 32-paired member of the Suaveolentes, referred to by Kostoff as "*N. eastii*" (95), is not here recognized as a species, since it has not been formally described and for other reasons referred to below. Chromosome numbers for 11 of these 13 species have already been reported (48, 50, 98), and the remaining two have been determined by Wheeler (154). She also has determined as 19-paired both *N. benthamiana* and *N. excelsior* which were cited by Kostoff as 18-paired (98). Descriptions of the karyotype pattern of five species have been given (50, 152) and are confirmed by more recent observations made on pollen grain mitoses (154).

Although there is a considerable size range (*ca.* 2–5 μ in length) and the extremes may occur within a single species, close intergradation in size even in the species of greatest range occurs. The genom of every species of the Suaveolentes possesses subterminal chromosomes, as do the genoms of all species (except *N. undulata*) of the five other sections of subgenus Petunioides already reviewed. Apparently the relative number of subterminal chromosomes increases with increase of chromosome number, and correspondingly, the average chromosome length decreases.

Although distinctions in chromosome morphology are difficult to make in some instances, Wheeler's studies (154) of karyotypes in the 13 species of the Suaveolentes which she has investigated cytologically may be interpreted as follows. The four 16-paired species show $8^1\,m + 4^1\,sm + 4\,st$ (*N. suaveolens* and *N. maritima*), $8^1\,m + 4\,sm + 4^1\,st$ (*N. velutina*) or $6^1\,m + 5\,sm + 5^1\,st$ (*N. exigua*); the 18-paired species (*N. gossei*) $5\,m + 4^1\,sm + 9^1\,st$. Of the two 19-paired species *N. excelsior* shows $5\,m + 4^1\,sm + 10^1\,st$ and *N. benthamiana* $1\,m + 4\,sm + 14^1\,st$; of the two 20-paired species *N. megalosiphon* shows $2\,m + 4^1\,sm + 14^1\,st$ and *N. goodspeedii* $6^1\,m + 4^1\,sm + 10\,st$. The 21- and 22-paired species (*N. occidentalis* and *N. rotundifolia*) have much the same karyotype, $4\,m + 3$

sm + 14^1 *st* and 4^1 *m* + 3 *sm* + 15^1 *st*, respectively, while the two 24-paired species (*N. debneyi* and *N. fragrans*) consist of 4^1 *m* + 1 *sm* + 19^1 *st* and 2^1 *m* + 6 *sm* + 16^1 *st*, respectively. Thus the karyotypes of *N. suaveolens* and *N. maritima, N. benthamiana, N. megalosiphon* and *N. debneyi* are most extreme in that they are composed predominantly of chromosomes of one morphological type or the other. The occurrence of one pair of satellited subterminal chromosomes has in the above formulae been noted for every species except *N. suaveolens, N. maritima* and *N. goodspeedii.* In these three species the chromosome pair involved has been classified as submedian by Wheeler, although it appears to be on the borderline between submedian and subterminal. Apparently there is some evidence (154) that a second subterminal pair is satellited in *N. debneyi* and in *N. fragrans.* In one to several median or submedian pairs in every species except *N. benthamiana* Wheeler notes an additional constriction (*cf.* p. 540), and in *N. benthamiana* she has observed one submedian pair possessing a large chromosomal segment, simulating the large satellite in *N. langsdorffii* (*cf.* p. 563), separated by a distinct thread from the body of the chromosome.

b. Meiotic chromosome behavior in F_1 *interspecific hybrids.* The extent and character of pairing at MI has been studied in a total of 63 F_1 hybrids involving 12 of the species of this section, the karyotypes of which were described above. Thirty of these hybrids are intrasectional, 26 are intersectional and 7 involve species of the two other subgenera. Reference to the appended list of F_1 interspecific hybrids (p. 579) will permit identification of those the meiotic behavior of which will be briefly referred to below. The evidence in this connection is derived from the work of Wheeler upon 26 intrasectional hybrids (154), that of Goodspeed (50, 60) upon nine intersectional and intersubgeneric hybrids, and that of Kostoff (98) upon 13 of the same hybrids and upon 28 additional ones. In four instances (16, 44) others have provided information on meiotic chromosome behavior in F_1 hybrids investigated by Wheeler, Goodspeed or Kostoff. In general, the results of the various investigators confirm one another, except that Kostoff in certain cases reports a somewhat higher degree of pairing in intrasectional hybrids than is found by Wheeler.

Of the six hybrids which have been studied between the four 16-paired species, all show complete or almost complete pairing.

In F_1 *N. maritima* × *N. suaveolens* 16 pairs occur, and for F_1 *N. exigua* × *N. suaveolens* and F_1 *N. exigua* × *N. maritima* up to 16 pairs have been reported. Of the remaining three hybrids—*N. velutina* with *N. maritima, N. exigua* and *N. suaveolens*—the first two, according to Wheeler's evidence, exhibit 12 to 15 pairs and the last 13 to 15 pairs, while Kostoff reports for these three hybrids 13 to 16, 15 to 16 and 14 to 16 pairs, respectively. The four hybrids between 16- and 18-paired species show "Drosera scheme" pairing or an approximation thereof. The same is true of three of the six F_1 hybrids between 16- and 20-paired species, of one of the two between 18- and 19-paired species, and of a single hybrid in each case between 16- and 22-, 16- and 24-, 20- and 22-, and 20- and 24-paired species. Among these Wheeler notes the highest degree of pairing in the following three F_1 hybrids: *N. suaveolens* × *N. maritima, N. goodspeedii* × *N. suaveolens* and *N. maritima* × *N. debneyi.* An amount of pairing somewhat less complete than in the foregoing hybrids but still in the high variable category obtains for the 11 remaining F_1 intrasectional hybrids investigated. Of these, four involving *N. benthamiana* with *N. suaveolens, N. gossei, N. megalosiphon* and *N. debneyi* show the lowest amount of pairing among all the intrasectional hybrids of the Suaveolentes, with a combined range of 6 to 17 and a mode at 10 or 11 pairs. Five other hybrids in this category involve *N. megalosiphon* with *N. suaveolens, N. exigua, N. velutina, N. gossei* and *N. debneyi,* and the remaining two hybrids showing high variable pairing are F_1 *N. occidentalis* × *N. gossei* and that between the two 20-paired species, F_1 *N. goodspeedii* × *N. megalosiphon,* where the pairing ranges from 12 to 16 pairs. Wheeler finds a high degree of multivalent formation in most of the 26 intrasectional hybrids she has studied. Indeed, of 30 such hybrids, in all but four one or more trivalents were present in a high percentage of the PMC. In one hybrid between two 16-paired species up to five trivalents occurred. A quadrivalent has occasionally been seen in certain of the hybrids.

By contrast with the high degree of homology exhibited by the genoms of the parents of all the F_1 hybrids made between the species of section Suaveolentes, is the almost complete lack of pairing consistently shown in intersectional and intersubgeneric F_1 hybrids involving species of that section. As found principally by

Kostoff (98), but also by Goodspeed (50, 60), a pairing range of zero to 2 or 3 is characteristic of 22 such hybrids, a range of zero to 4 or 5 of ten, and in one case a range of zero to 6. These results refer to nine hybrids between species of section Alatae (*N. alata, N. sanderae, N. langsdorffii, N. longiflora, N. plumbaginifolia,* and *N. repanda*) and 16-paired species, three of the same species (*N. alata, N. sanderae,* and *N. longiflora*) and an 18-paired species, and two (*N. longiflora* and *N. plumbaginifolia*) and a 20-paired species. Similarly, ten F_1 hybrids involve 16-, 19-, 20- and 24-paired species and *N. attenuata, N. miersii, N. nudicaulis, N. bigelovii* and *N. clevelandii,* members of the Acuminatae. Hybrids between a 24-paired species and *N. trigonophylla* and *N. palmeri* of section Trigonophyllae have also been studied. Intersubgeneric hybrids, all showing lack of pairing, include four involving a member of subgenus Rustica (F_1 *N. suaveolens* x *N. solanifolia,* x *N. glauca* and x *N. rustica,* and F_1 *N. glauca* x *N. megalosiphon*) and three in which a species of subgenus Tabacum is concerned (F_1 *N. suaveolens* x *N. tabacum* and x *N. glutinosa,* and F_1 *N. debneyi* x *N. tabacum*).

An additional series of F_1 hybrids of which a 32-paired member of the Suaveolentes is a parent has been reported principally by Kostoff. He has applied the designation "*N. eastii*" to this 32-paired race, since he considers that he has demonstrated its origin as a probable amphidiploid of F_1 *N. suaveolens* x *N. maritima.* Wheeler (153), on the other hand, refers to a 32-paired race which is probably equivalent, as a tetraploid of *N. suaveolens.* Hybrids between "*N. eastii*" and *N. alata, N. sanderae, N. longiflora, N. plumbaginifolia* (98) and *N. bigelovii* (60) show 16 pairs. Since in F_1 *N. suaveolens* x *N. maritima* complete pairing occurs, such consistent evidence of autosyndesis within the gamete set of "*N. eastii*" might be considered indicative of its origin through either allo- or autopolyploidy. In F_1 hybrids of "*N. eastii*" with the 16-paired species *N. suaveolens* and *N. maritima* Kostoff finds 14 to 16 bivalents and zero to three trivalents, and for F_1 "*N. eastii*" x *N. suaveolens* Wheeler reports up to 15 trivalents. Kostoff refers to pairing in F_1 "*N. eastii*" x *N. velutina,* x *N. gossei* and x *N. megalosiphon* as variable with some trivalency. For convenience of reference F_1 hybrids involving this 32-paired race of section Suaveolentes are included in the subjoined list of interspecific hybrids under "*N. eastii*".

Comment. For subgenus Petunioides cytological information in general confirms and amplifies the morphological and distributional evidence which contributed largely to the formulation of the sectional arrangement. Thus, almost identical karyotypes of uniformly subterminal chromosomes characterize the three species which have been studied in the 12-paired section Noctiflorae, and complete pairing occurs in an F_1 hybrid between them. Similarly, chromosome conjugation is complete in the F_1 hybrid between the two species of the Trigonophyllae. The close relationship of the seven 12-paired species of the Acuminatae is clearly shown by their possession of a similar karyotype pattern and by the extensive pairing in F_1 hybrids involving them, and equivalent cytological evidence relates *N. bigelovii* and *N. clevelandii,* two of the three 24-paired species of that section. Finally, and in particular, similarities in karyotype patterns and extensive pairing in F_1 intrasectional hybrids confirm the evidence from external morphology which indicates the close relationship of the species of the Suaveolentes. The species of the remaining two sections (Undulatae and Alatae) show in some instances an equivalent and in others a less close integration on the basis of the cytological as well as the morphological and distributional information available.

In the Undulatae the 12-paired species, *N. undulata* and *N. wigandioides,* exhibit a certain morphological resemblance to members of subgenus Rustica and subgenus Tabacum, respectively. Occurrence of "Drosera scheme" pairing in F_1 *N. rustica* × *N. undulata* and similarity of the karyotypes of *N. undulata* and members of subgenus Rustica, together with the extent of pairing in F_1 *N. wigandioides* × *N. glutinosa* (Tomentosae) contribute to the impression that the Undulatae may represent a transitional section somewhat uniting subgenus Petunioides with the two other subgenera. The remaining member of the Undulatae is 24-paired *N. arentsii.* To the parentage of this amphidiploid species progenitors of the two other species of the Undulatae entered in as shown, first, by "Drosera scheme" relations on the two sides of the triangle uniting as hybrids *N. arentsii* with *N. undulata* and *N. wigandioides* and, second, by the almost complete reflection of the karyotypes of the latter two species in that of the former.

Morphologically, section Alatae contains a nucleus of five 9- or 10-paired species, the relationships of certain of them being obviously close. In terms of karyotype patterns there are essential identities and also dissimilarities among these five species, whereas the high degree of pairing and multivalency in F_1 hybrids between them indicates their fundamental relationships as well as the occurrence of quantitative chromosomal alterations during their evolution. In addition to these nucleus species there are in the Alatae the three 24-paired species, *N. repanda, N. stocktonii* and *N. nesophila.* Similar karyotypes and complete pairing in F_1 hybrids between them indicate, as does the morphological and distributional evidence, the closeness of their relationship. In floral and in chromosomal morphology they are somewhat allied to the 9- and 10-paired species. In view of this fact and because of the approximation of "Drosera scheme" pairing in F_1 *N. repanda* × *N. palmeri* it is suggested that the 24-paired species of the Alatae represent amphidiploids, progenitors of a 12-paired ancestor of the 9- and 10-paired members of the Alatae and of members of the Trigonophyllae having entered into their origin. The remaining species, 12-paired *N. sylvestris,* although somewhat outlying in many respects, is, however, included in the Alatae because of morphological resemblances to other members of this section. Its karyotype is not unrelated to the basic karyotype of the 9-paired species. Only in the case of F_1 *N. tabacum* × *N. sylvestris,* where "Drosera scheme" relations obtain, is there significant pairing in the relatively few F_1 hybrids involving *N. sylvestris* which it has been possible to obtain.

Cytological evidence indicates in some instances considerable and in others little relationship between sections of subgenus Petunioides. Thus, high variable pairing in an F_1 hybrid between *N. petunioides* (Noctiflorae) and *N. corymbosa* (Acuminatae) points to genic similarities between members of different sections which also find expression in the possession by the two species of certain morphological characters in common. The occurrence of amphidiploid species and the presence in different sections of the modern descendants of their putative ancestors also serve to relate certain subdivisions of this subgenus. The evidence in this connection concerning the amphidiploid origin of these 24-paired species of the Alatae has been noted above. In the amphidiploid origin of the two closely related 24-paired species of the Acuminatae (*N. bigelovii* and *N. clevelandii*) a progenitor of the 12-paired members of the same section is involved, as evidenced by "Drosera scheme" pairing exhibited by their F_1 hybrids with such 12-paired species. Equally significant cytological information pointing to present day species, an ancestor of which represented the other parent of these two amphidiploid species of the Acuminatae, is not available. Possibly modern descendants of such a parental species are no longer in existence. It is to be noted, however, that we find as many as ten bivalents in F_1 *N. bigelovii* x *N. tabacum,* and since *N. sylvestris,* a member of the Alatae, is represented in the amphidiploid origin of *N. tabacum* it may be that a 12-paired progenitor of the Alatae entered into the origin of *N. bigelovii* and *N. clevelandii.* The third 24-paired member of the Acuminatae, *N. nudicaulis,* apparently involved in its amphidiploid origin a progenitor of *N. trigonophylla* (Trigonophyllae), since the karyotypes of the two species are similar and their F_1 hybrid shows "Drosera scheme" pairing. Our observation of up to 12 bivalents in F_1 *N. nudicaulis* x *N. bigelovii* and up to nine in F_1 *N. nudicaulis* x *N. clevelandii,* together with morphological characters which *N. nudicaulis* has in common with other species of the Acuminatae, suggests that a progenitor of some member of the Acuminatae represents the other parental species concerned in the amphidiploid origin of *N. nudicaulis.*

By contrast with such direct and indirect indications of relationship between sections of subgenus Petunioides is the cytological indication of a genic isolation (lack of pairing in intersectional and intersubgeneric F_1 hybrids) of the Suaveolentes equivalent to its geographic isolation. However, there is, according to Wheeler (154), some evidence that the Suaveolentes may have been derived from the source from which the 9- and 10-paired species of the Alatae, the 12-paired Noctiflorae and the 12-paired Acuminatae have differentiated.

The aneuploid series—16, 18, 19, 20, 21, 22, 24—into which fall the chromosome numbers of the 13 species investigated cytologically points to a fundamental hybrid structure for the Suaveolentes, and the increase in number of subterminal chromosomes with increasing chromosome number might be reconciled with this concept. If the hybrids involving "*N. eastii*" are included, meiotic chromosome behavior is known in a total of 73 F_1 interspecific hybrids of which members of the Suaveolentes are parents. As already noted all these hybrids except those in which *"N. eastii"* is concerned exhibit four of the five types of pairing defined in the résumé of the cytology of the genus (*cf.* p. 542). Thus in the majority of the intrasectional hybrids either complete or "Drosera scheme" pairing or approximations thereof occur, with the remaining hybrids showing high variable pairing, while all intersectional and intersubgeneric hybrids show lack of pairing. If *"N. eastii"* is to be considered a species, a sixth category of meiotic behavior at MI in interspecific hybrids of *Nicotiana* must be added, since in five intersectional and intersubgeneric hybrids involving *"N. eastii"* autosyndesis occurs.

SUMMARY

Current morphological and distributional information indicates that the genus *Nicotiana* consists of somewhat less than 60 valid species, the natural distribution of which is confined to temperate South America, western North America, Australia and a few South Pacific islands. From the beginning, efforts to organize the genus taxonomically resulted in the recognition of at least three major subdivisions, relationships within each of which were more or less clearly expressed morphologically. Less than half the total number of species now known were considered in these older taxonomic organizations. Addition of numerous new species, increased knowledge of the morphology, range of distribution and variation of the classical species, and, in particular, information concerning chromosome number and morphology and chromosome behavior in F_1 interspecific hybrids have resulted in revaluations of previous taxonomic concepts.

Efforts to correlate accumulating morphological, distributional and cytological evidence revealed the presence of a series of genetic centers or groupings of species within the genus. The members of these "genetic groups" were considered to possess distinctive morphological, distributional and cytological characters sufficiently in common, on the one hand, to demonstrate that phylogenetic relationships within the individual groups were relatively intimate, and, on the other, to set apart the groups themselves one from another. Somewhat later most of these "genetic groups" were referred to as sections in the taxonomic sense. In a recent taxonomic revision of the genus three of the major subdivisions of the classical taxonomic organizations were retained as subgenera (Rustica, Tabacum, Petunioides) under which a total of 11 sections were described.

Available cytological information concerning the genus consists of determination by a number of investigators of chromosome morphology in 55 of the 58 recognized species of *Nicotiana* and of the amount and quality of pairing in some 210 F_1 interspecific hybrids involving as parents 53 species. This information can be organized as follows to reflect the extent to which the most recent taxonomic arrangement is expressive of what appear to be fundamental phylogenetic relationships.

In terms of chromosome morphology it has been shown that there is no common karyotype pattern for *Nicotiana,* but in the genus as a whole median and submedian predominate over subterminal chromosomes in the ratio of 5:3. Certain other cytological features are characteristic: (*a*) the possession by considerably over one-half the species of genoms containing 12 or 24 pairs of chromosomes; (*b*) the relatively small to medium size of the chromosomes; (*c*) the presence in every genom of at least one pair of satellited chromosomes, usually a subterminal pair with proximal satellites. In addition, distinctions in chromosome morphology characterize individual species, sections and even subgenera, as follows: (*a*) a characteristic ratio of median and submedian to subterminal chromosomes serves to distinguish the three subgenera from one another—Rustica 9:1, Tabacum 5:2, Petunioides 4:3; (*b*) in subgenus Rustica, which contains three sections, the karyotype formulae are practically identical for all but one of the nine species; in the two other subgenera there is no comparable uniformity in karyotype, although, as just noted, their basic karyotype patterns set them apart from subgenus Rustica and from each other; (*c*) in section Tomentosae of subgenus Tabacum, three of the five species closely related morphologically possess practically identical karyotypes, the remaining section consisting of a single amphidiploid species; (*d*) each of four of the six sections of subgenus Petunioides possesses a particular karyotype characteristic in each case of the nucleus species; thus these four sections are set apart from one another in terms of chromosome morphology; (*e*) the karyotypes of the nine amphidiploid species reflect in more or less detail the karyotypes of the modern relatives of their postulated ancestors.

From the point of view that chromosome homology reflected in the formation of bivalents (or higher valencies) at MI is indicative of the extent to which genes and their arrangement in the conjugating chromosomes are common or similar, the amount of pairing in F_1 interspecific hybrids has been employed to provide evidence concerning the relationships of the species in each case involved: (*a*) approximately 90% of the F_1 intrasectional hybrids indicate close relationship of the species concerned by exhibiting complete or almost complete pairing of their genoms;* (*b*) by contrast, in only 10% of the hybrids between species of different sections of a given subgenus does appreciable pairing occur; (*c*) corresponding to the more distant relationship of the species concerned which is postulated in the taxonomic arrangement, none of the F_1 hybrids which involve species of different subgenera shows significant pairing, except certain of those in which *N. glauca* or a species of the Alatae is a parent; (*d*) in all intra- and intersubgeneric F_1 hybrids between any of the nine amphidiploid species and the descendants of their putative progenitors, "Drosera scheme" pairing or an approximation thereof occurs; (*e*) on the other hand, when these same amphidiploid species are crossed with species other than those related to their parentage, 85% of the F_1 hybrids exhibit little or no pairing.

Thus, the evidence of phylogenetic relationships in *Nicotiana* which is intrinsic in the morphological and distributional data appears to be sufficiently confirmed and amplified by the cytological conclusions to justify the current taxonomic arrangement of the genus.

LIST OF F_1 INTERSPECIFIC HYBRIDS OF NICOTIANA INVESTIGATED CYTOLOGICALLY

Hybrids are listed alphabetically; reciprocals not listed. Page references are to the present article; others are to the bibliography. Except in a few instances the results of Kostoff and of Goodspeed are referred to only in articles containing résumés of cytological behavior.

N. alata × *N. bonariensis*—p. 566 (3, 60, 97, 98)
N. alata × *N. langsdorffii*—p. 566 (3, 16, 60, 83, 98)

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* Hybrids involving amphidiploid species are obviously not included in points (*a*), (*b*) and (*c*).

N. arentsii x *N. wigandioides*—p. 561 (57, 60)
N. attenuata x *N. glauca*—p. 549 (60)
N. attenuata x *N. miersii*—p. 568 (60, 98)
N. benavidesii x *N. glutinosa*—pp. 549, 555 (60)
N. benavidesii x *N. raimondii*—p. 548 (60)
N. benavidesii x *N. solanifolia*—p. 548 (60)
N. benthamiana x *N. debneyi*—p. 572 (154)
N. benthamiana x *N. megalosiphon*—p. 572 (154)
N. benthamiana x *N. suaveolens*—p. 572 (154)
N. bigelovii x *N. attenuata*—p. 569 (50, 60, 97, 98)
N. bigelovii x *N. benthamiana*—pp. 569, 573 (98)
N. bigelovii x *N. clevelandii*—p. 569 (60)
N. bigelovii x *N. glauca*—pp. 549, 569 (50, 60, 97, 98)
N. bigelovii x *N. glutinosa*—pp. 555, 569 (17, 60, 68, 97, 98)
N. bigelovii x *N. megalosiphon*—pp. 569, 573 (97, 98)
N. bigelovii x *N. nudicaulis*—pp. 569 (16, 60, 97, 98)
N. bigelovii x *N. paniculata*—pp. 549, 569 (97, 98)
N. bigelovii x *N. solanifolia*—pp. 549, 569 (50, 60)
N. bigelovii x *N. suaveolens*—pp. 569, 573 (50, 60, 68, 92, 97, 98)
N. bigelovii x *N. sylvestris*—pp. 566, 569 (50, 60, 98)
N. bigelovii x *N. tabacum*—pp. 556, 569 (17, 60, 97, 98)
N. bigelovii x *N. tomentosa*—pp. 555, 569 (97, 98)
N. bigelovii x *N. tomentosiformis*—pp. 555, 569 (97, 98)
N. bonariensis x *N. plumbaginifolia*—pp. 566 (98)
N. clevelandii x *N. attenuata*—p. 569 (60, 98)
N. clevelandii x *N. exigua*—pp. 569, 573 (98)
N. clevelandii x *N. nudicaulis*—p. 569 (60)
N. corymbosa x *N. acuminata*—p. 568 (60)
N. corymbosa x *N. linearis*—p. 568 (60)
N. corymbosa x *N. petunioides*—pp. 567, 569 (60)
N. debneyi x *N. nudicaulis*—pp. 569, 573 (50, 60, 97, 98)
N. debneyi x *N. palmeri*—pp. 562, 573 (98)
N. debneyi x *N. suaveolens*—p. 572 (98)
N. debneyi x *N. tabacum*—pp. 556, 573 (98)
N. debneyi x *N. trigonophylla*—pp. 562, 573 (60, 97, 98)
"*N. eastii*" x *N. alata*—p. 573 (97, 98)
"*N. eastii*" x *N. bigelovii*—p. 573 (60)
"*N. eastii*" x *N. longiflora*—p. 573 (97, 98)
"*N. eastii*" x *N. gossei*—p. 573 (98)

N. knightiana x *N. cordifolia*—p. 548 (60)
N. langsdorffii x *N. bonariensis*—p. 566 (3, 60, 97, 98, 117)
N. langsdorffii x *N. sanderae*—p. 566 (83, 97, 98)
N. longiflora x *N. alata*—p. 566 (3, 60, 98)
N. longiflora x *N. bonariensis*—p. 566 (3, 60, 98)
N. longiflora x *N. langsdorffii*—p. 566 (3, 60, 97, 98)
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N. noctiflora x *N. petunioides*—p. 567 (60)
N. noctiflora x *N. sanderae*—pp. 566, 567 (97, 98)
N. nudicaulis x *N. glauca*—pp. 549, 569 (50, 60, 97, 98)
N. nudicaulis x *N. glutinosa*—pp. 555, 569 (17, 97, 98)
N. nudicaulis x *N. palmeri*—pp. 562, 569 (97, 98)
N. nudicaulis x *N. rustica*—pp. 551, 569 (97, 98)
N. nudicaulis x *N. suaveolens*—pp. 569, 573 (44, 97, 98)
N. nudicaulis x *N. tabacum*—pp. 556, 569 (44, 97, 98)
N. nudicaulis x *N. tomentosa*—pp. 555, 569 (97, 98)

N. tabacum × *N. sylvestris*—pp. 556, 557, 567 (20, 50, 70, 83, 97, 98, 132, 157)
N. tabacum × *N. tomentosa*—pp. 555, 556, 557 (20, 50, 70, 83, 97, 98)
N. tabacum × *N. tomentosiformis*—pp. 555, 556, 557 (5, 6, 10, 20, 50, 83, 97, 98)
N. thyrsiflora × *N. glauca*—pp. 549, 550 (60)
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N. velutina × *N. megalosiphon*—p. 572 (154)
N. velutina × *N. rotundifolia*—p. 572 (154)
N. velutina × *N. suaveolens*—p. 572 (97, 98, 154)

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