Chapter 2
Classification of the Genus *Nicotiana* and Origin of *Nicotiana* Species

2.1 Classification of the Genus *Nicotiana*

The name *Nicotiana* was invented by Francis the duke of Guise, a French aristocrat and general, to honor his countryman Jean Nicot, the French ambassador to Portugal. In 1559, Nicot sent dried leaves of tobacco to the king Francis II and his mother Catherine de Medici as a presumed miracle cure against persistent headaches. In 1565, the German botanist and physician Adam Lonitzer gave the name *Nicotiana* to the whole genus of tobacco, the name recognized later by Linnaeus (Julio, 2005). In the meantime, the French botanist Jacques Dalechamps listed tobacco under the name *Herba nicotiana*. Another Frenchman, Paul Reneaulme, a physician of Blois, in his *Specimen Historiae Plantarum* issued in 1611 was the first to call tobacco by its present name of *Nicotiana tabacum*. In the eighteenth century, Linnaeus described, along with *N. tabacum*, three more species of *Nicotiana*: *N. rustica*, *N. paniculata* and *N. glutinosa* (Knapp et al., 2004). Some nineteenth century classifications included up to 41 species (East, 1928). The first attempt to systematize the growing number of *Nicotiana* species was made by George Don in 1838 (Knapp et al., 2004). Don divided the *Nicotiana* species known to him into four sections: Tabacum, Rustica, Petunioides and Polydiclia. In 1912, William Setchell modified Don's division by dropping the section Polydiclia (Knapp et al., 2004). Edward M. East (East, 1928) adopted Setchell's classification scheme but gave his recognition to only 27 species, "*upon which some genetic work has been accomplished*", as he explained his criterion. The foundations for the present-day classification of *Nicotiana* were laid down by Doncho Kostoff (Kostoff, 1943) and Thomas H. Goodspeed (1954). Kostoff divided the genus into 8 sections and 47 species. Goodspeed's taxonomical division of the genus, while not differing much in its essentials from Kostoff's, was nevertheless much larger. It included 60 species divided into three subgenera and 14 sections, and that classification continued to provide the basic framework for subsequent additions and revisions. Based on

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previous molecular studies (Aoki & Ito, 2000; Chase et al., 2003; Clarkson et al., 2004), Knapp and her coworkers (Knapp et al., 2004) proposed some significant modifications to Goodspeed's classification. In the revised systematics, the division into three subgenera was dropped, as it was not supported by DNA sequencing evidence (Aoki & Ito, 2000). Two of Goodspeed's 14 sections (Thyrsiflorae and Nudicaules) were removed, a new section was added (Sylvestres), and three sections were renamed (Genuinae to Nicotiana, Bigelovianae to Polydicliae, Acuminatae to Petunioides). Several species (*N. glauca*, *N. thyrsiflora*, *N. glutinosa*, *N. sylvestris*, and *N. nudicaulis*) were shifted from one section to another. The current inventory of *Nicotiana* species is considerably enlarged compared to the original Goodspeed's list: earlier additions included those by Burbidge (1960), Smith and Downs (1964), Merxmueller and Buttler (1975), D'Arcy (1976), Symon (1984), Clarkson and Symon (1991); more recent additions were contributed by Symon (1998, 2005), Stehmann et al. (2002), and Scarpa and Rosso (2011). In conformance with the rules laid down in the International Code of Botanical Nomenclature (Greuter cited after Knapp et al., 2004), the number of recognized *Nicotiana* species can be further enlarged by the inclusion of *N. monoschizocarpa*, a new taxon separated from *N. debneyi* (Horton, 1981; Symon & Lepschi, 2007). Another addition was proposed by Marks (2010), who produced evidence for separating *N. fatuhivensis* from the already recognized *N. fragrans,* although she had not supplied a regular description of the new species. Further additions included *N.* 'rastroensis' and *N.* 'Corunna'. *N.* 'rastroensis' lacks a valid description but was the object of several studies (Descorbeth, 2004; Descorbeth & McClure, 2005; Lee et al., 2008; Jimenez-Duran et al., 2013). 'N. Corunna' was first discovered by E. Symon and was renamed 'N. symonii' by Dodsworth (2015) and finally described as a new species under the name of *N. paulineana* by Bally et al. (2021). *N. leguiana* was separated from N. *tomentosa* by Knapp (2020).

Starting in 2018, there has been a virtual rash of new *Nicotianae*, which were discovered in the Australian outback by essentially the same team of scientists and classified in the section Suaveolentes. Their long succession was opened by *N. yandinga*, *N. faucicola*, *N. karijini*, *N. gascoynica*, *N. notha, N. truncata, N. hoskingii, N. walpa, and N. pila* (Chase & Christenhusz, 2018a, b, 2021a, b; Chase et al., 2018a, b, 2021a, b, c, d, e, f). In several cases, the newly announced species were separated from previously known taxa, *N. insecticida, N. murchisonica, and N. salina,* and were included by their discoverers in what the authors described as the *N. occidentalis* group (Chase & Christenhush, 2021a); *N. hoskingii* was separated from *N. debneyi* (now *N. forsteri*) (Chase et al., 2021f); and *N. gascoynica* and *N. walpa* were recognized as distinct from *N. simulans* Chase et al. (2021g). *N. pila* was found to be distinct from both *N. rosulata* and *N. ingulba,* although in some aspects, it resembled one or the other (Chase & Christenhush, 2021b). Likewise, *N. karijini* bore resemblance to both *N. umbratica* and *N. benthamiana* (Chase & Christenhusz, 2018a). *N. notha* is considered a product of hybridization between *N. suaveolens* and *N. velutina* (Chase et al., 2021c). The most recent additions include four species that have been separated from *N. benthamiana*: *N. candelabra*, *N. bilybara*, *N. rupestris*, and *N. scopulorum* (Chase et al., 2021h; Chase et al., 2022a). Chase et al. (2021g) were themselves surprised at the wealth of *Nicotiana* species they had been able to discover and wondered why such a great level of diversity had passed unnoticed by their predecessors in their studies of Australian *Nicotianae*. The authors named several reasons for that underestimation. Among them were the very size of the continent, few side-by-side comparative studies of sufficiently large numbers of collected accessions and peculiar germination requirements of some species that persist in the soil as seed banks most of the time and only occasionally germinate, come to flower, and can be collected for study. Chase et al. (2021g) estimated that currently recognized Suaveolentes species may account for approximately 60% of their actual total number. It remains to be seen how many of these newly recognized species will stand the test of time and to what extent the predictions regarding the extraordinary specific diversity of the Suaveolentes will materialize.

Another recent addition to the genus is *N. gandarela*, found in a single location in Brazil. Like the other species native to that country, it was classified in the section Alatae. According to the researchers who described *N. gandarela*, the species is under threat of extinction from extensive iron mining in the area (Augsten et al., 2022).

Essentially, the list in Table 2.1 is a compilation of those published recently by Knapp (2020) and Berbeć and Doroszewska (2020). The five species not listed by Knapp but included by Berbeć and Doroszewska are *N.* sp. ‘Rastroensis’, *N. sanderae*, *N. eastii*, *N.* sp. ‘Corunna’ (now *N. paulineana*) and *N. palmeri*. Table 2.1 also lists eleven Suaveolentes species most recently reported by Prof. Chase and his collaborators (Anon, 2021) and discussed in one of the previous paragraphs and the previously mentioned *N. gandarela*. The largest part of the *Nicotiana* species, approaching half of their total number, is classified within the section Suaveolentes. The classification presented in Table 2.1 is fairly liberal for it includes both those taxa which are known from collections and natural sites as well as those of which only herbarium specimens exist. The status of the latter is also liable to change, e.g., *N. ameghinoi* was rediscovered in the wild (Knapp 2013). Knapp’s classification comprised eighty-two species, and the list in Table 2.1 of this volume elevates their number to 99. Not included in Table 2.1 are the most recent discoveries in the family Suaveolentes made by Prof. Chase and his associates (Chase et al., 2023). The newest nine species include: *N. bungonia* Chase & Tereski, *N. clarksonii* Chase & Christenhush ($2n = 36$), *N. erytheia* Chase & Christenhush, *N. gibosa* Chase, Andrew & Brull, *N. karakara* Chase & Christenhush, *N. latifolia* Chase & Christenhush ($2n = 36$), *N. latzii* Chase, Jobson & Christenhush, *N. olens* Chase & Christenhush ($2n = 30$), and *N. praecipitis* Chase & Durban.

The basic framework of the revised classifications, their differences in recognized taxa notwithstanding, has been accepted as standard by most researchers writing on *Nicotiana* (Lewis & Nicholson, 2007; Doroszewska et al., 2009; Lewis, 2011; Knapp, 2020), although some continued to use Goodspeed’s original systematics (Khan & Narayan, 2007).

Table 2.1 Species of the genus *Nicotiana* classified by sections and in alphabetical order within a section

Species name	Described by	Origin	Year of first description	Diploid chromosome number	Genome size (pg) 2C DNA content after Hussain et al., 2023 (H2C); 1C and 4C DNA contents after Leitch et al., 2008 (L1C and 4C, respectively)
Alatae					
N. alata Link et Otto	Goodspeed (1954)	SAm	1828	18	4.53, 5.49 (H2C)
N. azambujae Sm. et Down	Smith and Downs (1964)[17]	SAm	1964	?	
N. bonariensis Lehm.	Goodspeed (1954)	SAm	1818	18	4.45 (H2C)
N. forgetiana Hemsl	Goodspeed (1954)	SAm	1905	18	
N. gandarela Augsten & Stehmann[1]	Augsten et al. (2022)	SAm	2022	?	
N. langsdorffii Weinm.	Goodspeed (1954)	SAm	1819	18	6.82 (H2C)
N. longiflora Cav	Goodspeed (1954)	SAm	1802	20	5.74 (H2C)
N. mutabilis Stehmann et Samir	Stehmann et al. (2002)[18]	SAm	2000	18[20]	
N. plumbaginifolia Viv. *	Goodspeed (1954)	SAm	1802	20	5.46 (H2C)
N. sp. ‘Rastroensis’[2]	Murfett et al. (2005)	SAm	2005	?	
(*N.* x *sanderae*) Watson	Christoff (1928), Daly (1959)[19]	SAm	1904	18	
Nicotiana					
N. tabacum L.	Goodspeed (1954)	SAm	1753	48	5.2, 20.70 (L1C, 4C), 9.77 (H2C)
Noctiflorae					
N. acaulis Speg.	Goodspeed (1954)	SAm	1902	24	6.20 (H2C)
N. ameghinoi Speg.	Goodspeed (1954)	SAm	1902	12[20]	
N. glauca Graham	Goodspeed (1954)	SAm	1828	24	6.85 (H2C)

N. noctiflora Hock. *	Goodspeed (1954)	SAm	1827	24	9.53 (H2C)
N. paa MartCrov.	Martinez-Croveto (1978), Scarpa and Rosso (2011)	SAm	1978	?	
N. petunioides (Griseb.) Millán*	Goodspeed (1954)	SAm	1928	24	5.30 (H2C)
Paniculatae					
N. benavidesii Goodsp.	Goodspeed (1954)	SAm	1938	24	6.11 (H2C)
N. cordifolia Phil.	Goodspeed (1954)	SAm	1856	24	
N. cutleri D'Arcy	D'Arcy (1976)	Sam	1976	?	
N. knightiana Goodsp.	Goodspeed (1954)	SAm	1938	24	3.2, 12.64 (L1C, 4C) 6.57 (H2C)
N. paniculata Goodsp.	Goodspeed (1954)	SAm	1753	24	3.0, 11.78 (L1C, 4C) 6.40 (H2C)
N. raimondii Macbr.	Goodspeed (1954)	SAm	1930	24	
N. solanifolia Goodsp.	Goodspeed (1954)	SAm	1844	24	
Petunioides					
N. attenuata Wats.	Goodspeed (1954)	CAm, NAm	1871	24	2.5, 9.93 (L1C, 4C) 6.95 (H2C)
N. corymbosa Rémy	Goodspeed (1954)	SAm	1849	24	
N. longibracteata Phil.	Goodspeed (1954)	SAm	1891	?	
N. linearis Phil.	Goodspeed (1954)	SAm	1895	24	6.50 (H2C)
N. miersii Rémy	Goodspeed (1954)	SAm	1849	24	5.82 (H2C)
N. pauciflora Remy*	Goodspeed (1954)	SAm	1849	24	
N. spegazzinii Millán	Goodspeed (1954)	SAm	1926	24[20]	7.11 (H2C)
Polydicliae					
N. clevelandii Gray	Goodspeed (1954)	NAm	1878	48	4.1, 16.56 (L1C, 4C) 7.76 (H2C)
N. quadrivalvis Pursh*	Goodspeed (1954)	NAm	1871	48	4.3, 17.01 (L1C, 4C) 10.50 (H2C)

(continued)

Table 2.1 (continued)

Species name	Described by	Origin	Year of first description	Diploid chromosome number	Genome size (pg) 2C DNA content after Hussain et al., 2023 (H2C); 1C and 4C DNA contents after Leitch et al., 2008 (L1C and 4C, respectively)
Repandae					
N. nesophila Jonst.	Goodspeed (1954)	CAm (I)	1931	48	5.0, 20.13 (L1C, 4C) 10.33 (H2C)
N. nudicaulis Wats.	Goodspeed (1954)	CAm	1883	48	3.6, 14.22 (L1C, 4C) 7.05 (H2C)
N. repanda Wild.	Goodspeed (1954)	NAm	1818	48	5.4, 21.76 (L1C, 4C) 9.98 (H2C)
N. stocktonii Brandegee	Goodspeed (1954)	CAm (I)	1899	48	5.0, 19.99 (L1C, 4C) 10.00 (H2C)
Rusticae					
N. rustica L.	Goodspeed (1954)	CAm	1753	48	5.3, 21.19 (L1C, 4C) 10.82 (H2C)
Suaveolentes					
N. africana Merxm.	Merxmueller and Buttler (1975)	SAm	1975	46	9.66 (H2C)
N. amplexicaulis Burb.	Burbidge (1960)	Africa (Namibia)	1960	36	6.92 (H2C)
N. benthamiana Domin.	Goodspeed (1954)	Au	1929	38	6.92 (H2C)
N. bilybara M.W. Chase and Christenh[3]	Chase et al. (2022a)	Au	2022	36, 38[21]	
N. burbidgeae Symon	Symon (1984)	Au	1984	42	
N. cavicola Burb	Burbidge (1960)	Au	1960	40[22]	
N. candelabra M.W. Chase and Christenh[3]	Chase et al. (2022a)	Au	2022	36, 38[21]	

N. debneyi (*N. forsteri*) Roem et Schult. [4]	Goodspeed (1954), Marks (2010)	Su	1929	48	9.15 (H2C)
N. eastii Kostoff[5]	Kostoff (1939, 1943)	Au	1939	64	
N. excelsior (J.M. Black) J.M. Black	Goodspeed (1954)	Au	1926	38	6.65 (H2C)
N. exigua Wheeler	Goodspeed (1954)	Au	1936	32	6.95 (H2C)
N. fatuhivensis F. Br.	Marks (2010)	FPol	2010	?	
N. faucicola Chase and Christenh.[6]	Chase et al. (2018b)	Au	2018	30	
N. fragrans Hock.	Goodspeed (1954)	Au (I NC)	1855	48	
N. gascoynica M.W. Chase and Christenh.[7]	Chase and Christenhusz (2018b)	Au	2018	40, 44	
N. goodspeedii Wheeler	Goodspeed (1954)	Au	1935	40[23]	6.31 (H2C)
N. gossei Domin.	Goodspeed (1954)	Au	1929	36	6.89 (H2C)
N. hesperis Burbidge	Burbidge (1960)	Au	1960	42	
N. heterantha Kenneally and Symon	Symon and Keneally (1994)	Au	1994	48[24]	
N. hoskingii Chase, Palsson & Christenh [8]	Chase, Palsson, and Christenhush (2021f)		2021	?	
N. ingulba Black	Goodspeed (1954)	Au	1933	32	
N. insecticida Chase and Christenh.[9]	Chase and Christenhush (2021a)	Au	2021	42	
N. karijini Chjini,). Chase and Christenh. [10]	Chase and Christenhusz (2018a)	Au	2018	?	
N. maritima Wheeler	Goodspeed (1954)	Au	1932	32[25]	
N. megalosiphon Van Huerck and Müll.	Goodspeed (1954)	Au	1870		
N. monoschizocarpa (P. Horton) Symon and Lepschi	Horton (1981)	Au	1981	48[24]	

(continued)

Table 2.1 (continued)

Species name	Described by	Origin	Year of first description	Diploid chromosome number	Genome size (pg) 2C DNA content after Hussain et al., 2023 (H2C); 1C and 4C DNA contents after Leitch et al., 2008 (L1C and 4C, respectively)
N. murchisonica Chase and Christenh.[9]	Chase, Przeslawski, Falvey, and Christenhusz (2021d)	Au	2021	?	
N. notha Chase and Christenh.[11]	Chase et al. (2021c)	Au	2021	64	
N. occidentalis Wheeler	Burbidge (1960)	Au	1935	42	5.83 (H2C)
N. paulineana Newbegin & Waterh[12]	Bally et al. (2021)	Au	2021	32[24]	
N. pila Chase & Christenh[13]	Chase and Christenhush (2021b)	Au	2021	?	
N. rosulata Wheeler	Chase and Christenhush (2021c)	Au	1929	20	5.42 (H2C)
N. rotundifolia Lindl.	Goodspeed (1954)	Au	1838	44[26]	5.44 (H2C)
N. rupestris M.W. Chase and Christenh[3]	Chase et al. (2022)		2022	38[21]	
N. salina Chase and Christenh.[9]	Chase, Fay, and Christenhusz (2021b)	Au	2021	42	
N. scopulorum M.W. Chase and Christenh[3]	Chase et al. (2022a)	?	2022	?	
N. simulans Burbidge	Burbidge (1960)	Au	1960	40	3.28 (H2C)
N. stenocarpa Wheeler	Goodspeed (1954), Chase and Christenhush (2018c)	Au	1935	40	
N. suaveolens Goodsp.	Goodspeed (1954) He et al. (2019)	Au	1818	32 64[27]	11.88 (H2C)

N. truncata Symon	Symon (1998), Chase, Conran, and Christenhusz (2021a)	Au	1998	36	
N. umbratica Burbidge	Burbidge (1960)	Au	1960	46	
N. velutina Wheeler	Goodspeed (1954)	Au	1935	32	
N. walpa Chase, Dodsworth & Christenh.[14]	Chase, Dodsworth, and Christenhush (2021e)	Au	2021	20	
N. wuttkei Clarkson and Symon	Clarkson and Symon (1991)	Au	1991	28, 32 [28]	
N. yandinga[15] Chase and Christenh.	Chase et al. (2018a)	Au	2018	42	
Sylvestres					
N. sylvestris Speg.	Goodspeed (1954)	Sam	1899	24	2.7, 10.78 (L1C, 4C) 5.81 (H2C)
Tomentosae					
N. kawakamii Ohashi	Ohashi (1976)	Sam	1976	24	6.34 (H2C)
N. leguiana Macbride[16]	Macbride (1930)		1930		
N. otophora Griseb.	Goodspeed (1954)	Sam	1879	24	5.99 (H2C)
N. setchellii Goodsp.	Goodspeed (1954)	Sam	1941	24	
N. tomentosa Macbride	Goodspeed (1954)	Sam	1759	24	
*N. tomentosiformis** Goodsp.	Goodspeed (1954)	Sam	1933	24	2.7, 10.97 (L1C, 4C) 5.52 (H2C)
Trigonophyllae					
N. obtusifolia Martens and Galeotti*	Goodspeed (1954)	Cam, Nam	1852	24	1.5, 6.18 (L1C, 4C)
N. palmeri Gray	Goodspeed (1954)	NAm	1878	24	
Undulatae					
N. arentsii Goodsp.	Goodspeed (1954)	Sam	1944	48	5.1, 20.22 (L1C, 4C) 4.71 (H2C)

(continued)

Table 2.1 (continued)

Species name	Described by	Origin	Year of first description	Diploid chromosome number	Genome size (pg) 2C DNA content after Hussain et al., 2023 (H2C); 1C and 4C DNA contents after Leitch et al., 2008 (L1C and 4C, respectively)
N. glutinosa L.	Goodspeed (1954)	Sam	1753	24	2.2, 8.94 (L1C, 4C)
N. thyrsiflora Goodsp.	Goodspeed (1954)	Sam	1938	24	
N. undulata Ruiz and Pay	Goodspeed (1954)	Sam	1759	24	2.4, 9.66 (L1C, 4C) 10.30 (H2C)
N. wigandioides Koch and Fintelm.	Goodspeed (1954)	Sam	1858	24	2.9, 11.38 (L1C, 4C)

The data include systematic status, provenance, somatic chromosome number and estimated genome size. Based on the lists of *Nicotiana* species by Berbeć and Doroszewska (2020) and Knapp (2020), expanded and updated

In early literature on *Nicotiana* the following species are sometimes referred to under the following names:*N. alata* = *N. affinis*; *N. plumbaginifolia N.* = *cavanillesii*; *N. noctiflora* = *N. cavanillesii*; *N. petunioides* = *N. pampasana*, *N. pauciflora* = *N. caudigera*; *N. quadrivalvis* = *N. bigelovii*; *N. tomentosiformis* = *N. Rusbyi*; *N. obtusifolia* = *N. trigonophylla* (see Kostoff, 1943; Goodspeed, 1954; Knapp, 2020)

Abbreviations: SAm (South and Central America including offshore islands), NAm (North America), CAm (Central America), CAm (I) (Revilla Gigedo islands southwest of Baja California) Au (Australia), Au (NC) (New Caledonia archipelago, Au (M) (Marquesas archipelago)

[1] discovered in Brazil by Augsten et al. (2022)

[2] described by Murfett et al. (2005) as closely related to *N. bonariensis* and reported in two other studies on hybrids within the section *Alata*e (Descorbeth, 2004; Lee et al., 2008);

[3] separated from *N. benthamiana* (Chase et al. 2022a)

[4] according to Marks (2010) the name *N. forsteri* takes rightful precedence over *N. debneyi,* the name *N. debneyi*, as universally adopted, is also used throughout this review

[5] separate taxonomic status not recognized by Wheeler (1945) and Goodspeed (1954) who considered *N. eastii* a tetraploid race of *N. suaveolens*

[6] similar to and confused with *N. maritima*, apparently closely related to *N. suaveolens* (Chase et al., 2018b); raised from seeds supplied by C. Marks and studied by Dodsworth (2015); description provided by (Chase & Christenhusz, 2018c)

[7] confused earlier with *N. simulans* from which it differs for some morphological features (Chase & Christenhusz, 2018b)

[8] morphologically similar but not closely related to *N.* debneyi (Chase, Palsson, & Christenhush, 2021f)

[9] separated from ‘the *N. occidentalis* group’ as a distinct taxon

[10] confused earlier with *N. benthamiana* and *N. umbratica*, found distantly related to *N. umbratica* by restriction site-associated DNA sequencing (RAD-seq), but distinguishable from either by size and other morphological features (Chase & Christenhusz, 2018a)

[11] most similar to western forms of *N. suaveolens*, probably an allotetraploid of *N. suaveolens* and *N. velutina* (Chase, Fay, Nollet, & Christenhusz, 2021c)

[12] discovered by D. E. Symon in early 2010s (Bally et al., 2021) and named provisionally "Corunna". Studied by Dodsworth (2015) who proposed the name "*N. symonii*" for the new accession. Finally, the species was named *N. paulineana* and described as related to *N. maritima* and *N. velutina*

[13] related to *N. rosulata* and *N. ingulba* (Chase & Christenhush, 2021b)

[14] similar to *N. gossei* but related to *N. stenocarpa* Chase, Dodsworth, and Christenhush (2021e)

[15] closely related to *N. maritima* from which it differs for somatic chromosome number (Chase et al., 2018a)

[16] described by Macbride (1930) as a species closely related to *N. tomentosa* and recognized as a separate species by Knapp (2020); a botanical variety of *N. tomentosa* according to Goodspeed (1954)

[17] no valid description; reported by Smith and Downs (1964), included in the list of *Nicotiana* species by Knapp et al. (2004), known from a single herbarium specimen (Lim et al., 2006)

[18] no valid description; reported by Smith and Downs (1964), included in the list of *Nicotiana* species by Knapp et al. (2004), known from a single herbarium specimen (Lim et al., 2006)

[19] in this work referred to as '*N. sanderae'* throughout the text and in tables to avoid graphic ambiguity when giving hybrid formulas involving this form; description by Christoff cites an earlier work by WB Hemsley published in 1905; no recognized status; a polymorphic form and ephemeral form—in most publications treated as an artificial horticultural hybrid of *N. alata* with *N. forgetiana* (Daly, 1959); in some strains of *N. sanderae* involvement of *N. langsdorffii* and other 18-chromosome members of the section Alatae is also possible

[20] after Knapp (2020)

[21] Chase et al. (2022a) give what seems to be dual monoploid chromosome numbers (18 and 19) for *N. bilybara* and *N. candelabra*. They follow their statements on chromosome numbers by a reference to another paper (Chase et al. 2022b). However, in the latter paper no direct mention is made of either of these two new species or of the third one (*N. rupestris*)

[22] originally reported by Burbidge (1960) as $2n = 46$, corrected by Williams (1975) to $2n = 40$, correction confirmed by Tatemichi (1990)

[23] as reported by Goodspeed (1945) and confirmed by Tatemichi (1990), Marks et al. (2011) report the number as $2n = 32$

[24] reported by Marks et al. (2011)

[25] reported by Goodspeed (1954) and confirmed by Tatemichi (1990); Marks et al. (2011) report the number as $2n = 30$

[26] reported by Goodspeed (1954) and confirmed by Tatemichi (1990); Marks et al. (2011) report the number as $2n = 32$

[27] tetraploid lineages reported to occur (Wheeler, 1945; He et al. 2019)

[28] as reported by Clarkson and Symon (1991) and Laskowska and Berbeć (2003), respectively

2.2 Spontaneous Interspecific Hybridization and Origin of Allopolyploid *Nicotiana* Species

2.2.1 Introductory Notes

Spontaneous interspecific hybridization is considered the basic evolutionary mechanism that led to the formation of today's *Nicotiana* species having from 9 to 24 chromosome pairs (Table 2.1). According to Goodspeed (1954), the genus *Nicotiana* originated from some hypothetical ancestors of the genera *Cestrum* and *Petunia* that gave rise to the "pre-*Nicotiana*" complex. More precisely, Goodspeed's theory postulated a dual ancestry of *Nicotiana*—the existence of two centers of divergence: "precestroid" and "prepetunioid" from which "petunioid" and "cestroid" present-day *Nicotiana* species had evolved. Goodspeed theorized that the base chromosome number of pre-*Nicotiana* was $n = 6$. Indeed, early researchers (Kostoff, 1943) found haploids of certain 12-paired *Nicotiana* species to form up to 5 chromosome pairs in meiosis, which was interpreted as remnants of ancient homology between two ancestral six-chromosome sets.

Plastid DNA analyses and molecular trees constructed by Clarkson et al. (2004) demonstrated that the involvement of *Cestrum* and *Petunia* in the origin of *Nicotiana* is unlikely and that it is the Australian tribe Anthocercidae that shows the closest affinity to *Nicotiana*.

The genus *Nicotiana* is now thought to have emerged as a separate solanaceous group approximately 24–28 million years ago (Särkinen et al., 2013). The evolution and speciation within the genus *Nicotiana* is considered to be largely driven by hybridization. Interspecific hybridization is thought to have contributed to the speciation process in *Nicotiana* species along two different routes, heteroploid and homoploid. Hybridization followed by chromosome doubling (heteroploid route) ultimately results in an allopolyploid species. The homoploid speciation process involves hybridization between two species with equal chromosome numbers and—essentially through recombination—ultimately generates a new species that retains the chromosome number of its parents (Goodspeed, 1954; Kelly et al., 2010; Runemark et al., 2019). Homoploid evolution is much more difficult to trace than the allopolyploid origin, and tentative evidence for homoploid hybrid ancestry was suggested for a few species only (Kelly et al., 2010; Clarkson et al., 2010; McCarthy et al., 2015). Of the present-day *Nicotianae*, the homoploid pathway of evolution has been proposed for *N. glauca*, *N. linearis* and *N. glutinosa*. The first two evolved following matings between the ancestral forms of *N. noctiflora* and *N. petunioides* on one side and a group involving *N. acuminata*, *N. attenuata*, *N. miersii* and *N. pauciflora* on the other. The origin of *N. glutinosa* appears to be equally complex and ensuing from ancient hybridization events that involved three tomentosoid species (*N. otophora*, *N. setchellii* and *N. tomentosiformis*) and two species of the section Undulatae (*N. undulata* and *N. wigandioides*) (McCarthy et al., 2015).

The major tenets of Goodspeed's theory on the origin and evolution of the genus and the arrangement of its species into groups of lower order have thus retained their validity, but numerous revisions, corrections and clarifications based on biochemical and, first of all, molecular methods, unavailable at the time of Goodspeed and his contemporary *Nicotiana* researchers, have since been introduced.

Over the next decades, a strong body of evidence has accumulated supporting the allopolyploid origin of several 24-chromosome pair species, including the most important one, the cultivated tobacco *N. tabacum*.

2.2.2 Origin of **Nicotiana Tabacum**

Anastasia, Christoff and Hachaturov (Kostoff, 1943) published the first views on the origin of *N. tabacum* in the early twentieth century, but their speculations were subsequently disproved. The first scientifically sound theories concerning the origin of *N. tabacum* were essentially built around the hypothesis advanced earlier by Winge (Barker et al., 2016), who proposed that the species with polyploid chromosome number had evolved from spontaneous sterile interspecific hybrids to which chromosome pairing and fertility were restored by chromosome doubling. These early hypotheses, based on morphological and cytogenetic evidence, pointed to *Nicotiana sylvestris* and one of the *Tomentosa* group species as the likely progenitors of *N. tabacum*: *N. tomentosa* (Clausen, 1928; Goodspeed & Clausen, 1928) and *N. otophora* (Goodspeed & Bradley, 1942; Goodspeed, 1945). Clausen (1932) was the first to propose *N. tomentosiformis* as the putative tomentosoid parent of *N. tabacum,* and the proposition was supported by Kostoff (1936, 1938). Kostoff based his assertion on the striking morphological similarity of his amphidiploid 4x (*N. sylvestris* × N. *tomentosiformis*) to *N. tabacum* and on its self-fertility (Kostoff, 1936). Incidentally, the 'Kostoff's amphidiploid' or 'Kostoff's hybrid', as it came to be known in later times, was demonstrated to carry a considerable amount of introgression from *N. tabacum* (Sheen, 1972; Lim et al., 2006; Moon et al., 2008). A hypothesis was also advanced regarding the involvement of *N. tomentosa* rather than *N. tomentosiformis* in Kostoff's amphidiploid (Slana et al., 1977; Stavely, 1979; Stavely et al., 1977). Those controversies notwithstanding, Gerstel (1960) furnished strong cytogenetic evidence in favor of *N. tomentosiformis* based on comparative homologies, pairing rates and segregation ratios in the offspring of allopolyploids of 6x (*N. tabacum* × *N. otophora*) and 6x (*N. tabacum* × *N. tomentosiformis*) when the two alloploids were backcrossed to *N. tabacum*. The segregation ratios for recessive markers were consistently lower for the alloploids involving N. *tomentosiformis* than for those involving *N. otophora*. These data indicated that *N. tomentosiformis* showed a greater homology with the tomentosoid subgenome of *N. tabacum* than did *N. otophora*. Hence, *N. tomentosiformis* was more likely to be the tomentosoid parent of *N. tabacum* even though the amphidiploid 4x (*N. sylvestris* × *N. otophora*) was fully self-fertile and the areas of the natural occurrence of those two species overlapped, while *N. tomentosiformis* was known to occur further up north (Goodspeed, 1954).

Cameron (1965) demonstrated that the interactions of the *N. sylvestis* plasmon with the *N. tabacum* genome and *vice versa* did not produce flower modifications commonly associated with the effect of alien cytoplasmic factors (see Chap. 5), indicating that *N. tabacum* and *N. sylvestris* shared the same type of cytoplasm. This provided a clue that *N. sylvestris* was the likely maternal parent in the amphidiploid from which *N. tabacum* evolved. Sheen (1972) compared *N. tabacum*, amphihaploids 2x (*N. sylvestris* × *N. otophora*) and 2x (*N. sylvestris* × *N. tomentosiformis*), and Kostoff's hybrid (alleged amphidiploid 4x (*N. sylvestris* × *N. tomentosiformis*) for the similarity index of their eight isoenzyme systems. The index was higher for *N. sylvestris* × *N. tomentosiformis* than for *N. sylvestris* × *N. otophora* but was the highest for Kostoff's amphidiploid. Sheen concluded that *N. tomentosiformis* is the likely progenitor of *N. tabacum* and explained the very high similarity between *N. tabacum* and Kostoff's hybrid by inadvertent introgression from *N. tabacum* in the latter, a possibility indirectly admitted by Kostoff himself (Kostoff, 1938) and confirmed later (see the previous paragraph but also Sect. 4.6.4 where Kostoff's hybrid is discussed at some length).

In the 1970s, a series of studies lent more support to *N. sylvestris and N. tomentosiformis* as respective putative maternal and paternal parents of *N. tabacum*. Gray et al. (1974) compared *N. tabacum*, *N. sylvestris*, *N. otophora* and *N. tomentosiformis* for the polypeptide composition of ribulose bisphosphate carboxylase-oxygenase (RuBisCO), an enzyme performing a dual function of oxygenation and carboxylation and a vital component of both photosynthetic and photorespiratory systems (Kung, 1977). The enzyme, the most abundant protein in higher plants, previously commonly known as Fraction 1 protein, is also unique in that it is composed of two subunits, the larger being coded by chloroplast DNA and the smaller by nuclear DNA. Thus, it can be used as a marker for both cytoplasmic and nuclear genomes (Kung, 1977). Gray et al. (1974) found that the polypeptide compositions of the large subunits in *N. tabacum* and *N. sylvestris* were identical. The small subunits were identical in *N. tomentosiformis* and *N. tabacum* and different in *N. otophora*. The parentage of *N. tabacum* originating from ancestral forms of *N. sylvestris* and *N. tomentosiformis* was confirmed in similar RuBisCO composition studies by Iwai et al. (1976) and Kawashima et al. (1976) and by comparing the content of soluble b-proteins induced by TMV infection in *N. tabacum* and in the amphidiploid 4x (*N. sylvestris* x *N. tomentosiformis*) (Ahl et al., 1982).

The major argument against *N. tomentosiformis* as the paternal progenitor of *N. tabacum* was that the synthetic amphidiploids 4x (*N. sylvestris* × *N. tomentosiformis*) produced by Greenleaf (1941), Lilienfeld (1952, 1953), Gerstel (after Sheen, 1972), Burk (1973), Lim et al. (2006) including those produced somatically by protoplast fusion (Liao & Lai, 1994a, b, c) were consistently reported to be pollen fertile but sterile on the female side due to the collapse of embryo sac development during meiotic divisions (Greenleaf, 1942; Ar-Rushdi, 1955; Liao & Lai, 1994a, b). Obviously, the original amphidiploid must have retained some ability to perpetuate itself as a lineage from which a new species could have evolved. Burk (1973) found his original amphidiploid plant of *N. sylvestris-tomentosiformis* to show vestigial self-fertility that tended to increase slightly over subsequent generations. He speculated that the spontaneous ancestral amphidiploid may have also had a chance to produce some selfed offspring, given that its self-fertility levels and longevity were approximately equal to those of his synthetic hybrid. Over generations, the fertility of the amphidiploid would have progressively increased owing to reverse mutations of the recessive sterility system (Clausen, 1941; Ar-Rushdi, 1955) as well as because of other genomic rearrangements as it continued to evolve into a new species (Bindler et al., 2011). Indeed, after several generations of selfing, Burk's amphidiploid regained enough fertility for its seeds to be eventually pooled into a seed stock designated the 'Th line' (Skalicka et al., 2003, 2005). The accession of 4x (*N. sylvestris* x *N. tomentosiformis*) of undeclared provenance used in the study by Sievert (1972) was implied by the author to be "fertile".

Since the advent of molecular methods, a sizeable new body of evidence on the ancestry of *N. tabacum* has accumulated. On the maternal side, *N. sylvestris* remained an undisputed candidate, and DNA sequencing data reconfirmed its status (Bland et al., 1986; Yukawa et al., 2006). Most of the molecular evidence, starting with the measurements of DNA content in *N. tabacum* and in its putative progenitors (Narayan & Rees, 1974), supported *N. tomentosiformis* as the donor of the tomentosoid (T) subgenome of *N. tabacum* (Okamuro & Goldberg, 1985; Murad et al., 2002; Lim et al., 2006; Khan & Narayan, 2007; Sierro et al., 2014). Some studies, while confirming the major role of *N. tomentosiformis*, also yielded evidence for introgression from *N. otophora* (Kenton et al., 1993; Riechers & Timko, 1999; Kitamura et al., 2001; Ren & Timko, 2001; Raju et al., 2012). Based on the transferability of *N. tabacum* microsatellite (SSR) probes, Moon et al. (2008) found *N. kawakamii* and *N. tomentosiformis* to show the greatest similarity to *N. tabacum* but also did not exclude the possible involvement of *N. otophora* in the evolution of the cultivated species. The issue of whether *N. tomentosiformis* alone or with some participation from other tomentosoid species contributed to the T subgenome of *N. tabacum* may be difficult to resolve. From the evolutionary perspective, *N. tabacum* is a relatively young species, although the estimates regarding its age differed substantially: from less than 6 million years (Okamuro & Goldberg, 1985) to approximately 200 thousand years (Clarkson et al., 2005; McCarthy et al., 2015) or even much less than that (Petit et al., 2007). The most recent investigations put the age of *N. tabacum* ca. 600 thousand years (Clarkson et al., 2017). The actual time span notwithstanding, since its inception, *Nicotiana tabacum* has undergone substantial changes in its genomic structure and organization (Bombarely et al., 2012). Analysis of repetitive DNA sequences indicated that the T genome of *N. tabacum* has experienced greater sequence loss than the S genome (Macke et al., 2004; Renny Byfield et al., 2011). Skalicka et al. (2003, 2005), in their studies of the 'synthetic tobacco' (amphidiploid hybrid 4x (*Nicotiana sylvestris* × *N. tomentosiformis*)), found the changes in DNA sequences to be very rapid and preferentially targeted to the *N. tomentosiformis* genome. Since the polyploidization event, the *N. tabacum* genome has decreased by 3.7–8% of its original size, and the sylvestroid and tomentosoid subgenomes now account for 53 and 47%, respectively, of the total *N. tabacum* genome (Wang & Bennetzen, 2015). This notwithstanding, due to its young age, *N. tabacum* still retains a sizeable and complex genome, with repeat elements accounting for approximately 70% of 4.5 Gb of its total size (Bromley et al., 2017). On the other hand, the changes that the *N. tabacum* genome has undergone may have blurred to some extent the molecular fingerprints originally left by its ancestral parents, especially by the tomentosoid one, the more so as the genomes of the putative parents themselves must have undergone some significant alterations during that time (Bindler et al., 2011).

The evolution of *Nicotiana tabacum* into contemporary market types of cultivated tobacco was summarized by Lewis and Nicholson (2007).

2.2.3 Polyploid Origin of some Other Nicotianae

There are several other 24-pair chromosome *Nicotiana* species, the origin of which has been traced to particular 12-chromosome-pair progenitors.

Origin of N. rustica Another cultivated tobacco (*N. rustica*) is recognized as the product of hybridization between the direct ancestors of *N. paniculata* and *N. undulata* (Goodspeed, 1954; Gray, 1978; Lim et al., 2004, 2005; Kovarik et al., 2008; Leitch et al., 2008). With the exception of Gray (1978), who, based on serological evidence, pointed to the female parentage of *N. undulata* in the hybrid, all subsequent reports agree on *N. paniculata* as the closest extant relative of the maternal parent of *N. rustica* (Lim et al., 2004; Kovarik et al., 2008; Knapp, 2020). This notwithstanding, introgression from *N. knightiana* (Aoki & Ito, 2000; Chase et al., 2003) on the maternal side and from *N. glutinosa* on the paternal side (Chase et al., 2003) was also suggested. Indeed, according to a more recent study by Sierro et al. (2018), present-day *N. rustica* inherited ca. 60% of its genome from the common ancestor of both *N. paniculata* and *N. knightiana*, and of the two, *N. knightiana* seems to be more closely related to *N. rustica*. The maternal parentage of *N. rustica* is also indirectly suggested by the similarity of flower morphologies in alloplasmic lineages of *N. tabacum* that carry the cytoplasm of *N. rustica*, *N. paniculata* or *N. knightiana* (Hart, 1965; Kubo, 1985; Berbeć, 2001; Nikova & Vladova, 2002). The cytoplasm of *N. undulata* affects the floral development in *N. tabacum* in a markedly different way (see Chap. 5). *N. rustica* is a relatively young allopolyploid whose age is estimated to range from 200 thousand (Mac McCarthy et al., 2015) to 700 thousand years (Clarkson et al., 2017).

Origin of *N. arentsii* N. *arentsii* is considered to be the youngest natural allopolyploid of *Nicotiana* that arose approximately 400 thousand years ago (Clarkson et al., 2017) and was determined to have descended from the union of *N. undulata* and *N. wigandioides* (Goodspeed, 1954, Gray, 1978, Leitch et al., 2008, Mc McCarthy et al., 2015). Spontaneous amphidiploid hybrids between some ancestral 24-chromosome forms of *N. obtusifolia* and *N. attenuata* gave rise to two separate 48-chromosome lineages from which contemporary species of the section

Polydicliae, *N. clevelandii* and *N. quadrivalvis* evolved (Lim et al., 2004; Anssour et al., 2009) approximately 1.2 million years ago (Clarkson et al., 2017).

Origin of the Repandae Species The four extant species of the Repandae section (*N. nudicaulis*, *N. repanda*, *N. nesophila* and *N. stocktonii*) have differentiated from the ancestral hybrid of *N. sylvestris* with *N. obtusifolia*: (Leitch et al., 2008; Clarkson et al., 2017), and those events are estimated to have taken place approximately 4 million years ago.

Origin of the Suaveolentes Species The most ancient allopolyploids in *Nicotiana* belong to the present-day section of Suaveolentes (Leitch et al., 2008; Liu & Marubashi, 2014; Clarkson et al., 2017), and their age is estimated at 6–10 million years (Clarkson et al., 2017; McCarthy et al., 2015; D'Andrea et al., 2023). While the majority of *Nicotiana* allopolyploids retained their original chromosome number, most species in the section Suaveolentes underwent a reduction in chromosome number (Gottula et al., 2014). That most numerous and most highly diversified group in the genus probably originated from a sequence of hybridization events that involved the hybrid between two ancient and as yet undetermined species of the sections Noctiflorae and Suaveolentes on the maternal side and the progenitor of *N. sylvestris* on the paternal side (Clarkson et al., 2017; Schiavinato et al., 2020). The most recent data also suggest contributions from some ancestral forms of the sections Alatae and Petunioides. According to the study by d'Andrea et al. (2023), the single hybridization event that ultimately gave rise to the section Suaveolentes occurred before the split between the ancient clades of Alatae/Sylvestres and Noctiflorae/Petunioides. Thus, the ancestral progenitor of Suaveolentes was a hybrid between the common ancestor of the sections Noctiflorae and Petunioides as the female parent and the progenitor of the sections Alatae and Sylvestres as the male parent (D'Andrea et al., 2023).

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