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A REVIEW OF CHROMOSOME NUMBERS IN THE GENUS *TILIA* (*TILIACEAE*)

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Chromosome numbers for all 23 species of *Tilia* recognized by the author, and for several subspecies and hybrids, are reviewed. Counts or cytometric analyses for 14 species are reported for the first time; of these, eight species are diploid ($2n=82$): *T. amurensis* subsp. *taquetii*, *T. chingiana*, *T. endochrysea*, *T. kiusiana*, *T. mandshurica*, *T. caroliniana*, *T. heterophylla* and *T. mexicana*, five are tetraploid ($2n=164$): *T. chinensis*, *T. henryana*, *T. miqueliana*, *T. mongolica* and *T. paucicostata*, and one is octoploid ($2n=328$): *T. nobilis*. *T. japonica*, previously reported as diploid, is tetraploid in Japan (Hokkaidō) and in China. Three new combinations are created: *T. amurensis* Rupr. subsp. *taquetii* (C.K. Schneid.) Pigott, *T. cordata* Mill. subsp. *sibirica* (Bayer) Pigott and *T. platyphyllos* Scop. subsp. *corinthiaca* (Bosc ex K. Koch) Pigott.

Keywords. Checklist, chromosome numbers, lime, linden, *Tilia*.

Introduction

Recent molecular studies (Bayer *et al.*, 1999) have shown that the genus *Tilia* probably separated at an early stage in the evolution of the *Malvales* and is now very isolated, not only from other families within the order, but also from other genera traditionally included in the *Tiliaceae*. The high basic number of chromosomes ($x=41$) is evidence of a polyploid origin of the genus.

Associated with this isolation is the very distinctive and relatively uniform morphology of *Tilia*, but consequently the separation of species within the genus is often unclear and there is no consensus on the number of species which should be recognized. A large number of species have been described but present studies, based largely on analyses of variation within natural populations, indicate that the number of species is relatively small and probably not more than 20–25. These species now occupy three separate areas in the temperate zone of the Northern Hemisphere: Europe and adjacent parts of Asia, eastern Asia, and eastern North America and Mexico. Two species just enter the tropics, one in Vietnam and the other in Mexico.

There is at present no satisfactory subdivision of the genus, although one species, *T. endochrysea*, in which the fruits split along the sutures when ripe, seems to stand apart. It is hoped that current molecular studies will fill this gap.

The earliest published chromosome counts for *Tilia* appear to be those of Wallisch (1930), who reported $n=36$–40 for three species native to central Europe. Two years later Dermen (1932) published counts for 15 taxa and established with certainty

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$n=41$ for the diploid taxa and the existence of several tetraploid taxa. Subsequently, numbers for five further taxa have been published (Khlonov, 1965; Stepanov, 1993; Pigott & Francis, 1999).

As part of a taxonomic revision of the genus, chromosome counts or cytometric estimates are now available for all the taxa which it is proposed to treat as species and for some subspecies. Consequently this review is also a checklist of the 23 species recognized. Taxonomic uncertainties remain and can be resolved only by further analyses of the ranges of variation in natural populations and by experimental investigations. This applies particularly to the many species related to *T. tuan* which are included in Chang (1993) but seem to be based on individuals within the range of variation of normal populations of the species. The American taxa present similar problems, but for the present the four species recognized by Jones (1968) have been accepted, even though his treatments of *T. caroliniana* and *T. mexicana* are unsatisfactory. Three new combinations are published here but the full synonymy is omitted and will be published elsewhere.

Methods

For all but one of his counts Dermen (1932) used aceto-carmine squashes of pollen mother cells during meiosis, and he based his counts on drawings. All new counts reported here were made on squashes of root-tips of plants which were either 1–4 years old and grown from seed (described as seedlings), or from cuttings rooted under mist and taken from older trees. The plants were lifted from their pots in early summer and a thin layer of peat-based compost was placed below and surrounding the soil mass. They were then kept in a heated glasshouse (20–25°C) for 3–4 weeks to allow the roots to grow into the grit-free soil. The pretreatment with colchicine, staining by the Feulgen method and preparation are described in Pigott & Francis (1999). Counts were based on both drawings and photographs. Some species were available only as grafts and, because cuttings repeatedly failed, the level of ploidy of these was measured by cytometry, using four plants with known chromosome numbers as standards. Cytometry measures the DNA content of individual cell nuclei and was done commercially by Gerard Greenen of Plant Cytometry Services, 's-Hertogenbosch, the Netherlands.

Results and Review

The chromosomes of *Tilia* are very small, about lμm long and 0.5μm wide (Dermen, 1932), and very little structure can be resolved optically. Like many plants with small chromosomes, parts of chromosomes close to the centromere are condensed at prophase and clearly visible as small, dispersed, stained bodies ('prochromosomes'). Their number in *Tilia* appears to be more or less equal to the number of chromosomes. All new counts were made at metaphase but, even following pretreatment with colchicine, complete separation of the chromosomes during squashing

was rarely achieved, so that it was difficult to obtain completely unambiguous counts. In these cases the actual number counted is placed in parentheses after the probable number. The type of count is indicated as pollen mother cells (pmc), root-tips (rt) and cytometry (ctm).

No voucher specimens were preserved by Dermen but, for the then newly introduced Asiatic species, it is possible to be reasonably certain which trees were used. All were growing in the Arnold Arboretum; some are still alive and there are specimens of others in the herbarium at the Arboretum. Voucher specimens are preserved for all the new counts reported here, either of the actual plant (v) or of the parent from which seedlings were grown (vp). These are in the author's herbarium and will be transferred to national herbaria. They are identified by a double number (year and serial number, e.g. 98-52). Trees in the University Botanic Garden, Cambridge (referred to as Cambridge), the Royal Botanic Gardens, Kew and the Arnold Arboretum of Harvard University are identified by their reference numbers, and trees whose chromosomes were actually counted by the author's serial cultivation numbers (e.g. DP445). Cartmel refers to the author's living collection in Cumbria.

Europe and western and central Asia

Tilia cordata Mill. subsp. **cordata**. $n=36$ (pmc): tree in the Botanic Garden, University of Vienna, Austria (Wallisch, 1930). $n=41$ (pmc): tree in the Arnold Arboretum (Dermen, 1932). $2n=82$ (rt): northern Germany (Tischler, 1935). $2n=82$ (rt): Slovakia (Májovský & Murín, 1987). $2n=82(-86)$ (rt): trees at six native sites in England (Pigott, 1991). $2x$ (ctm): cutting from tree (v94-31, DP445) at Chalkney Wood, Essex, which is the source of the neotype (Pigott, 1997).
Subsp. **sibirica** (Bayer) Pigott. $2n=82$ (rt?): Tomsk, Siberia (Khlonov, 1965). $2n=36$ (rt): as *T. nasczokinii* Stepanov from Krasnoyarsk (56°05′N, 92°46′E) (Stepanov, 1993): plant (DP495) raised from seed supplied by Stepanov gave $2x$ (ctm), indicating $2n=82$.

T. dasystyla Steven subsp. **dasystyla**. $2n=164$ (three plants), $2n=150–160$ (three plants) (rt): one plant $4x$ checked (ctm): four native localities in Crimea, Ukraine (Pigott & Francis, 1999); vouchers of parent trees (vp92-20, 92-63, 92-66).
Subsp. **caucasica** (V. Engl.) Pigott. $2n=164$ (159) (rt): (DP302), seedling from woodland at Lagodekhi (41°50′N, 46°16′E), Great Caucasus, Georgian Republic (Pigott & Francis, 1999).

T. × euchlora K. Koch. $4x$ (ctm), indicating $2n=164$; tree (v88-51, DP507) in Cambridge.

T. × europaea L. $n=41$ (pmc): tree in Arnold Arboretum (Dermen, 1932).

T. platyphyllos Scop. subsp. **platyphyllos**. $2x$ (ctm): tree (DP006: 95–97) in

Cambridge from Bohinska Bela (46°21′N, 14°04′E), Slovenija, which is the source of the holotype (Pigott, 1997). $n=40$ (pmc): tree in the Botanic Garden, University of Vienna, Austria (Wallisch, 1930); if this is the large old tree still present in the Garden in 1999, then this count applies to subsp. *pseudorubra*.
Subsp. **cordifolia** (Besser) C.K. Schneid. $n=41$ (pmc): tree identified as *T. cordata* '*cordifolia*' in the Arnold Arboretum (Dermen, 1932). $2n=82$ (rt): seedlings (DP010, 440, 201) from native sites in Surrey and Sussex, England, and near Castellane (43°50′N, 6°29′E), Alpes de Provence, France.
Subsp. **pseudorubra** C.K. Schneid. $2n=82$ (rt): seedling (DP408) from tree in woodland near Cumbels (46°43′N, 9°12′E), Lugnezertal, Graubünden, Switzerland (vp92-36, 92-37).
Subsp. **corinthiaca** (Bosc ex K. Koch) Pigott. $2n=82$ (76+) (rt): seedling (DP496) from tree (vp94-7) at Trikala (38°0′N, 22°26′E), Peloponnisos, Greece.

T. tomentosa Moench. $n=40$ (pmc): tree in the Botanic Garden, University of Vienna, Austria (Wallisch, 1930). $n=41$ (pmc): tree labelled *T. petiolaris* in the Arnold Arboretum (Dermen, 1932). $2n=82$ (rt): seedling (DP244, v00-45) from tree in churchyard near the rue Gay Lussac, Paris, France. $2n=82$ (76+) (rt): tree (DP230) at Cartmel, seed from tree (vp86-16) on karst, near Lijeva Rijeka (42°39′N, 19°29′E) between Kolasin and Podgorica (Titograd), Montenegro.

Eastern Asia

T. amurensis Rupr. subsp. **amurensis**. $n=82$ (pmc): tree in the Arnold Arboretum (Dermen, 1932): almost certainly 6783 from Regel and Kesselring (St Petersburg, Russia), possibly from Manchuria, planted in 1900 and flowering by 1924 (vp94-80, 00-81). $n=82$ (pmc): tree in the Arnold Arboretum (Dermen, 1932) as *T. insularis* Nakai (Pigott, 2000): this was 10859 collected by E.H. Wilson from Ullung-do, Korea (vp00-75, 00-76). $2n=164$ (rt): seedling from tree at München-Nymphenburg, Germany (as *T. insularis* but source unknown) (vp91-12, v99-1).
Subsp. **taquetii** (C.K. Schneid.) Pigott. $2n=$c.82 (rt): $2x$ (ctm): seedling (DP339) at Cartmel from near Mikhaylovka (43°58′N, 132°01′E), near Ussuriyisk, Primorsk Kray, eastern Siberia. $2n=$c.82 (75–77) (rt): seedling (DP270) in Cambridge from Chiri-san (35°13′N, 127°38′E), Kuri-gun, Korea, supplied by Chollipo Arboretum, Taejon as *T. koreana* Nakai (v97-1, 99-7).

T. chinensis Maxim. $2n=164$ (rt): seedling (DP438) from tree (vp93-105) at Long-chi ('Dragon Lake'), Qing-chen-shan (31°01′N, 103°40′E), near Du Jiang Yan (Guan Xian), Sichuan, China. $2n=$c.200 (?164, several preparations with poor separation of chromosomes). $2n=164$ (rt): seedling (DP197) from a second tree (vp93-93) at Long-chi. $2x$ and $4x$ chimaera (ctm): seedling (DP343) from tree (vp93-96) at Da-wang-zi, above Bai-shui-he on Yu-long xue-shan (27°08′N, 100°17′E), Li-jiang, Yunnan, China.

T. chingiana Hu & Cheng. $2n = 82$ (79) (rt): seedling (DP508, vp96-114) from tree on cliff at Guling on Lushan (29°29′N, 116°02′E), Jiangxi, China, the *locus classicus* of the species (Cheng, 1935). $2x$ (ctm): tree (DP337, v91-71) in Cambridge; scion wood originally from a tree at Birr Castle, Offaly, Eire, grown from seed from Lushan.

T. endochrysea Hand.-Mazz. $2x$ (ctm), indicating $2n = 82$; grafted tree (DP320, v93-45); scion wood collected from woodland near Bai-nan-kin, Wu-zhu-shan (30°15′N, 113°03′E), Ru-yuan xian, Guangdong, China.

T. henryana Szyszyl. $2n = 164$ (170) (rt): tree (DP300) in Cambridge; seedling from old tree (ref. P27-056, vp92-111) at the Arboretum national des Barres, Nogent-sur-Vernisson, France.

T. japonica (Miguel) Simonkai. $2n = 82$: quoted in Funabiki (1958) without source. $2n = 164$ (rt): seedling (DP347) from seed collected by F.M. van Eck beside Lake Ōnuma (41°58′N, 140°41′E), Oshima, Hokkaidō, Japan. Numbers close to 164 were counted in three other plants (DP318, 383, 436) of the same provenance (v99-2). In a fifth (DP435), a count of 122 indicated a triploid. $4x$ (ctm): plant (v99-19); seed from tree at Vácrátót, Budapest, Hungary (vp92-107) originally from Nanjing and of Chinese origin (syn.: *T. eurosinica* Croizat).

T. kiusiana Makino & Shirasawa. $2n = 82$ (rt): tree (DP165) in Cambridge; seedling from the old tree (vp91-61) at the Arboretum national des Barres.

T. mandshurica Maxim. $2n = 82$ (78+), $2n = 82$ (76+) (rt): two young trees (DP143, v00-38; DP176); seed from Xiang-shan Botanic Garden, Beijing, China. Tree DP176 in Cambridge.

T. maximowicziana Shirasawa. $n = 82$ (pmc): tree in the Arnold Arboretum (Dermen, 1932). This was 5739, now lost (vp in GH); seed collected by J.G. Jack near Sapporo, Hokkaidō, Japan in 1905 and flowering in 1928.

T. miqueliana Maxim. $4x$ (ctm), indicating $2n = 164$: grafted tree at Cartmel, scion from 69-16884 Royal Botanic Gardens, Kew (v95-39, 98-60), source recorded as America. It closely matches tree 12211 in the Arnold Arboretum (wrongly labelled *T. mandshurica*); the original label states 'Hers 1068' and his catalogue shows that seed was sent in 1920 from Kiangsu (Jiangsu). A specimen in GH with the same number (presumably the parent tree) is *T. miqueliana*; it was collected by Joseph Hers from Suzhou-fu, Huang Tsang Yu (this is Xuzhou at 34°17′N, 117°18′E in Jiangsu).

T. mongolica Maxim. $2n = 164$ (rt): plants DP245 and 246 (vp95-4); seeds from Xiangshan Botanic Garden, Beijing, China, but of local origin. $2n = 164$ (DP158);

cutting of tree (DP134; v88-37, 97-57) in Cambridge, from Hillier; original provenance unknown but probably the Beijing area.

T. nobilis Rehder & Wilson. 8*x* (ctm), indicating 2*n* = 328: graft at Cartmel, scion from tree KR266 (v96-49), collected in 1980 by K. Rushforth at 2100m on Emeishan (29°36′N, 103°11′E), Sichuan, China, now in his living collection at Cullompton, Devon.

T. oliveri Szyszyl. *n* = 41 (pmc): tree in the Arnold Arboretum (Dermen, 1932), now lost but one of two (17541, 17452) from Veitch's Nursery, Chelsea, England, grown from seeds collected at Hsing-shou Hsien, W Hupeh (Xing-shan, 31°15′N, 119°49′E, Hubei) by E.H. Wilson in 1911 and flowering in 1927. 2*n* = 82 (rt): cutting of tree (v92-8, 95-51; DP144) in Cambridge, a graft from the tree (96-69) on the site of Hillier's old nursery in Winchester, England, also from Veitch but not identical to the trees at the Arnold Arboretum.

T. paucicostata Maxim. 2*n* = 164 (rt): seedling (DP434) from tree (10589, vp94-78) at the Arnold Arboretum, from the Parks Department, Rochester, New York, original provenance unknown.

T. tuan Szyszyl. *n* = 82 (pmc): tree, now lost, in the Arnold Arboretum (Dermen, 1932), almost certainly one of those grown from seed collected by E.H. Wilson in 1910 (4409, 4449) from Fang Hsien, western Hupeh (Fang Xian, Hubei) (Sargent, 1916). These trees (17560, 17510), of which there are herbarium specimens at the Arnold, were flowering by the 1920s. 4*x* (ctm): graft at Cartmel; scion from tree in Stephanie's Glade at Borde Hill, Sussex (v90-79, 91-31). 2*n* = 164 (159+): seedling (DP190) grown from tree (vp93-97) on Jizu-shan (25°55′N, 100°20′E), Bin-chuan, Yunnan, China, the type locality of *T. chenmouii* Cheng, here treated as a synonym of *T. tuan* (Cheng, 1936).

North America

T. americana L. var. **americana**. *n* = 41 (pmc): tree labelled *T. glabra* Vent. in the Arnold Arboretum (Dermen, 1932). 2*n* = 82 (rt): plant (DP259, v00-44); seed from a native tree in Kane County (39°12′N, 90°21′W), Illinois, USA, supplied by the Morton Arboretum, Lisle.
Var. **neglecta** (Spach) Fosberg. *n* = 41 (pmc): tree labelled *T. neglecta* Spach in the Arnold Arboretum (Dermen, 1932). 2*n* = 82 (rt): plant (DP271, v00-46); seed supplied by the Jagellonian Botanic Garden, Kraków, Poland.

T. caroliniana Mill. 2*n* = 82 (rt): plant (DP326); seed from tree (vp94-80) at Colkin's Neck (33°53′N, 78°38′W), between Calabash and Little River, Brunswick County, North Carolina, USA.

T. heterophylla Vent. $2n = 82$ (rt): three plants grown from seeds: two from trees (vp94-61 and 94-62) in ravine west of Tye River Gap (37°56′N, 79°11′W), Vesuvius, and one from a tree at Salt Log Gap, Coffeetown, Amherst County, Virginia, USA.

T. mexicana Schlecht. $2n = 82$ (rt): seedling (DP236, v95-117); seed collected by J. Compton and M. Rix east of Saltillo at 25°22′N, 100°10′W in the Sierra Madre Oriental, Nuevo Leon, Mexico.

New Combinations

Tilia amurensis Rupr. subsp. **taquetii** (C.K. Schneid.) Pigott, **comb. et stat. nov.**
Basionym: *Tilia taquetii* C.K. Schneid., Repert Spec. Nov. Regni Veg. 7: 200 (1909) ('*taqueti*').
Type: Korea: Quelpaert in Hallaisan (Cheju-do, Halla-san, 33°14′N, 126°37′E), 1500m in petrosis torrentium, vii 1907, *U. Faurie* 1615 (holo. LE; iso. BM, E, K, KYO).
Syn.: *Tilia koreana* Nakai, Chosen Shokubutsu [Plants of Korea] 1: 174 (1914).

Tilia cordata Mill. subsp. **sibirica** (Bayer) Pigott, **comb. et stat. nov.**
Basionym: *Tilia parvifolia* f. *sibirica* Bayer, Verh. K.K. Zool. Bot. Ges. Wien 12, Abhandl.: 23 (1862).
Type: Prov. Tomsk, *C.A. Meyer* (holo. LE; herb. Ledebour); has *Tilia semicuneata* Rupr. on original label in Ruprecht's writing. A second label may be Bayer's and refers it to the numbers in his key.
Syn.: *T. cordata* var. *sibirica* (Bayer) Maxim., Bull. Acad. Imp. St. Pétersbourg 10: 584 (1880).
T. sibirica (Bayer) V. Engl., Monogr. Tilia: 83 (1909).

Tilia platyphyllos Scop. subsp. **corinthiaca** (Bosc ex K. Koch) Pigott, **comb. et stat. nov.**
Basionym: *Tilia corinthiaca* Bosc ex K. Koch, Dendrologie 1: 474 (1869).
Type: Not found: Koch's herbarium was at Berlin (B) and destroyed in 1943; duplicates of some of Koch's specimens are in several herbaria but none of *Tilia* have yet been discovered.

The name *T. rubra* DC. (1813: 215) has been widely misapplied to this taxon but the correct application is to *T. platyphyllos* Scop. subsp. *cordifolia* (Besser) C.K. Schneid. (Pigott & Francis, 1999: 172).

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