

Ploidy variation in *Buddleja* L. (Buddlejaceae) in the Sino-Himalayan region and its biogeographical implications

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The chromosome numbers of 27 populations of *Buddleja*, comprising 14 species, were counted. The basic chromosome number of all species was $x = 19$, confirming previous reports. Different ploidy levels ($2n = 38, 76, 114, 228$) were observed in these taxa, representing diploids, tetraploids, hexaploids, and dodecaploids, respectively. The chromosome numbers of *B. yunnanensis*, *B. brachystachya*, and *B. macrostachya* are reported for the first time. The tetraploid $2n = 76$ is a new ploidy level for *B. myriantha*. Particular attention was given to *B. macrostachya*, because of the variation in morphology and ploidy level between isolated populations of this species. Two types of interphase nuclei were recognized: the complex chromocentre type in *B. macrostachya* and the simple chromocentre type in the other species. Biogeographically, most of the polyploidy in the Asiatic species occurs in the Sino-Himalayan region. It seems to be associated with the uplift of the Himalayan Mountains, the orogeny of this region playing an important role in the evolution of polyploidy in these taxa. © 2007 The Linnean Society of London. *Botanical Journal of the Linnean Society*, 2007, **154**, 305–312.

ADDITIONAL KEYWORDS: basic chromosome number – biogeography – evolution – interphase nuclei.

INTRODUCTION

Buddleja is a cosmopolitan genus of Buddlejaceae consisting of about 100 species in the tropical and temperate zones in the world (Leeuwenberg, 1979; Li & Leeuwenberg, 1996; Oxelman, Backlund & Bremer, 1999; Norman, 2000). Bentham (1846) divided the genus into two sections based on the corolla shape and the ratio of the length of the corolla tube to that of the calyx. Marquand (1930) proposed four series (*Gynandrae*, *Alternifoliae*, *Curviflorae*, and *Rectiflorae*) in *Buddleja*, and Leeuwenberg (1979) recognized four sections (*Neemda*, *Buddleja*, *Nicodemia*, and *Chilianthus*), with over 80% of the species in section *Neemda*. However, Norman (2000) suggested that section *Chilianthus* was sufficiently distinct to be recognized at the generic level. Although taxonomic treatments of *Buddleja* are still subject to some dispute, its most probable centre of origin is South Africa (Moore, 1947; Leeuwenberg, 1979; Norman, 2000). The diversity

centres in the New World are in south-east Brazil, the Andes, Central America, and south-west USA, which together account for c. 63% of species (Norman, 2000). The diversity centres of Old World *Buddleja* are in Africa (South Africa and Madagascar) and the Sino-Himalayan region of South-east Asia, with c. 15% and c. 21% of species, respectively (Leeuwenberg, 1979; Li & Leeuwenberg, 1996).

The Himalayas constitute one of the world's youngest mountain ranges. Their origin was the result of the collision of the Eurasian and Indian tectonic plates, which started about 50 million years ago and continues to this day (Patriat & Achache, 1984; Tao, 1992, 2000; Wang & Ding, 1998). The Sino-Himalayan region has recently been recognized as the most biologically rich temperate region in the world (Myers *et al.*, 2000), with about 8000 species recorded from the 500 000 km² terrain. Over 90% of the Asian *Buddleja* species are distributed in this area (Li & Leeuwenberg, 1996; Nie, Gu & Sun, 2005).

Cytological data on Asian *Buddleja* species have been reported since the early 1940s (Moore, 1947,

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Table 1. Available cytological data on the Asian *Buddleja* species. All are based on $x = 19$

Species	Reference	2n	Ploidy	Locality
<i>B. alternifolia</i> Maxim.	Moore (1947)	38	2x	China, Kansu
	Moore (1947)	38	2x	China, Qinghai
<i>B. curvifolia</i> Hook.	Janaki Ammal (1954)	38	2x	Japan
<i>B. officinalis</i> Maxim.	Janaki Ammal (1954)	38	2x	China
<i>B. myriantha</i> Diels.	Janaki Ammal (1954)	38	2x	Burma
<i>B. paniculata</i> Wall.	Moore (1960)	38	2x	India, Calcutta
	Janaki Ammal (1954)	38	2x	Bhutan
	Sandhu & Mann (1988)	38	2x	India
<i>B. asiatica</i> Lour.	Moore (1947)	38	2x	Malaya
	Moore (1961)	38	2x	SE India
	Chatha & Bir (1987)	38	2x	S India
	Bir & Chatha (1983)	38	2x	India
	Sandhu & Mann (1988)	38	2x	India
	Gadella & Norman (1986)	38	2x	India
<i>B. delavayi</i> Gagne.	Moore (1960)	114	6x	China, Yunnan
<i>B. colvillei</i> Hook. f. & Thoms.	Moore (1947)	300	c. 16x	Himalayas
	Janaki Ammal (1954)	152	8x	NE India
	Gadella (1980)	456	24x	Nepal
<i>B. fallowiana</i> Balf. f. & Sm.	Moore (1947)	76	4x	China, Yunnan
<i>B. nivea</i> Duthie.	Moore (1947)	114	6x	China, Sichuan
	Moore (1960)	228	12x	China, Yunnan
<i>B. forrestii</i> Diels.	Moore (1947)	114	6x	China, Yunnan
<i>B. albiflora</i> Hemsl.	Moore (1947)	114	6x	China, Hubei
<i>B. candida</i> Dunn.	Moore (1960)	76	4x	China, Kansu
<i>B. crispa</i> Benth.	Moore (1961)	38	2x	NW India
	Bedi <i>et al.</i> (1981)	38	2x	Not given
	Khatoon & Ali (1993)	38	2x	Not given
<i>B. davidii</i> Franch.	Moore (1947)	76	4x	Central China
	Chatha & Bir (1987)	76	4x	S India
	Bir & Chatha (1983)	76	4x	Not given
<i>B. lindleyana</i> Fort.	Moore (1947)	38	2x	E China
<i>B. japonica</i> Hemsl.	Moore (1947)	38	2x	Japan

1960, 1961; Janaki Ammal, 1954; Gadella, 1980; Bedi, Bir & Gill, 1981; Bir & Chatha, 1983; Gadella & Norman, 1986; Chatha & Bir, 1987; Sandhu & Mann, 1988; Khatoon & Ali, 1993) (Table 1). These studies revealed that the basic chromosome number in the genus was $x = 19$, with several ploidy levels occurring. Cytologically, the Asian *Buddleja* species are better known, because many of the species are ornamentals, introduced into cultivation by European collectors. However, the relationship between the ploidy levels of the Asian *Buddleja* species and the orogeny of the Sino-Himalayan region has not been studied. Alpine and arctic floras have been reported to have high frequencies of polyploidy (Hanelt, 1966; Ohba, 1988; Abbott & Brochmann, 2003; Brochmann *et al.*, 2004). Therefore, as the Sino-Himalayan region consists of extensive alpine areas produced by the Tertiary Himalayan orogeny (Li, Shi & Li, 1995; Shi, Li & Li, 1998), there should be a high proportion of

plant polyploidy in this area. However, Nie *et al.* (2005) indicated that polyploidy may have played only a minor role in the evolutionary diversification in the Sino-Himalayan region, based on their cytological statistical analysis of 552 taxa. Therefore, we undertook an overall cytological study of the genus *Buddleja* in this region to determine whether the incidence of polyploidy supported the conclusion of Nie *et al.* (2005), and to discuss the possible trend of chromosome evolution of the genus in the Asian region.

In this study, 27 populations of *Buddleja*, comprising 14 species, were surveyed. These populations are as follows: five of *B. macrostachya*, three of each of *B. asiatica*, *B. davidii*, and *B. crispa*, two of each of *B. officinalis*, *B. forrestii*, and *B. fallowiana*, and one of each of the other species (Table 2). Together with the previously reported data, 20 of the 21 species occurring in Asia are discussed, with *B. macrostachya*,

Table 2. Chromosome numbers of the *Buddleja* species ($x = 19$), with localities and vouchers

Species	2n	Ploidy	Locality	Voucher
<i>B. asiatica</i> Lour.	38	2x	China, Yunnan, Dali	Sun 036
	38	2x	China, Yunnan, Chuxiong	Chen 034
	38	2x	China, Yunnan, Wenshan	Chen 015
<i>B. paniculata</i> Wall.	38	2x	China, Yunnan, Baoshan	Chen 031
<i>B. officinalis</i> Maxim.	38	2x	China, Yunnan, Kunming	Chen 012
	38	2x	China, Yunnan, Hekou	Chen 019
<i>B. brachystachya</i> Diels.	38	2x	China, Yunnan, Deqing	Chen 073
<i>B. macrostachya</i> Wall. ex Benth.	114	6x	China, Yunnan, Ximeng A	Sun 023
	228	12x	China, Yunnan, Simao D	Sun 029
	228	12x	China, Yunnan, Dali E	Chen 045
	114	6x	China, Yunnan, Bingchuan B	Chen 044
<i>B. fallowiana</i> Balf. f. & Sm.	114	6x	China, Yunnan, Dehong C	Sun 021
	76	4x	China, Yunnan, Dali	Chen 059
	76	4x	China, Yunnan, Zhongdian	Chen 054
<i>B. forrestii</i> Diels.	114	6x	China, Yunnan, Dali	Chen 047
	114	6x	China, Yunnan, Pianma	Chen 025
<i>B. myriantha</i> Diels.	76	4x	China, Yunnan, Daili	Sun 033
<i>B. crispa</i> Benth.	38	2x	China, Yunnan, Deqing	Chen 026
	38	2x	China, Yunnan, Kunming	Sun 030
	38	2x	China, Yunnan, Yimen	Sun 004
<i>B. davidii</i> Franch.	76	4x	China, Yunnan, Songming	Chen 051
	76	4x	China, Yunnan, Zhaotong	Sun 019
	76	4x	China, Yunnan, Suijiang	Wang s.h.
<i>B. yunnanensis</i> Rehd.	38	2x	China, Yunnan, Simao	Sun 028
<i>B. curviflora</i> Hook. ex Sith.	38	2x	Taiwan	Sun 010
<i>B. lindleyana</i> Fort.	38	2x	China, Hubei	Chen 053
<i>B. japonica</i> Hemsl.	38	2x	Japan	Sun 017

A–E in the Locality column indicate the different populations in Table 3.

in particular, being addressed, because of the variation in its morphology between populations.

MATERIAL AND METHODS

All cytological observations were made from root tip cells, with the exception of *B. macrostachya*, for which shoot tip cells were used. Most of the material was collected in the Sino-Himalayan region (Table 2). Vouchers of all collections and permanent slides are deposited in the herbarium of the Kunming Institute of Botany (KUN). Root and shoot tips were pretreated in 8-hydroxyquinoline solution (0.002 mol l^{-1}) at 23°C for 3 h, and then fixed in Carnoy (absolute ethanol:glacial acetic acid, 3:1, v/v) at 4°C for at least 1 h. The fixed root tips were hydrolysed in 1:1 1 M HCl:45% acetic acid at 60°C for 90 s, stained with 1% aceto-orcin, and squashed for cytological observation. Permanent slides were made using the standard liquid nitrogen method.

The number of somatic chromosomes at metaphase was determined from at least ten well-spread metaphases in three or more root tips of each species.

The karyomorphological classification of the mitotic interphase nuclei followed Tanaka (1971, 1977).

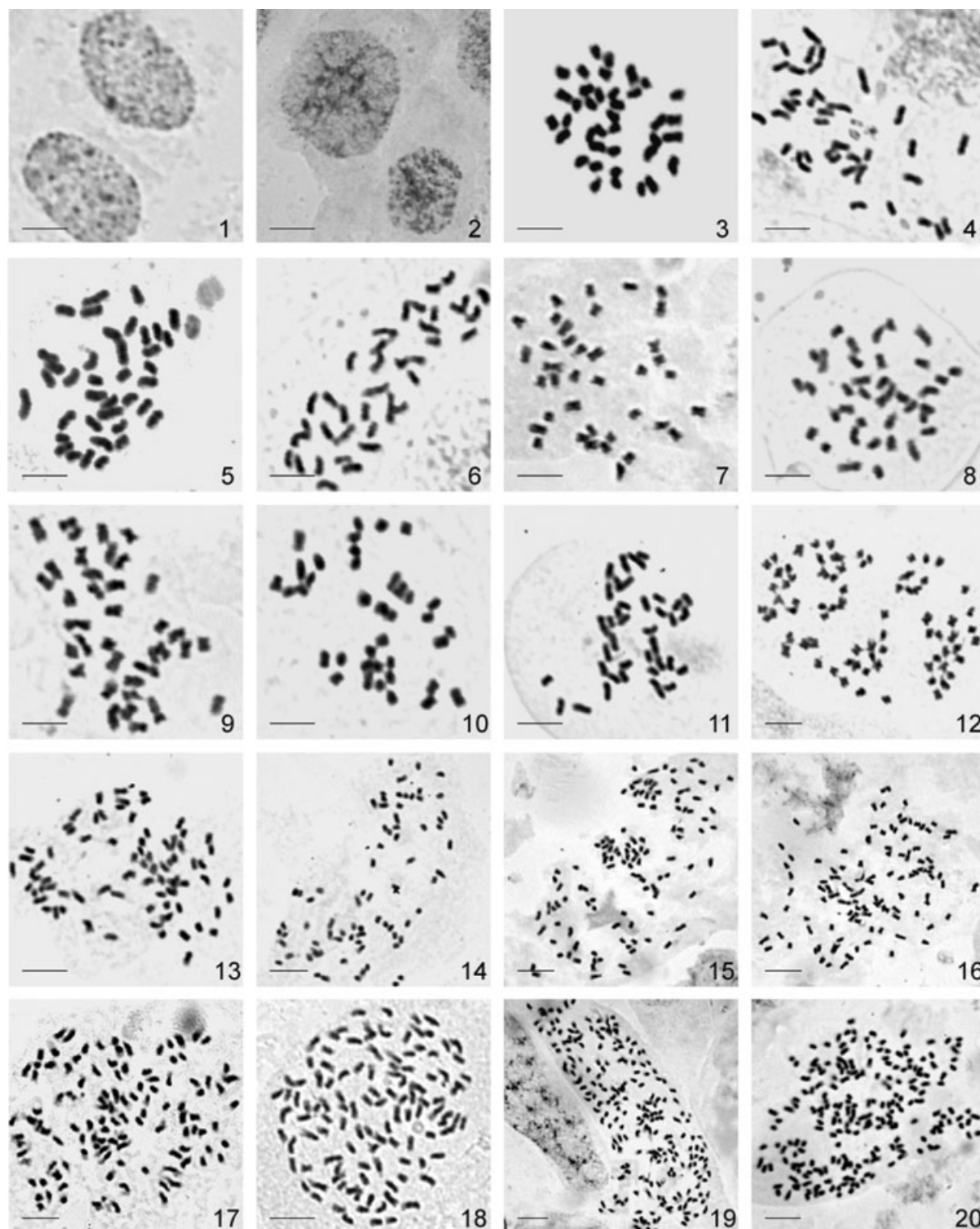
RESULTS

INTERPHASE NUCLEI

The interphase nuclei of the 14 *Buddleja* species were classified into two categories based on the shape and distribution pattern of chromatin (Figs 1, 2). The interphase nuclei of all species, except those of *B. macrostachya*, showed rounded prochromosomes dispersed evenly throughout the nuclei. According to Tanaka (1971, 1977), they are thus categorized as the round prochromosome type (Fig. 1). The interphase nuclei of *B. macrostachya* were of Tanaka's complex chromocentre type, characterized by darkly stained chromocentres of irregular shape and lightly stained chromatin threads (Fig. 2).

CHROMOSOME COUNTS

Table 2 and Figures 3–20 show the chromosome numbers observed in the study; the numbers for



Figures 1–20. Cytological features. Fig. 1. Interphase nuclei of smooth-faced round prochromosomes. Fig. 2. Interphase nuclei of complex chromocentre type. Figs 3–20. Mitotic metaphases. Fig. 3. *Buddleja asiatica*, $2n = 38$. Fig. 4. *B. officinalis*, $2n = 38$. Fig. 5. *B. paniculata*, $2n = 38$. Fig. 6. *B. brachystachya*, $2n = 38$. Fig. 7. *B. crispa*, $2n = 38$. Fig. 8. *B. curviflora*, $2n = 38$. Fig. 9. *B. yunnanensis*, $2n = 38$. Fig. 10. *B. japonica*, $2n = 38$. Fig. 11. *B. lindleyana*, $2n = 38$. Fig. 12. *B. davidii*, $2n = 76$. Fig. 13. *B. fallowiana*, $2n = 76$. Fig. 14. *B. myriantha*, $2n = 76$. Fig. 15. *B. forrestii*, $2n = 114$. Figs 16–18. *B. macrostachya*, $2n = 114$. Figs 19, 20. *B. macrostachya*, $2n = 228$. Scale bars, 5 μm .

Table 3. Comparison of morphological characters and polyploidy levels in different populations of *Buddleja macrostachya* Wall. ex Benth

	Population				
	A	B	C	D	E
Ploidy	6x	6x	6x	12x	12x
Altitude (m)	1730	1500	1840	1560	1920
Time of flowering (month)	2	1	1	1	1
Leaf width (mean, cm)	9.0	3.5	4.8	5.0	4.2
Inflorescence size (mean, cm)	27	24	9	25	21
Corolla colour*	RHS77C	RHS80A	RHS82C	RHS80B	RHS4C
Corolla length (mean, mm)	14.4	10.6	11.2	13.0	12.5
Trichome in corolla	Spiculate	Spiculate	Spiculate	Spiculate	Spiculate
Stamen position	In throat	In throat	In throat	In throat	In throat
Sexuality	Infertile	Semi-fertile	Fertile	Fertile	Infertile

*Measured with reference to the RHSCC (Royal Horticultural Society's Colour Chart). RHSCC is the standard reference for plant colour identification, published by Flower Council of Leiden, Holland.

B. yunnanensis, *B. brachystachya*, and *B. macrostachya* are reported for the first time. A new ploidy level of $2n = 76$ ($4x$) was observed in *B. myriantha*. The basic chromosome number of all the studied populations was $x = 19$. Three populations of *B. asiatica* were all diploid, with $2n = 38$ (Fig. 3); *B. officinalis*, *B. paniculata*, *B. brachystachya*, *B. crispa*, *B. curviflora*, *B. yunnanensis*, *B. japonica*, and *B. lindleyana* were also diploid (Figs 4–11). *B. davidii*, a species distributed widely in China and recognized as an invasive plant in Western countries (Zhang *et al.*, 1993), had a tetraploid number of $2n = 76$ (Fig. 12). *B. fallowiana* and *B. myriantha* were also tetraploid (Figs 13, 14). The chromosome number of $2n = 114$ (hexaploid) in *B. forrestii* (Fig. 15) was found to be the same as in a previous study by Moore (1947). However, as a morphologically variable species, *B. macrostachya* was found to be hexaploid or dodecaploid, with $2n = 114$ or 228 (Figs 16–20). The range of morphological variation in different populations is detailed in Table 3.

DISCUSSION

PLOIDY VARIATION OF *BUDDLEJA* IN THE SINO-HIMALAYAN REGION

Polyploidy has long been recognized as a prominent force in evolutionary diversification, and is one of the most important cytogenetic mechanisms in plant evolution and rapid speciation (Stebbins, 1971; Grant, 1981; Masterson, 1994; Levin, 2002). The capacity of angiosperms to form new combinations via polyploidy may help to explain the rapid diversification and high incidence of endemism in a given region (Levin, 2002). Alpine and arctic floras have been reported to have high frequencies of polyploidy (Hanelt, 1966; Löve &

Löve, 1975; Ohba, 1988; Abbott & Brochmann, 2003; Brochmann *et al.*, 2004). Thus, extreme environments in areas of high latitude or altitude have been suggested to increase the frequency of polyploidy (Stebbins, 1985; Thompson & Lumaret, 1992; Otto & Whitton, 2000; Levin, 2002). It is well known that extensive areas of the Sino-Himalayan region are alpine and that the Tertiary Himalayan orogeny has led to changes in climate and topography (Li *et al.*, 1995; Shi *et al.*, 1998). Therefore, the uplifting and advance and retreat of ice sheets and glaciers may have provided opportunities for polyploidy development through interspecies hybridization or other evolutionary mechanisms in the contact zones. Our study has indicated that almost 85% of the Asian *Buddleja* species occur in the Sino-Himalayan region, with ten of the 18 species in this region being polyploid (Tables 1, 2; Fig. 21). Moreover, these ten species present a wide range of ploidy levels ($4x$, $6x$, $8x$, $12x$, $16x$, and $24x$). Therefore, we consider that the high levels of speciation occurring in *Buddleja* in this area may be attributed to polyploidization, at least in part. From an analysis of the cytological data of Norman (2000), it was found that most polyploids of *Buddleja* in the New World are distributed along the Andes and adjacent regions. All of the above observations indicate that the polyploidization of *Buddleja* in South-east Asia is associated with the uplift of the Himalayan Mountains, with the Himalayan orogeny and Cenozoic ice-age cycles playing an important role in the evolution of polyploidy in the genus.

CHROMOSOME EVOLUTION OF *BUDDLEJA* IN ASIA

Chromosome data are usually used to discuss current distribution patterns and centres of variation and fre-

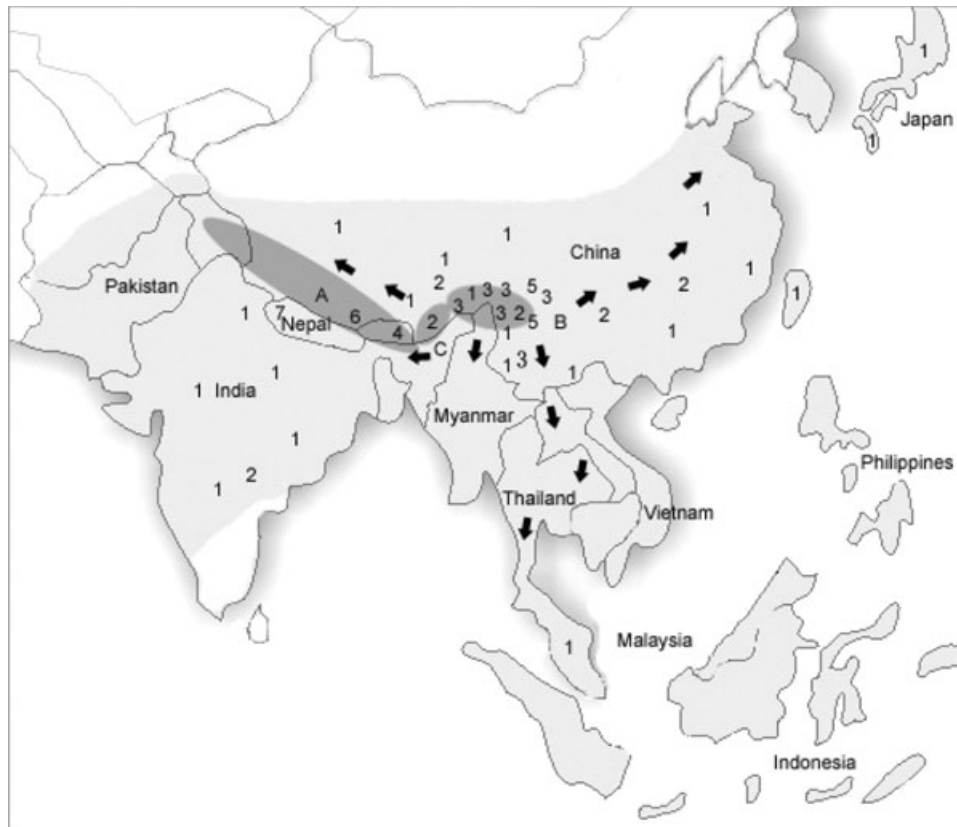


Figure 21. Distribution of *Buddleja* in Asia (light shade), modified from Leeuwenberg (1979) and Li & Leeuwenberg (1996). The total region of the three ellipses (A, B, C) represents the approximate range of the Sino-Himalayan region. Numbers 1–7 represent different ploidy levels of *Buddleja* and their localities. 1, $2n = 2x = 38$; 2, $2n = 4x = 76$; 3, $2n = 6x = 114$; 4, $2n = 8x = 152$; 5, $2n = 12x = 228$; 6, $2n = 16x = 304$; 7, $2n = 24x = 456$. Arrows indicate probable dispersal routes.

quency (Moore, 1947; Hong, 1990). Moore (1947, 1960) and Gadella (1980) found that Asian species of *Buddleja* had higher levels of ploidy and more polyploids than New World or African species, with an extreme ploidy level, $24x$, observed in a population of *B. colvilei*. As we know, most polyploids ultimately come from diploids (Stebbins, 1971; Raven, 1975; Hong, 1990; Stace, 2000). The diploid species of *Buddleja* have a relatively wide range of natural distribution. *B. asiatica* extends through India, Pakistan, Afghanistan, and China to the whole of South-east Asia, including the Philippines and other islands. *B. crispa* occurs from Afghanistan, through Kashmir, Nepal, and Tibet to Yunnan. *B. alternifolia*, a probable relict species with alternate leaves (other species in *Buddleja* have opposite leaves), is native to Tibet and Qinghai of China adjacent to the Sino-Himalayan region. *B. paniculata* occurs in north India, Nepal, Burma, and south-west China, and *B. officinalis* is found in south-west and central China. *B. brachystachya* and *B. yunnanensis* are restricted to Yunnan, China. Morphologically, *B. bhutanica* is very similar to

B. asiatica, except for its connate-perfoliate leaves, but it exists only in Bhutan. We have not yet examined the chromosome number of *B. bhutanica*, but we presume that it is also diploid. Because of their flower characters (Moore, 1960) and low ploidy level, all of the above eight diploid species are considered to be the ancestors of the Asian taxa, originating from ancestral species during the uplift of the Himalayan Mountains. Three other diploid species with curved flowers, *B. japonica*, *B. curviflora*, and *B. lindlejana*, occur in south Japan, Taiwan, and east China, respectively, and may be derived taxa (Li, 1982; Li & Leeuwenberg, 1996). This point has also been supported by the karyotype analysis of some diploid taxa (Chen & Sun, 2006). Although the evolution of these three taxa may not have been accompanied by polyploidy, structural intrachromosomal variation may have occurred at the same diploid level.

From the above discussion, it can be seen that both primitive taxa with lower ploidy levels and advanced species with higher ploidy levels are distributed in the Sino-Himalayan region. We believe that the region

may be the second variation centre of the genus *Buddleja* in Asia. From there, the genus may have spread via the following four pathways (Fig. 21): (1) southwards, generating a hexaploid and a 12-ploid of *B. macrostachya* in south Yunnan or Myanmar, Laos, and Thailand; (2) south-eastwards to Malaysia, Indonesia, and the Philippines, where *B. asiatica* is distributed; (3) eastwards, generating the tetraploid *B. davidii* and three derived diploid species in east China and Japan; (4) north-westwards, developing several diploids distributed in Pakistan and Afghanistan. Eight other polyploid species of *Buddleja* exist in restricted areas in the Sino-Himalayan and adjacent regions. This narrow distribution pattern could imply that they are still in a youthful developmental stage, spreading only a little beyond the original centre.

In summary, the differentiation of *Buddleja* possibly happened during the uplift of the Himalayan Mountains, which may have played a major role in the polyploid evolution of the genus. The polyploids in this area probably did not migrate from other regions, but developed there.

Buddleja macrostachya is a widely distributed polyploid species in the genus and presents a series of morphological variations in different populations (Table 3). As discussed above, its chromosome numbers ($2n = 6x$ or $12x$) and its interphase nucleus type (complex chromocentre) (Fig. 2) are unique when compared with the 13 other examined species, which are all of the smooth-faced round prochromosome type (Fig. 1). Li & Zhang (1991) indicated that the complex chromocentre type could mean that this species is in a process of rapid divergence. We consider that *B. macrostachya* may still be in a youthful developmental stage, and that its wide adaptability and variation may represent a rapid evolutionary phase.

The great range of ploidy levels found in the genus *Buddleja* makes it an ideal model for the investigation of polyploidization and speciation. Molecular markers have been applied to identify the nature of polyploid speciation in other taxa (Soltis & Soltis, 2000; Wendel, 2000), and similar work is required to clarify the mechanism of polyploidization in this genus. In addition, in order to test the relationship between the diversity of flower (tubular, campanulate, salverform, or funnellform corolla) and pollination mechanisms, to explain the wide geographical adaptation, and to measure accurately the origin and disjunction time of the species in the genus, further field observations and molecular clock data are certainly needed.

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