Karyomorphology and Its Evolution in Dipterocarpaceae (Malvales)

Kazuo Oginuma^{1*}, Shawn Y. K. Lum² and Hiroshi Tobe³

¹The Community Center for the Advancement of Education and Research at the University of Kochi,
5–15 Eikokuji-cho, Kochi 780–8515, Japan

²Asian School of the Environment, Nanyang Technological University, Singapore 639798

³Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606–8502, Japan

Received January 16, 2020; accepted February 9, 2020

Summary Previous chromosome information is restricted to Dipterocarpoideae, one of the two subfamilies of Dipterocarpaceae, and no chromosome information is available for another subfamily Monotoideae. Here we present the first karyomorphology of *Marquesia macroura* ($2n=22$) (Monotoideae), as well as of four species ($2n=22$) of four genera in tribe Dipterocarpeae and five species ($2n=14$) of tribe Shoreae in Dipterocarpoideae. Comparisons within Dipterocarpaceae and with Sarcolaenaceae ($2n=22$) sister to Dipterocarpaceae in the light of phylogenetic relationships show that the basic chromosome number $x=11$ is plesiomorphic and $x=7$ apomorphic in Dipterocarpaceae. Based on available information, tribe Shoreae ($x=7$) has a uniform karyotype where all chromosomes have a centromere at median position, while the rest of the family ($x=11$) have a diverse karyotype in terms of the frequency of chromosomes with a centromere at median, submedian and subterminal position. We discussed the meaning of lability of karyotype in chromosome evolution.

Keywords Basic chromosome number, Chromosome evolution, Dipterocarpaceae, Karyomorphology.

Dipterocarpaceae (Malvales) are a family of 16 genera and 680 species distributed in tropical regions of the Old World, especially in the rain forests of Malesia (Takhtajan 1986, Stevens 2001 onward: <http://www.mobot.org/MOBOT/research/APweb/>). Until recently, the family had been considered comprising three subfamilies Monotoideae, Pakaraimoideae, and Dipterocarpoideae (Maguire and Ashton 1977, Ashton 1982, 2003, Mabberley 2008), but recent molecular studies showed that *Pakaraimaea* (Pakaraimoideae) is placed near Cistaceae rather than near Dipterocarpoideae (Ducousso *et al.* 2004, Heckenhauer *et al.* 2017). Currently, APG IV (The Angiosperm Phylogeny Group 2016) accepts that Dipterocarpaceae comprises two subfamilies alone, *i.e.*, Monotoideae (three genera) and Dipterocarpoideae (13 genera), with Sarcolaenaceae as a sister group.

A larger subfamily Dipterocarpoideae is further subdivided into two tribes Dipterocarpeae (*Anisoptera*, *Cotylelobium*, *Dipterocarpus*, *Stemonoporus*, *Upuna*, *Vateria*, *Vateriopsis*, and *Vatica*) and Shoreae (*Dryobalanops*, *Hopea*, *Neobalanocarpus*, *Parashorea*, and *Shorea*) (Ashton 1982). As reviewed by Ashton (1982) and Heckenhauer *et al.* (2017), previous chromosome information on Dipterocarpaceae is restricted to ten genera of Dipterocarpoideae. Four genera *Anisoptera*, *Dipterocarpus*, *Upuna*, and *Vatica* of tribe Dipterocarpeae have $x=11$; *Vateria* of tribe Dipterocarpeae has

$x=10$, and five genera *Dryobalanops*, *Hopea*, *Neobalanocarpus*, *Parashorea* and *Shorea* of tribe Shoreae all have $x=7$. Ashton (1982) considered that the basic chromosome number of Dipterocarpeae is (probably) $x=11$, and that of Shoreae (probably) $x=7$. However, molecular phylogenetic analyses revealed that tribe Dipterocarpeae is polyphyletic with *Dipterocarpus* (Dipterocarpeae) (with $x=11$) sister to tribe Shoreae (Kajita *et al.* 1998, Gamage *et al.* 2006, Heckenhauer *et al.* 2017). In the light of such relationships, Kajita *et al.* (1998) discussed that the basic chromosome number changed from $x=11$ to $x=7$ after *Dipterocarpus* branched in a common clade with tribe Shoreae. In contrast, Bawa (1998) considered that $x=11$ may have been derived from $x=7$ through allopolyploidy, because several species in the genera with $x=7$ as the basic number have a somatic chromosome number of $2n=20$, 21 and 22.

Most of the previous chromosome studies were made by enumerating chromosome numbers alone without micrographs of the chromosomes. Exceptionally, Oginuma *et al.* (1998a) reported karyomorphology of *Anisoptera thurifera* Blume using a micrograph, and Heckenhauer *et al.* (2017) reported karyotypes of six species of four genera *Dipterocarpus*, *Hopea*, *Shorea*, and *Vatica*. According to Heckenhauer *et al.* (2017), “karyotypes were similar and symmetrical for all with small metacentric, submetacentric or subtelocentric chromosomes in all analyzed species in Dipterocarpaceae, which makes no identification of individual chromosome pairs difficult.” However, we have found a certain diversity in karyo-

* Corresponding author, e-mail: oginuma_kazuo@yahoo.co.jp
DOI: 10.1508/cytologia.85.141

Table 1. Studied taxa and their collection data of Dipterocarpaceae.

Taxon	Collection
Subfamily Monotoideae	
<i>Marquesia macroura</i> Gilg	Seeds from Zambia
Subfamily Dipterocarpoideae	
Tribe Dipterocarpeae	
<i>Anisoptera megistocarpa</i> V. Sl.	Cultivated, Singapore Bot. Gard.
<i>Dipterocarpus oblongifolius</i> Blume	Cultivated, Singapore Bot. Gard.
<i>Vateria indica</i> L.	Sri Lanka, Sinharaja Forest Reserve
<i>Vatica pauciflora</i> (Korth.) Bl.	Cultivated, Singapore Bot. Gard.
Tribe Shoreae	
<i>Dryobalanops aromatica</i> Gaertn. f.	Cultivated, Singapore Bot. Gard.
<i>Hopea odorata</i> Roxb.	Cultivated, Singapore Bot. Gard.
<i>H. sangal</i> Korth.	Cultivated, Singapore Bot. Gard.
<i>Neobalanocarpus heimii</i> (Korth.) Ashton	Malaysia, Johor Bahru, Panti, Mt. Gunung
<i>Shorea bracteolata</i> Dyer	Cultivated, Singapore Bot. Gard.
<i>S. macroptera</i> Dyer	Cultivated, Singapore Bot. Gard.

Fig. 1. Somatic chromosomes at metaphase of *Marquesia macroura* in Monotoideae of Dipterocarpaceae. (b) is a drawing of (a). Arrows indicate chromosomes with centromeres at subterminal position. Arrowheads indicate submetacentric chromosomes with a satellite. Scale bar=2 μ m.

morphology throughout the family.

In this paper, we provide the first report of the karyotype of subfamily Monotoideae using *Marquesia macroura*. We further report karyomorphology of four species from four genera of Dipterocarpeae and six species of four genera of Shoreae in subfamily Dipterocarpoideae. Based on the results, we will discuss chromosome evolution and the meaning of karyomorphology diversity in Dipterocarpaceae.

Materials and methods

The Dipterocarpaceae species investigated are presented in Table 1 along with collection data. Somatic chromosomes were examined using at least three to five cells of young leaves or root tips collected from wild-growing and cultivated plants. Methods of pretreatment, fixation, and staining for chromosome observations are described elsewhere (leaves: Oginuma *et al.* 1992, root tips: Oginuma and Nakata 1988). Categories of chromosome morphology on the basis of the position of centromeres followed Levan *et al.* (1964).

Results

Monotoideae

Marquesia macroura was studied for the first time. The variation of chromosome length is gradual; the longest chromosome is about 1.6 μ m, and the shortest about 0.9 μ m. Among the 22 chromosomes, 16 have centromeres at median position, two have submedian centromeres, and four have subterminal centromeres. Satellite chromosomes are observed at the proximal region of the short arms of two submedian chromosomes (Fig. 1a, b). The karyotype of the species is $2n=16m+2sm(sat)+4st$.

Dipterocarpoideae, Dipterocarpeae

Anisoptera megistocarpa was studied for the first time. Chromosomes at mitotic metaphase are $2n=22$. The variation of chromosome length is gradual; the longest chromosome is about 1.6 μ m, and the shortest about 1.1 μ m. Among the 22 chromosomes, 20 have median centromeres, and two have subterminal centromeres. Satellite chromosomes were not observed (Fig. 2a, b). The karyotype of $2n=20m+2st$ resembles that of *A. thurifera* reported earlier (Oginuma *et al.* 1998a).

Chromosomes at mitotic metaphase of *Dipterocarpus oblongifolius* are $2n=22$ as in previous reports (Somego

Fig. 2. Somatic chromosomes at metaphase of four genera in Dipterocarpeae of Dipterocarpoideae. (a) and (b): *Anisoptera megistocarpa* ($2n=22$). (c) and (d): *Dipterocarpus oblongifolius* ($2n=22$). (e) and (f): *Vateria indica* ($2n=22$). (g) and (h): *Vatica pauciflora* ($2n=22$). (b), (d), (f) and (h) are respective drawings of (a), (c), (e) and (g). Arrows indicate chromosomes with a centromere at subterminal position. Arrowheads indicate chromosomes with a centromere at submedian position. Arrowheads in (d) indicate submetacentric chromosomes with a satellite. Scale bar = $2\mu\text{m}$.

1978, Kaur *et al.* 1986). The variation of chromosome length is gradual: the longest chromosome is about $1.8\mu\text{m}$, and the shortest about $0.9\mu\text{m}$. Among 22 chromosomes, 20 have median centromeres, and two have submedian centromeres. Satellite chromosomes are observed in the interstitial region of the short arms in two submedian chromosomes (Fig. 2c, d). The karyotype of the species is $2n=20m+2sm$ (sat).

Chromosomes at mitotic metaphase of *Vateria indica*

are $2n=22$, a new count for the species in contrast to $n=10$ reported earlier (Mehra 1976). The variation of chromosome length is gradual; the longest chromosome is about $1.4\mu\text{m}$, and the shortest about $0.9\mu\text{m}$. All 22 chromosomes have centromeres at median position. Satellite chromosomes are not observed (Fig. 2e, f). The karyotype of the species is $2n=22m$.

Chromosomes at mitotic metaphase of *Vatica pauciflora* are $2n=22$ as reported earlier (Jong and Lethbridge

Fig. 3. Somatic chromosomes at metaphase of four genera in *Shorea* of Dipterocarpoideae. (a) and (b): *Dryobalanops aromatica* ($2n=14$). (c) and (d): *Hopea sangal* ($2n=14$). (e) and (f): *Neobalanocarpus heimii* ($2n=14$). (g) and (h): *Shorea bracteolata* ($2n=14$). (b), (d), (f) and (h) are respective drawings of (a), (c), (e) and (g). Scale bar= $2\mu\text{m}$.

1967, Somego 1978). The variation of chromosome length is gradual: the longest is about $2.0\mu\text{m}$, the shortest about $1.3\mu\text{m}$. Among 22 chromosomes, 16 have median centromeres, four have submedian centromeres, and two have subterminal centromeres. Satellites are not observed (Fig. 2g, h). The karyotype of the species is $2n=16m+4sm+2st$.

Dipterocarpoideae, Shoreae

Chromosomes at mitotic metaphase of *Dryobalanops aromatica* are $2n=14$ as reported earlier (Jong and Leithbridge 1967, Somego 1978). The variation of chromosome length is gradual; the longest chromosome is about $1.6\mu\text{m}$, and the shortest about $1.1\mu\text{m}$. All 14 chromosomes have median centromeres. Satellite chromosomes are not observed (Fig. 3a, b). The karyotype of the spe-

cies is $2n=14m$.

Two species, *Hopea odorata* and *H. sangal*, are studied. Chromosomes at mitotic metaphase of *H. odorata* are $2n=21$ as reported earlier (Somego 1978, Kaur *et al.* 1986), and $2n=14$ for *H. sangal* as reported earlier (Somego 1978). In *H. sangal*, the chromosome variation is gradual; the longest chromosome is about $1.8\mu\text{m}$, and the shortest about $1.2\mu\text{m}$. All 14 chromosomes have median centromeres. Satellite chromosomes were not observed (Fig. 3c, d). The karyotype of *H. sangal* is $2n=14m$.

Chromosomes of mitotic metaphase of *Neobalanocarpus heimii* are $2n=14$ as reported earlier (Somego 1978). The variation of chromosome length is gradual; the longest chromosome is about $1.7\mu\text{m}$, and the shortest about $1.1\mu\text{m}$. All 14 chromosomes have median centromeres.

Table 2. Chromosome data in Dipterocarpaceae.

Subfamily	Tribe	Species	<i>n</i>	<i>2n</i>	Reference
Monotoideae		<i>Marquesia macroura</i> Gilg		22	Present study
Dipterocarpoideae	Dipterocarpeae	<i>Anisoptera costata</i> Korth. (as <i>A. cochinchinensis</i> Pierre ex Laness.)		20	Tixier (1953)
		<i>A. laevis</i> Rindl.		22	Jong and Lethbridge (1967), Somego (1978)
		<i>A. megistocarpa</i> V. Sl.		22	Present study
		<i>A. scaphula</i> (Roxb.) Kurz (as <i>A. glabra</i> Kurz)		20	Tixier (1960)
				22	Somego (1978)
		<i>A. thurifera</i> Blume		22	Oginuma <i>et al.</i> (1998a)
		<i>Dipterocarpus alatus</i> Roxb.		20	Tixier (1953)
			11	22	Roy and Jha (1965)
		<i>D. artocarpifolius</i> Pierre		20	Tixier (1960)
		<i>D. baudii</i> Korth.		22	Roy and Jha (1965), Jong and Lethbridge (1967)
		<i>D. cornutus</i>		22	Somego (1978)
		<i>D. costatus</i> Gaertn. f. (as <i>D. artocarpifolius</i> Pierre ex Laness)		20	Tixier (1960)
		<i>D. elongatus</i> Korth. (as <i>D. warburgii</i> Brandis)		20	Pancho (1971)
		<i>D. intricatus</i> Dyer		20	Tixier (1953)
		<i>D. kunstleri</i> King (as <i>D. speciosus</i> Brandis)		20	Pancho (1971)
		<i>D. oblongifolius</i> Blume		22	Somego (1978), Kaur <i>et al.</i> (1986), Present study
		<i>D. obtusifolius</i> Teysm. Ex Miq.		20	Tixier (1953)
		<i>D. sarawakensis</i> Sloot.		22	Roy and Jha (1965), Jong and Lethbridge (1967)
		<i>D. tuberculatus</i> Roxb.		20, 30	Tixier (1960)
		<i>D. turbinatus</i> Gaertn. f.		20	Tixier (1960)
		<i>D. zeylanica</i> Thwaites		22	Heckenhauer <i>et al.</i> (2017)
		<i>Upna. borneensis</i> Sym.		22	Somego (1978)
		<i>Vateria indica</i> L.	10	22	Mehra (1976)
				22	Present study
		<i>Vatica cinerea</i> Dyer		22	Somego (1978)
		<i>V. endertii</i> Slooten		22	Heckenhauer <i>et al.</i> (2017)
		<i>V. odorata</i> (Griff.) Sym. (as <i>V. grandiflora</i> Dyer)	11	22	Roy and Jha (1965)
				22	Roy and Jha (1965), Somego (1978)
		<i>V. papana</i> Dyer		22	Jong and Lethbridge (1967)
		<i>V. pauciflora</i> (Korth.) Ashton (as <i>V. walichii</i> Dyer)	11	22	Jong and Lethbridge (1967)
				22	Present study
		<i>V. stapfiana</i> (King) V. Sl.		22	Jong and Lethbridge (1967)
	Shoreae	<i>Dryobalanops aromatica</i> Gaertn. f.		14	Jong and Lethbridge (1967), Somego (1978), Present study
		<i>D. oblongifolia</i> Dyer		14	Jong and Lethbridge (1967), Somego (1978)
		<i>Hopea beccariana</i> Burck		20, 21, 22	Somego (1978)
		<i>H. glabra</i> W. & A. (as <i>H. wightiana</i> Wall ex W. & A.)		14	Jong and Lethbridge (1967)
		<i>H. jucunda</i> Thwaites		21	Heckenhauer <i>et al.</i> (2017)
		<i>H. latifolia</i> Sym. (as <i>H. beccariana</i> Burck)		21	Jong and Kaur (1979)
		<i>H. nervosa</i> King		14	Somego (1978), Kaur <i>et al.</i> (1986)
		<i>H. nutans</i> Ridl.	14	28	Jong and Lethbridge (1967) Somego (1978)
		<i>H. odorata</i> Roxb.	7	14	Roy and Jha (1965), Sarkar <i>et al.</i> (1982)
				20	Roy and Jha (1965)
				20	Tixier (1953)
			20, 21, 22	21	Somego (1978), Kaur <i>et al.</i> (1986) Present study
		<i>H. pubescens</i> Ridl.	c.7		Jong and Lethbridge (1967)
		<i>H. sangal</i> Korth.		14	Somego (1978), Present study
		<i>H. subalata</i> Sym.		21	Jong and Kaur (1979), Kaur <i>et al.</i> (1986)
			20, 21, 22	14	Somego (1978)
		<i>Neobalanocarpus heimii</i> (Korth.) Ashton		14	Jong and Lethbridge (1967), Somego (1978), Present study
		<i>Parashorea densiflora</i> Sym.		14	Somego (1978)

Table 2. Continued.

Subfamily	Tribe	Species	<i>n</i>	<i>2n</i>	Reference
Dipterocarpoideae	Shoreae	<i>Shorea acuminata</i> Dyer		14	Somego (1978), Kaur <i>et al.</i> (1986)
		<i>S. agami</i> Ashton		14	Kaur <i>et al.</i> (1986)
		<i>S. argentifolia</i> Sym.		14	Somego (1978), Kaur <i>et al.</i> (1986)
		<i>S. assamica</i> Dyer		14	Somego (1978), Present study
		<i>S. bracteolata</i> Dyer		14	Somego (1978)
		<i>S. contorta</i> Vidal		14	Kaur <i>et al.</i> (1986)
		<i>S. curtisii</i> Dyer ex King		14	Jong and Lethbridge (1967), Somego (1978)
		<i>S. gardneri</i> (Thw.) Ashton		14	Jong and Kaur (1979)
		<i>S. glauca</i> King		14	Somego (1978)
		<i>S. guiso</i> (Blco.) Bl.		14	Somego (1978)
		<i>S. hypochroa</i> Hance		14	Somego (1978)
		<i>S. lepidota</i> Miq.		14	Kaur <i>et al.</i> (1986)
		<i>S. leprosula</i> Miq.		14	Jong and Lethbridge (1967), Somego (1978)
		<i>S. macrophylla</i> (de Vr.) Ashton		14	Jong and Kaur (1979), Kaur <i>et al.</i> (1986)
		<i>S. macroptera</i> Dyer		14	Somego (1978), Kaur <i>et al.</i> (1986), Present study
		<i>S. maxwellana</i> King		14	Somego (1978)
		<i>S. mecistopteryx</i> Ridl.		14	Somego (1978)
		<i>S. megistophylla</i> Ashton		14	Heckenhauer <i>et al.</i> (2017)
		<i>S. multiflora</i> (Burck) Sym.		14	Somego (1978), Kaur <i>et al.</i> (1986)
		<i>S. oblongifolia</i> Thwaites		14	Heckenhauer <i>et al.</i> (2017)
		<i>S. obtusa</i> Wall		20	Tixier (1960)
		<i>S. ovalis</i> (Korth.) Bl.	14		Jong and Lethbridge (1967)
				21	Jong and Kaur (1979)
				28	Jong and Lethbridge (1967), Somego (1978), Kaur <i>et al.</i> (1986)
		<i>S. palembanica</i> Miq.		14	Somego (1978)
		<i>S. parvifolia</i> Dyer		14	Somego (1978)
		<i>S. pauciflora</i> King		14	Jong and Lethbridge (1967), Somego (1978), Kaur <i>et al.</i> (1986)
		<i>S. pinanga</i> Scheff. (as <i>S. compress</i> Burck)		12	Tixier (1960)
				14	Nanda (1962), Roy and Jha (1965), Kaur <i>et al.</i> (1986)
				21	Jong and Kaur (1979)
		<i>S. platyclados</i> Sloonten ex Foxw.		14	Somego (1978), Kaur <i>et al.</i> (1986)
		<i>S. resinosa</i> Foxw.		14	Jong and Kaur (1979)
				21	Kaur <i>et al.</i> (1986)
		<i>S. robustus</i> Gaertn. f.	7		Roy and Jha (1965), Mehra (1976), Mehra and Sareen (1969, 1973), Singhal and Gill (1984)
				14	Roy and Jha (1965), Pal <i>et al.</i> (1993)
		<i>S. roxburghii</i> G. Don		14	Heckenhauer <i>et al.</i> (2017)
		<i>S. siamensis</i> Miq. (as <i>Pentacme siamensis</i> (Miq.) DC.)		12	Tixier (1953)
		<i>S. singkang</i> (Miq.) Burck	7		Jong and Lethbridge (1967)
		<i>S. smithiana</i> Sym.	7		Jong and Lethbridge (1967)
		<i>S. splendida</i> (de Vries) Ashton		14	Kaur <i>et al.</i> (1986)
		<i>S. splendida</i> (de Vr.) Ashton (as <i>S. martiniana</i> Scheff.)		14	Somego (1978)
		<i>S. stenoptera</i> Burck		14	Jong and Kaur (1979), Kaur <i>et al.</i> (1986)
		<i>S. sumatrana</i> (Sl. ex Thor.) Sym.	7		Jong and Lethbridge (1967)
		<i>S. talura</i> Roxb.	7	14	Roy and Jha (1965)
		<i>S. trapezifolia</i> Ashton		14	Jong and Kaur (1979), Kaur <i>et al.</i> (1986)

Satellite chromosomes are not observed (Fig. 3e, f). The karyotype of the species is $2n=14m$.

Two species, *Shorea bracteolata*, and *S. macroptera*, were studied. Chromosomes at mitotic metaphase of *S. bracteolata* are $2n=14$ as reported earlier (Somego 1978), and $2n=14$ of *S. macroptera* as reported earlier (Somego 1978, Kaur *et al.* 1986). In *S. bracteolata*, the

variation of chromosome length is gradual; the longest chromosome is about $1.8\mu m$, and the shortest about $1.1\mu m$. All 14 chromosomes have median centromeres. Satellite chromosomes are not observed (Fig. 3g, h). The karyotype of the species is $2n=14m$. The karyotype of *S. macroptera* resembled that of *S. bracteolata*, and is $2n=14m$.

Discussion

By adding the results of the present study, we have updated chromosome information of Dipterocarpaceae (Table 2). Important information we have added include $2n=22$ of *Marquesia* (Monotoideae) as well as of *Vateria* (Dipterocarpoideae, Dipterocarpeae). By means of our study, we could show that, like Dipterocarpoideae, another subfamily Monotoideae also have $x=11$. As for *Vateria*, $n=10$ of *V. indica* was the only chromosome count (Mehra 1976), although Ashton (1982) described “The diploid numbers of one species of *Stenmonoporus*, and two of *Vateria*, all $2n = 22$ ” (see also Heckenhauer *et al.* 2017). In the present study, we explicitly showed using micrograph that *Vateria indica* has $2n=22$. Therefore, the genera (*Anisoptera*, *Dipterocarpus*, *Upuna*, *Vateria*, and *Vatica*) investigated for chromosome counts of tribe Dipterocarpeae all have $x=11$. A few species of tribe Dipterocarpeae are reported by the same authors (Tixier 1953, 1960, Pancho 1971) to have $2n=20$ (Table 2). They now need reconfirmation.

Evolution of karyotypes in Dipterocarpaceae

As stated in the Introduction, two contrasting hypotheses have existed about chromosome evolution in Dipterocarpaceae, one from $x=7$ to $x=11$ (Bawa 1998), and the other from $x=11$ to $x=7$ (Kajita *et al.* 1998). As in Kajita *et al.* (1998), almost all of molecular phylogenetic studies have indicated that *Dipterocarpus* ($x=11$) forms a common clade with tribe Shoreae (with $x=7$) rather than with the rest of tribe Dipterocarpeae (with $x=11$) (Tsumura *et al.* 1996, Ducousso *et al.* 2004, Gamage *et al.* 2006, Heckenhaur *et al.* 2017). Our study showed that *Marquesia macroura* (Monotoideae) has $2n=22$, a fact suggesting that another subfamily Monotoideae likely has $x=11$ as in Dipterocarpeae. Also, Sarcolaenaceae, which are considered sister to Dipterocarpaceae (Ducousso *et al.* 2004, Heckenhauer *et al.* 2017), also have $x=11$ because both *Leptolaena* and *Sarcolaena* were reported to have $2n=22$ (Goldblatt and Dorr 1986). A distribution of $x=7$ and 11 on the phylogenetic tree shows that $x=11$ is plesiomorphic with $x=7$ apomorphic in Dipterocarpaceae, and that the basic chromosome

Fig. 4. Evolution of basic chromosome numbers in Dipterocarpaceae. Phylogenetic tree is modified from Heckenhauer *et al.* (2017). An arrow indicates an evolution of $x=7$ from $x=11$. Chromosome data of Bixaceae and Cistaceae are from Orunduff (1967, 1968), Moore (1973, 1974, 1977), Fedorov (1974), Goldblatt (1981, 1984, 1985, 1988), and Goldblatt and Johnson (1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006, 2010).

Table 3. A summary of karyotype of Dipterocarpaceae.

Subfamily/Tribe/Genus	2n (x)	Chromosome morphology ¹⁾		
		m	sm	st
Monotoideae				
<i>Marquesia</i>	22 (11)	16 (73%)	2 (9%) ²⁾	4 (18%)
Dipterocarpoideae				
Dipterocarpeae				
<i>Anisoptera</i>	22 (11)	20 (91%)	0 (0%)	2 (9%)
<i>Dipterocarpus</i>	22 (11)	20 (91%)	2 (9%) ²⁾	0 (0%)
<i>Vateria</i>	22 (11)	22 (100%)	0 (0%)	0 (0%)
<i>Vatica</i>	22 (11)	16 (73%)	4 (18%)	2 (9%)
Shoreae				
<i>Dryobalanops</i>	14 (7)	14 (100%)	0 (0%)	0 (0%)
<i>Hopea</i>	14 (7)	14 (100%)	0 (0%)	0 (0%)
<i>Neobalanocarpus</i>	14 (7)	14 (100%)	0 (0%)	0 (0%)
<i>Shorea</i>	14 (7)	14 (100%)	0 (0%)	0 (0%)

¹⁾ Abbreviations; m, chromosomes with a centromere at median position; sm, chromosomes with a centromere at submedian position; st, chromosomes with a centromere at subterminal position. ²⁾ Chromosomes with a satellite.

number evolved from $x=11$ to $x=7$ in tribe Shoreae within the family (Fig. 4).

Evolution toward a markedly discontinuous basic chromosome number as in Dipterocarpaceae is not rare. For example, in Hamamelidaceae $x=8$ and $x=12$ are known (Oginuma and Tobe 1991); likewise, $x=8$, $x=11$, $x=14$ are known in Betulaceae (Oginuma *et al.* 1998b), and $x=7$, $x=9$, $x=12$ in Stemonaceae (Oginuma *et al.* 2001).

In the present study, we further showed that diversity exists in karyotype throughout Dipterocarpaceae (for a summary see Table 3). The karyotype of tribe Shoreae ($2n=14$) is very uniform, consistently comprising seven pairs of chromosomes with a centromere at median position. In contrast, the karyotypes of tribe Dipterocarpeae ($2n=22$) are diverse in the frequency of chromosomes with a centromere at median, submedian, and subterminal position. Except in *Vateria*, one pair of chromosomes has a centromere at the submedian and/or subterminal position in tribe Dipterocarpeae, *i.e.*, *Anisoptera*, *Dipterocarpus*, and *Vatica*. This is also the case of *Marquesia*, in which one pair of chromosome has a centromere at submedian position, and another two pairs have a centromere at subterminal position. As far as based on data available from nine genera of Dipterocarpaceae, lability of karyotype is plesiomorphic in the family. The uniform karyotype of $x=7$ as in tribe Shoreae may have been derived from $x=11$ showing a labile karyotype by chromosome fusion.

References

- Ashton, P. S. 1982. Dipterocarpaceae. In: Van Steenis C. G. G. J. (ed.). Flora Malesiana. Series I, Spermatophyta. Flowering Plants, vol. 9, part 2, Martinus Nijhoff Pub., Leiden. pp. 237–552.
- Ashton, P. S. 2003. Dipterocarpaceae. In: Kubitzki, K. and Bayer, C. (eds.). The Families and Genera of Vascular Plants. Vol. 5. Flowering Plants Dicotyledons. Malvales, Caprales, Non-betain Caryophyllales. Springer, Berlin. pp. 182–197.
- Bawa, K. S. 1998. Conservation of Genetic Resources in the Dipterocarpaceae. In: Appanah, S. and Turnbull, J. M. (eds.). A Review of Dipterocarps, Taxonomy, Ecology and Silviculture. Center for Forest Research Institute, Bogor. pp. 45–56.
- Ducousso, M., Béna, G., Bourgeois, C., Buyck, B., Eyssartier, G., Vincelette, M., Rabevoitra, R., Randrihasipara, L., Dreyfus, B. and Prin, Y. 2004. The last common ancestor of Sarcolaenaceae and Asian dipterocarp trees was ectomycorrhizal before the India–Madagascar separation, about 88 million years ago. *Mol. Ecol.* **13**: 231–236.
- Fedorov, A. A. 1974. Chromosome Numbers of Flowering Plants. Otto Koeltz, Koenigstein.
- Gamage, D. T., de Silva, M. P., Inomata, N., Yamazaki, T. and Szmidt, A. E. 2006. Comprehensive molecular phylogeny of the sub-family Dipterocarpoideae (Dipterocarpaceae) based on chloroplast DNA sequences. *Genes Genet. Syst.* **81**: 1–12.
- Goldblatt, P. 1981. Index to Plant Chromosome Numbers 1975–1978. *Monogr. Syst. Bot. Mo. Bot. Gard.* **5**: 1–553.
- Goldblatt, P. 1984. Index to Plant Chromosome Numbers 1979–1981. *Monogr. Syst. Bot. Mo. Bot. Gard.* **8**: 1–427.
- Goldblatt, P. 1985. Index to Plant Chromosome Numbers 1982–1983. *Monogr. Syst. Bot. Mo. Bot. Gard.* **13**: 1–224.
- Goldblatt, P. 1988. Index to Plant Chromosome Numbers 1984–1985. *Monogr. Syst. Bot. Mo. Bot. Gard.* **23**: 1–264.
- Goldblatt, P. and Dorr, L. J. 1986. Chromosome number in Sarcolaenaceae. *Ann. Mo. Bot. Gard.* **73**: 828–829.
- Goldblatt, P. and Johnson, D. E. 1990. Index to Plant Chromosome Numbers 1986–1987. *Monogr. Syst. Bot. Mo. Bot. Gard.* **30**: 1–243.
- Goldblatt, P. and Johnson, D. E. 1991. Index to Plant Chromosome Numbers 1988–1989. *Monogr. Syst. Bot. Mo. Bot. Gard.* **40**: 1–238.
- Goldblatt, P. and Johnson, D. E. 1994. Index to Plant Chromosome Numbers 1990–1991. *Monogr. Syst. Bot. Mo. Bot. Gard.* **51**: 1–267.
- Goldblatt, P. and Johnson, D. E. 1996. Index to Plant Chromosome Numbers 1992–1993. *Monogr. Syst. Bot. Mo. Bot. Gard.* **51**: 1–267.
- Goldblatt, P. and Johnson, D. E. 1998. Index to Plant Chromosome Numbers 1994–1995. *Monogr. Syst. Bot. Mo. Bot. Gard.* **58**: 1–276.
- Goldblatt, P. and Johnson, D. E. 2000. Index to Plant Chromosome Numbers 1996–1997. *Monogr. Syst. Bot. Mo. Bot. Gard.* **69**: 1–208.
- Goldblatt, P. and Johnson, D. E. 2003. Index to Plant Chromosome Numbers 1998–2000. *Monogr. Syst. Bot. Mo. Bot. Gard.* **81**: 1–188.
- Goldblatt, P. and Johnson, D. E. 2006. Index to Plant Chromosome Numbers 2001–2003. *Monogr. Syst. Bot. Mo. Bot. Gard.* **94**: 1–297.
- Goldblatt, P. and Johnson, D. E. 2010. Index to Plant Chromosome Numbers 2004–2006. A. R. G. Gartner Verlag, Ruggell.
- Heckenhauer, J., Samuel, R., Ashton, P. S., Turner, B., Barfuss, M. H. J., Jang, T.-S., Temsch, E. M., Mccann, J., Salim, K. A., Atanayake, A. M. A. S. and Chase, M. W. 2017. Phylogenetic analyses of plastid DNA suggest a different interpretation of morphological evolution than those used as the basis for previous classifications of Dipterocarpaceae (Malvales). *Bot. J. Linn. Soc.* **185**: 1–26.
- Jong, K. and Lethbridge, A. 1967. Cytological studies in the Dipterocarpaceae. I. Chromosome numbers of certain Malaysian genera. *Notes Roy. Bot. Gard. Edinb.* **27**: 175–184.
- Jong, K. and Kaur, A. 1979. A cytotaxonomic view of Dipterocarpaceae with some comments on polyploidy and apomixes. *Mem. Mus. Natl. Hist. Nat., Ser. B. Bot.* **26**: 41–49.
- Kajita, T., Kamiya, K., Nakamura, K., Tachida, H., Wickneswari, R., Tsumura, Y., Yoshimura, H. and Yamazaki, T. 1998. Molecular phylogeny of Dipterocarpaceae in Southeast Asia based on nucleotide sequences of *matK*, *trnL* intron, and *trnL-trnF* intergenic spacer region in chloroplast DNA. *Mol. Phylogenet. Evol.* **10**: 202–209.
- Kaur, A., Johb, K., Sands, V. E. and Soepadmo, E. 1986. Cytoembryology of some Malaysian dipterocarps, with some evidence of apomixis. *Bot. J. Linn. Soc.* **92**: 75–88.
- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position of chromosomes. *Hereditas* **52**: 201–220.
- Mabberley, D. J. 2008. Mabberley's Plant-Book. A Portable Dictionary of Plants, Their Classification and Use. 3rd edn. Cambridge University Press, New York.
- Maguire, B. P. C. and Ashton, P. S. 1977. Pakaraimoideae, Dipterocarpaceae of the Western Hemisphere II. Systematic, geographic and phyletic considerations. *Taxon* **26**: 341–368.
- Mehra, P. N. 1976. Cytology of Himalayan Hardwoods. Sree Saraswati Press, Calcutta.
- Mehra, P. N. and Sareen, T. S. 1969. In: Löve, V. (ed.). IOPB Chromosome Number Report XXII. *Taxon* **18**: 433–442.
- Mehra, P. N. and Sareen, T. S. 1973. Cytology of some Himalayan

- trees. *Silvae Genet.* **22**: 66–70.
- Moore, R. J. 1973. Index to Plant Chromosome Numbers 1967–1971. Oosthoek's Uitgeversmaatschappij B. V., Utrecht.
- Moore, R. J. 1974. Index to Plant Chromosome Numbers for 1972. Oosthoek's Uitgeversmaatschappij B. V., Utrecht.
- Moore, R. J. 1977. Index to Plant Chromosome Numbers for 1973–1974. Bohn, Scheltema and Holkema, Utrecht.
- Nanda, P. C. 1962. Chromosome number of some trees and shrubs. *J. Indian Bot. Soc.* **41**: 271–277.
- Oginuma, K. and Nakata, M. 1988. Cytological studies on phanerogams in Southern Peru, I. Karyotype of *Acaena ovalifolia*. *Bull. Natl. Sci. Mus. Tokyo. Ser. B* **14**: 53–56.
- Oginuma, K. and Tobe, H. 1991. Karyomorphology and evolution in some Hamamelidaceae and Platanaceae (Hamamelididae; Hamamelidales). *Bot. Mag. Tokyo* **104**: 115–135.
- Oginuma, K., Ibarra-Manriquez and Tobe, H. 1992. Chromosomes of *Tuxtla pittieri* (Asteraceae; Heliantheae). *Acta Phytotax. Geobot.* **43**: 135–137.
- Oginuma, K., Klaptrianis, R., Damas, K. and Tobe, H. 1998a. A cytological study of some plants from Papua New Guinea. *Acta Phytotax. Geobot.* **49**: 105–114.
- Oginuma, K., Kato, H. and Tobe, H. 1998b. Chromosome base number of *Corylus* (Betulaceae): Correction, and evolution. *Acta Phytotax. Geobot.* **49**: 99–104.
- Oginuma, K., Horiuchi, K. and Fukuhara, T. 2001. Karyomorphology of two genera in Stemonaceae. *Acta Phytotax. Geobot.* **52**: 57–63.
- Ornduff, R. 1967. Index to Plant Chromosome Numbers for 1965. The International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
- Ornduff, R. 1968. Index to Plant Chromosome Numbers for 1966. The International Bureau for Plant Taxonomy and Nomenclature, Utrecht.
- Pal, J. K., Mandal, S. and Bhattacharya, G. N. 1993. Cytopalynological studies in *Shorea robusta* Gaertn f. *Sci. Cult.* **59**: 55–57.
- Pancho, J. V. 1971. In: Löve, V. (ed.). IOPB chromosome number reports XXXIV. *Taxon* **20**: 785–787.
- Roy, R. P. and Jha, P. R. 1965. Cytological studies in Dipterocarpaceae. *I. J. Indian Bot. Soc.* **44**: 387–397.
- Sarkar, A. K., Datta, N., Chatterjee, U. and Hazra, D. 1982. In: Löve, V. (ed.). IOPB chromosome number reports LXXVI. *Taxon* **32**: 576–579.
- Singhal, V. K. and Gill, B. S. 1984. SOCGI plant chromosome number reports—II. *J. Cytol. Genet.* **19**: 115–117.
- Somego, M. 1978. Cytogenetical study of the Dipterocarpaceae. *Malays. Forester* **41**: 358–366.
- Takhtajan, A. 1986. *Floristic Regions of the World*. University of California Press, Berkeley.
- The Angiosperm Phylogeny Group 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* **181**: 1–20.
- Tixier, P. 1953. Donnees cytologiques sur quelques Guttiferales du Viet-Nam. *Rev. Cytol. Biol. Veg.* **14**: 1–12.
- Tixier, P. 1960. Donnees cytologiques sur quelques Guttiferales du recoltees au Raos. *Rev. Cytol. Biol. Veg.* **22**: 65–70.
- Tsumura, Y., Kawahara, T., Wickneswari, R. and Yoshimura, K. 1996. Molecular phylogeny of Dipterocarpaceae in Southeast Asia using RFLP of PCR-amplified chloroplast genes. *Theor. Appl. Genet.* **93**: 22–29.