Botanical Journal of the Linnean Society, 2005, **149**, 451–455. With 9 figures

Karyotype differentiation between *Koelreuteria bipinnata* and *K. elegans* ssp. *formosana* (Sapindaceae) based on chromosome banding patterns

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Received January 2005; accepted for publication April 2005

Koelreuteria Laxm., a genus in the tribe *Koelreuterieae* (Sapindaceae), includes three tree species that are widely recognized as having horticultural merit. The two more closely related species, *K. bipinnata* Franch. and *K. elegans* (Seem.) A. C. Sm., are easily distinguished from *K. paniculata* Laxm. by their bipinnate leaves. In this study, both species were investigated cytogenetically and their karyotypes and heterochromatic patterns were compared. *Koelreuteria bipinnata* and *K. elegans* ssp. *formosana* (Hayata) F. G. Mey. both have $2n = 32$ but their karyotypes present slight morphological differences when observed using conventional staining. Chromosome banding patterns are reported for the first time for this genus. Both species exhibit terminal heterochromatic blocks, as revealed by C-Giemsa and C-CMA_3, but the band size varies between the species. *Koelreuteria bipinnata* has larger heterochromatic blocks and more GC-rich segments, while in *K. elegans* ssp. *formosana* these bands are smaller. The relationship between the karyotype features in these closely related species is discussed.

ADDITIONAL KEYWORDS: chromosome numbers – fluorochromes – Giemsa C-banding – interphase nuclei – karyosystematics.

INTRODUCTION

The family Sapindaceae is distributed worldwide and possesses 136 genera with 2000 species. The systematic arrangement, as circumscribed by Radlkofer, 1931–34), comprises 14 tribes. *Koelreuteria* Laxm. belongs to tribe Koelreuterieae in subfamily Dodonaeoideae (Radlkofer's Dyssapindaceae). The tribe, as defined by Radlkofer (1890; Radlkofer, 1931–34), includes the genera *Stocksia* Benth. and *Erythrophysa* E. Mey. in addition to *Koelreuteria*. The genus *Sinoradlkofera* F. G. Mey. was later incorporated into the tribe because of its possession of features such as inflated and membranaceous capsules and monosymmetrical flowers (Meyer, 1977). Within the Dodonaeoideae, Koelreuterieae presents a plesiomorphic state in most of its morphological characters (Muller & Leenhouts, 1976) and is closely related to Cossinieae and Dodonaeeae (Radlkofer, 1931–34).

Koelreuteria includes three species, native to China, Taiwan and Fiji, which are found in parks, arboreta and gardens in many countries, and are cultivated in Europe, Africa, Australia and the USA (Meyer, 1976). This genus is separated from the other genera of the tribe because of its large leaves and paniculiform inflorescences of yellow flowers. In Argentina only *K. paniculata* Laxm. (Parodi, 1980) and *K. elegans* (Seem.) A. C. Sm. ssp. *formosana* (Hayata) F. G. Mey. (Ferrucci & De Pompert, 1996) have been recorded, the latter being known in Argentina as 'soap stick from China'. Subspecies *formosana* is distinguished from the type species by minor details of the leaflet margins, petiolule length and flowers (Meyer, 1976).

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Until now, cytological studies in Sapindaceae have concentrated mainly on chromosome number and size diversity. Reported numbers have varied from $2n = 14$ in *Cardiospermum integerrimum* Radlk. (Ferrucci, 1989) to $2n = 96$ in *Melicoccus lepidopetalus* Radlk. (Ferrucci & Solís Neffa, 1997). The chromosome length varies from 0.6 to 1.5 μm in *C. halicacabum* L. var. *microcarpum* (Kunth) Blume (Hemmer & Morawetz, 1990) and 2.5–6.57 μm in *Urvillea laevis* Radlk. (Ferrucci, 1997).

Earlier cytogenetical studies in *Koelreuteria* have been based on chromosome number and interphase nucleus structure. The species possess relatively small chromosomes, comprising karyotypes with $2n = 30$ (Huang *et al*., 1989) and 32 (Hemmer & Morawetz, 1990) in *K. bipinnata*, $2n = 22$ (Bowden, 1945) and 32 (Hemmer & Morawetz, 1990; Ferrucci & De Pompert, 1996) in *K. elegans* ssp. *formosana*, and $2n = 22$ (Bowden, 1945) and 30 (Eichhorn & Franquet, 1936; Guervin, 1961; Huang *et al*., 1986) in *K. paniculata*. Based on these chromosome counts, the basic numbers proposed for the genus are $x = 15$ and 16, and the results of Bowden (1945) being discounted as probable misidentifications (Ferrucci, 2000).

In the present study the chromosome numbers of two new South American populations of *K. bipinnata* and *K. elegans* ssp. *formosana* are reported, together with an analysis of the interphase nuclei, karyotypes and heterochromatin patterns of these two closely related species. Our results are discussed in relation to previously published data in the light of the current taxonomy of the genus.

MATERIAL AND METHODS

Seeds of *K. bipinnata* and *K. elegans* ssp. *formosana* were obtained from specimens cultivated in Argentina and were grown in a greenhouse at the Laboratório de Biodiversidade Restauração de Ecossistemas (LABRE) of the Universidade Estadual de Londrina, Brazil.

The following voucher specimens are deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES), Argentina:

K. bipinnata: Argentina, Buenos Aires. Capital Federal, cultivated in the Jardín Botánico 'Carlos Thays', 21.iv.2004, Bello s.n., ex BAA 25365.

K. elegans ssp. *formosana*: Argentina, Corrientes. Dpto. Capital, cultivated in the Facultad de Ciencias Agrarias, UNNE, 28.iv.2004, Ferrucci 2119.

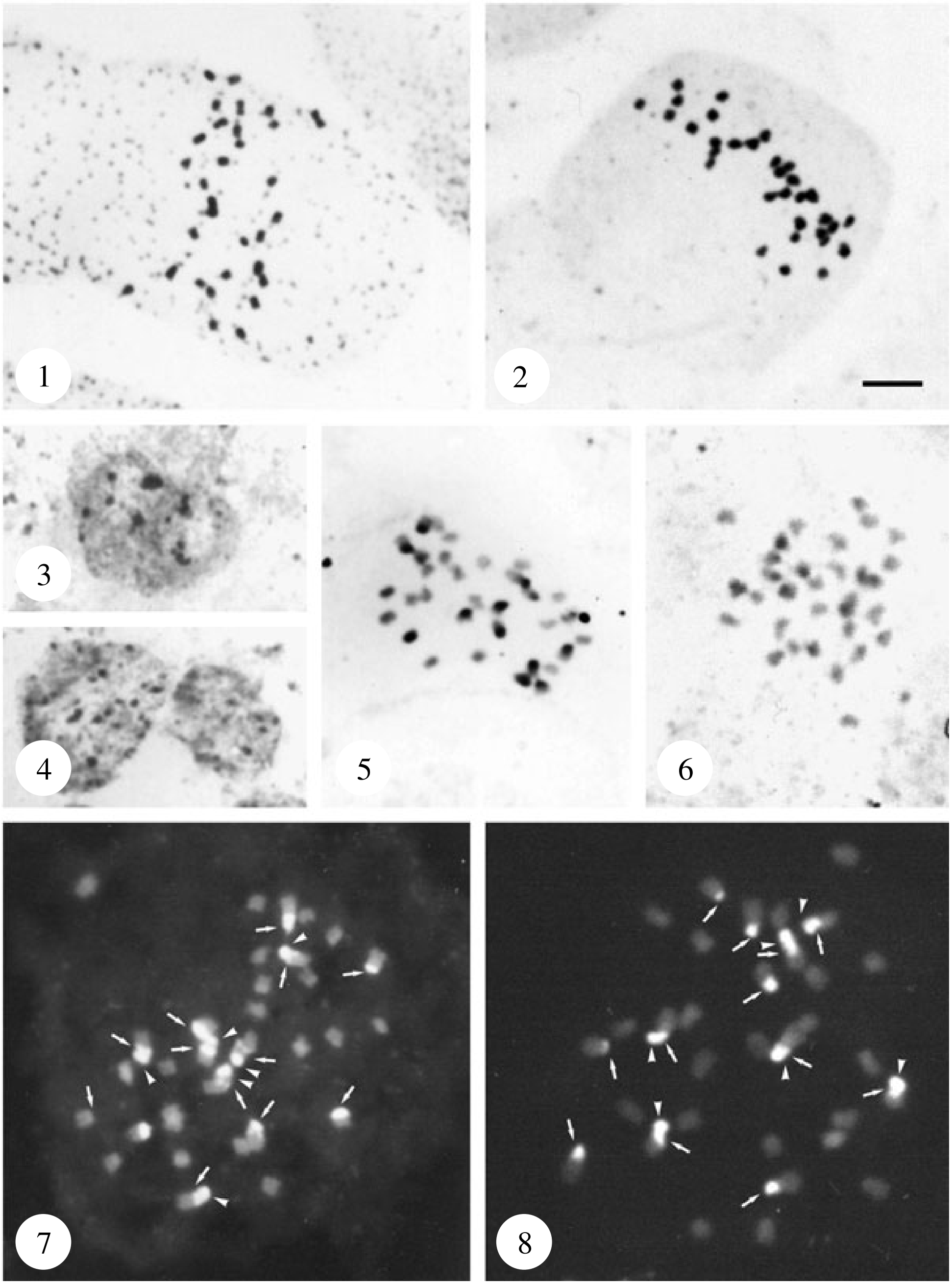
Chromosome preparations were made from root tips pretreated in 2 mM 8-hydroxyquinoline for 4–5 h at 15 °C, fixed in ethanol:acetic acid (3:1, v:v) for 12 h and stored at –20 °C until use. For conventional analysis of chromosomes, the HCl/Giemsa technique of Guerra (1983) was used. C-Giemsa banding followed Schwarzacher, Ambros & Schweizer (1980) and C-CMA_3/DAPI banding followed Schwarzacher & Schweizer (1982), both with some modifications (see Vanzela *et al*., 2002). Roots were digested in an enzyme solution comprising 4% cellulase and 40% pectinase at 37 °C, and were dissected in a drop of 45% acetic acid. Slides were frozen in liquid nitrogen and the cover slips removed. They were aged for three days at room temperature and treated with 45% acetic acid, 5% barium hydroxide, and 2× SSC, pH 7.0, then stained with 2% Giemsa or aged for three days at room temperature and sequentially stained with 0.5 mg/mL CMA_3 for 1.5 h and 2 μg/mL DAPI for 30 min. Samples stained with Giemsa were mounted with Entellan, but those stained with the fluorochromes were mounted in a medium composed of glycerol/McIlvaine buffer pH 7.0, 1:1 (v:v), plus 2.5 mM $MgCl_2$. The cells were photographed with Imagelink HQ ASA 25 or T-max ASA 100, both from Kodak. Analyses were based on five well-spread metaphase plates for each treatment.

RESULTS AND DISCUSSION

Both *K. bipinnata*, and *K. elegans* ssp. *formosana* possess bi-compound leaves, a character that separates them from *K. paniculata* Laxm., which has compound leaves. Characteristics of the leaflets distinguish the species treated here. *Koelreuteria bipinnata* has weakly oblique leaflets, acute to shortly acuminate, opaque above, with secondary veins prominent below, while *K. elegans* has strongly oblique leaflets, sometimes caudate, lustrous above and with secondary veins weakly impressed below (Meyer, 1976).

In the *Koelreuterieae*, only *Koelreuteria* has been analysed cytologically to date. The chromosome counts for *K. bipinnata* and *K. elegans* ssp. *formosana* showed $2n = 32$ (Figs 1, 2; Table 1). Our counts for these species disagree with Huang *et al*. (1989), who cited $2n = 30$ for *K. bipinnata* (syn *K. integrifolia* Merr.) and Bowden (1945), who registered $2n = 22$ for *K. elegans* ssp. *formosana* (syn *K. formosana*). However, we agree with Hemmer & Morawetz (1990; without citation of voucher) and with Ferrucci & De Pompert (1996), who reported $2n = 32$ for *K. elegans* ssp. *formosana*, and $2n = 32$ for *K. bipinnata* (Hemmer & Morawetz, 1990; also without citation of voucher, see Table 1).

A major difficulty in the analysis of the material is the high incidence of chromosome adhesion, even after treatment with antimitotic agents. This feature might have contributed to the reports of varying chromosome numbers for these species, e.g. *K. bipinnata* with $2n = 30$ (Huang *et al*., 1989) and *K. paniculata* with $2n = 30$ (Eichhorn & Franquet, 1936; Guervin, 1961; Huang *et al*., 1986). The occurrence of different numbers might also the result of differing cytotypes or mis-



Figures 1–8. Interphase nuclei and chromosomes of *Koelreuteria*. Fig. 1. Mitotic metaphase of *K. bipinnata*, $2n = 32$. Fig. 2. Mitotic metaphase of *K. elegans* ssp. f*ormosana*, $2n = 32$. Fig. 3. C-banded interphase nuclei of *K. bipinnata*. Fig. 4. C-banded interphase nuclei of *K. elegans* ssp. *formosana*. Fig. 5. Giemsa C-banding in *K. bipinnata*. Fig. 6. Giemsa C-banding in *K. elegans* ssp. *formosana*. Fig. 7. C-CMA_3 banding in *K. bipinnata*. Fig. 8. C-CMA_3 banding in *K. elegans* ssp. *formosana*. Scale bar = 5 µm.

identification of the plants assayed. For instance, the chromosome counts reported for *K. elegans* ssp. *formosana* and *K. paniculata*, both with $2n = 22$ (Bowden, 1945), are considered to be erroneous counts.

The basic chromosome numbers proposed for the genus, and probably for the tribe, are $x = 15$ and 16 (Ferrucci, 2000), which are related through dysploidy mechanisms. Most of the genera in this family have diploid numbers between $2n = 28$ and 32 (Hemmer & Morawetz, 1990). According to the available data, $x = 14$, 15 and 16 occur more frequently. Considering that $x = 7$ is probably the primitive base number in the

Table 1. Chromosome and karyotype features of *Koelreuteria* species

Species	2*n*	TCL (μm)	BN	% Hc (σ)	Interphase nucleus	C-Giemsa band	C-CMA_3	References
K. bipinnata	30							Huang *et al*. (1989).
	32							Hemmer & Morawetz (1990).
	32	1.05–0.62 (26.14)	12	29.03 (0.70)	Areticulate	+++ terminal	12 +++	This paper
K. elegans	22							Bowden (1945).
ssp. *formosana*	32							Hemmer & Morawetz (1990)
								Ferrucci & De Pompert (1996)
	32	0.98–0.61 (24.83)	12	21.18 (0.46)	Areticulate	+ terminal	12 +	This paper
K. paniculata	22							Bowden (1945)
	30							Eichhorn & Franquet (1936)
								Guervin (1961)
								Huang *et al*. (1986)

TCL, total karyotype length; BN, band numbers; %Hc, percentage of heterochromatin per diploid complement.

family (Ferrucci, 1989), the other numbers would have been derived by polyploidy and aneuploidy. The basic number $x = 16$ in the tribe Koelreuterieae would represent a plesiomorphic character shared with Cupanieae, Harpullieae, Lepisantheae, Melicocceae, Nephelieae, Sapindeae, Schleichereae and Thouinieae.

Conventional staining showed that karyotypes have similar characteristics, with small chromosomes varying gradually from meta- to submetacentric (Table 1; Figs 1,2). The studied species show great similarity in their interphase nucleus structure, which is always areticulate, in agreement with Eichhorn & Franquet (1936) and Guervin (1961). *Koelreuteria bipinnata* has a larger number of chromocentres, between 10 and 12 per nucleus, which can fuse to form circular chromocentres. *Koelreuteria elegans* ssp. *formosana* has a variable number of 6–10 chromocentres, which can be fused in threes or fours (Figs 3, 4). *Koelreuteria* shows the generally accepted correlation between interphase nucleus structure and chromosome size (Nagl, 1979).

The Giemsa-C banding revealed heterochromatin distribution pattern differences between the studied species. While *K. elegans* ssp. *formosana* presented terminal bands of different size in the short arms of four chromosome pairs (Fig. 6), *K. bipinnata* showed major terminal blocks in short arms of six chromosome pairs (Fig. 5).

In contrast, *K. bipinnata* had strongly Giemsa-positive bands, almost as long as the short arms. The larger size of heterochromatic blocks observed in mitotic chromosomes was correlated with the larger number of chromocentres observed in this species (Figs 3, 5).

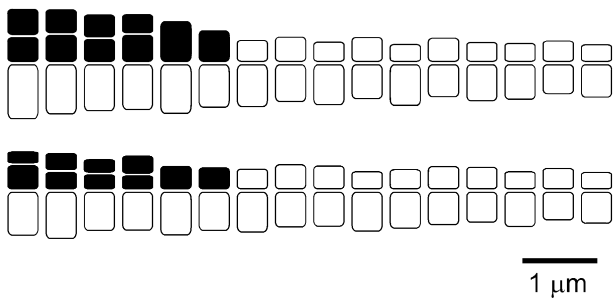


Figure 9. C-CMA_3 idiograms of *Koelreuteria*. A, *K. bipinnata*. B, *K. elegans* ssp. *formosana*.

The C-CMA_3 banding revealed, in both species, bands in six chromosome pairs, which appeared mainly as terminal GC-rich blocks in the short arms. *Koelreuteria bipinnata* exhibited C-CMA_3 blocks larger than those of *K. elegans* ssp. *formosana*, occupying about 29% (Fig. 7) and 21% (Fig. 8) of total karyotype length, respectively. This difference is demonstrated in Figure 9. Additionally, these GC-rich blocks are coincident with the C-Giemsa banding pattern and to some terminal blocks associated with secondary constrictions (Figs 7, 8). DAPI bands were not detected in either species, indicating the absence of AT-rich heterochromatin, at least with the use of this technique.

The chromosome banding showed that heterochromatic blocks found in *K. bipinnata* and *K. elegans* ssp. *formosana* were not distributed randomly (Guerra, 2000). Heterochromatic bands usually appear in equidistant or equilocal positions in the chromosome complement, but preferentially in the terminal regions. The heterochromatic pattern observed in both species, with GC-rich heterochromatin distributed preferentially in terminal positions, suggests that both species share the same repetitive DNA family. The isolation and characterization of these repeated segments would be an interesting research area. *Koelreuteria bipinnata* and *K. elegans* ssp. *formosana* are closely related species and their karyotypes observed by conventional Giemsa staining also showed a close similarity. Differences between them were detectable only with fluorochrome staining. Nevertheless, the band size and number differences found between *K. bipinnata* and *K. elegans* ssp. *formosana* were clearly associated with morphological differences presented by these species. The karyotype variations are important not only as a source of genetic variability, but also because they represent an important micromorphological feature for this plant group. The results obtained in this work support the affinities of both species, but reveal that changes in karyotype structure and heterochromatin patterns have occurred, features that are frequently associated with species differentiation.

ACKNOWLEDGEMENTS

We thank Gabriel Rua for supplying the *Koelreuteria bipinnata* seeds used in this work, Lic. Ernestina Galdeano for improving the English version and the Brazilian agencies CAPES and ProPPG-UEL for financial support.

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