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Paternity Study of an Interspecific Natural Hybrid of the Genus *Eucalyptus* L'Hér (Myrtaceae) Based on Cytogenetic Data

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Summary Three species of the genus *Eucalyptus* (*E. dunni*, *E. grandis*, *E. saligna*) and interspecific hybrid were studied cytogenetically. The *Eucalyptus* species and the hybrid showed a symmetrical karyotype with $2n=22$ chromosomes, with chromosome length ranging from 0.67 to 1.39 μm. Karyotypic analysis indicated a homogenous morphology and chromosome number for the species and the hybrid studied here. Based on the karyotype asymmetry data, together with the chromosome morphology results, the hybrid presented close similarity to *E. saligna*, suggesting that the latter is one of the parental species involved in the production of the hybrid.
Key words Karyomorphology, Chromosome, *Eycalyptus*.

Cytogenetic analysis is an important instrument in taxonomic and phylogenetic studies and, for some groups, is extremely useful for the determination of genetic mechanisms related to speciation (Stebbins 1971, Guerra 1986, Matsumoto *et al.* 2000).

Analyses of chromosome number and morphology and banding patterns are used for the characterization of species since these traits are associated with genetic data which remain unchanged during the different phases of development of the organism, and therefore efficiently characterize the individual, species or population analyzed.

The aim of the present study was to karyomorphologically characterize an interspecific natural F_1 hybrid of the genus *Eucalyptus* and 3 species of the same genus (*E. dunni*, *E. grandis*, *E. saligna*) probably involved in the paternity of the hybrid to determine the species involved in the production of this hybrid.

Materials and methods

The plants were obtained from a previous selection made by the paper and cellulose industry-Klabin-PR, Southern Brazil. The following species were analyzed: *E. dunni*, *E. grandis*, *E. saligna* and a natural hybrid which emerged between the culture plots of the 3 *Eucalyptus* species. The interest in the determination of the paternity of this hybrid resulted from its superior conditions in terms of fiber production.

Root tips were collected for mitotic analysis, preferably from 11.5 h to 12.5 h, as a greater mitotic incidence was observed at this time. The meristems were pretreated with 0.002 M 8-hydroxyquinoline (8HQ) and 0.5 ml dimethylsulphoxide (DMSO) per 100 ml of 8HQ solution over a period of 4.5 h, involving 1 h at room temperature and 3.5 h at 8°C. The roots tips were then fixed in Carnoy solution (3 parts ethyl alcohol: 1 part acetic acid) for 12 h at room temperature and later conserved in the same fixer, at −20°C.

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The staining methodology described by Feulgen and Rossembeck (1924) (Mello and Vidal 1978) was used, with modifications. The material was hydrolyzed in 1 N HCl at 60°C for 11 min, and then stained with the Schiff reagent for 90 min. The material was then squashed on slides containing a drop of 1% acetic carmine.

Cells of the *Eucalyptus* species and the hybrid in mitotic metaphases were analyzed to establish the number, size and morphology of the chromosome. The values obtained for chromosome morphology were absolute length of each chromosome pair, length of haploid chromosome and arm ratio (AR) calculated by the following formula:

$$AR = \text{long arm length/short arm length}$$

The relative length was calculated by the following formula:

$$CR = \text{absolute length} \times 100/\text{total haploid lot length}$$

The values obtained were used to determine the chromosome type, as described by Levan *et al.* (1964). The asymmetry indices were also assessed according to Zarco (1986).

$$A1 = \Sigma b/B/\text{number of pair}$$

$$A2 = \text{SD of absolute length } (\mu m)/\text{mean of absolute lingth } (\mu m)$$

Where A1 represents the intra-chromosome asymmetry index and A2 represents the inter-chromosome asymmetry index: B represents the mean of the largest arms and b the mean of the smallest arms. The Huziwara (1962) test was used to assess the Karyotypic asymmetry, using the following formula:

$$TF = \Sigma \text{ short arm length} \times 100/\text{total chromosome length}$$

The genetic proximity between the species analyzed was established by calculation of the taxonomic distance as described by Sokal (1961), based on the intrachromosomal and interchromosomal asymmetry presented by the species and the hybrid.

Results

Chromosome characterization of the 3 *Eucalyptus* species and the interspecific natural hybrid was carried out by cytogenetic analysis. The karyomorphological analyses, including chromosome length (μm), relative length (%), arm ratio and karyotype formula, for the *Eucalyptus* species and the hybrid are shown in Table 1. The metaphases of the studied species are illustrated in Fig. 1. The karyotypes are shown in Fig. 2 and the idiogram is shown in Fig. 3.

All 3 species and the natural hybrid showed the same chromosome number of $2n=22$.

The results of the chromosome analyses obtained for the 3 species and the hybrid are present-

Table 1. Chromosome numbers ($2n$), Karyotype formulae, total set chromosome length (TCL) and TF% index of species of *Eucalyptus*

Species	$2n$	Karyotype formulae	TCL	TF%
E. dunni	22	1AM+1BM+1Am+2Bm+2Cm+3Dm+1Em	22.80	47.14±1.12
E. grandis	22	2Am+3Bm+1Cm+5Dm	22.80	46.89±0.27
E. saligna	22	1AM+1Am+3Bm+3Cm+1Dm+1Em+1Dsm	23.00	46.03±0.42
Hybrid	22	1AM+1BM+1Csm+1Am+1Bm+4Cm+2Dm	23.20	45.96±0.84

Chromosome length, in μm: A=1.3–1.5; B=1.1–1.13; C=0.9–1.1; D=0.7–0.9; E=0.5–0.7. Centromeric position M=metacentric (1 : 1), m=metacentric and sm=sub-metacentric.

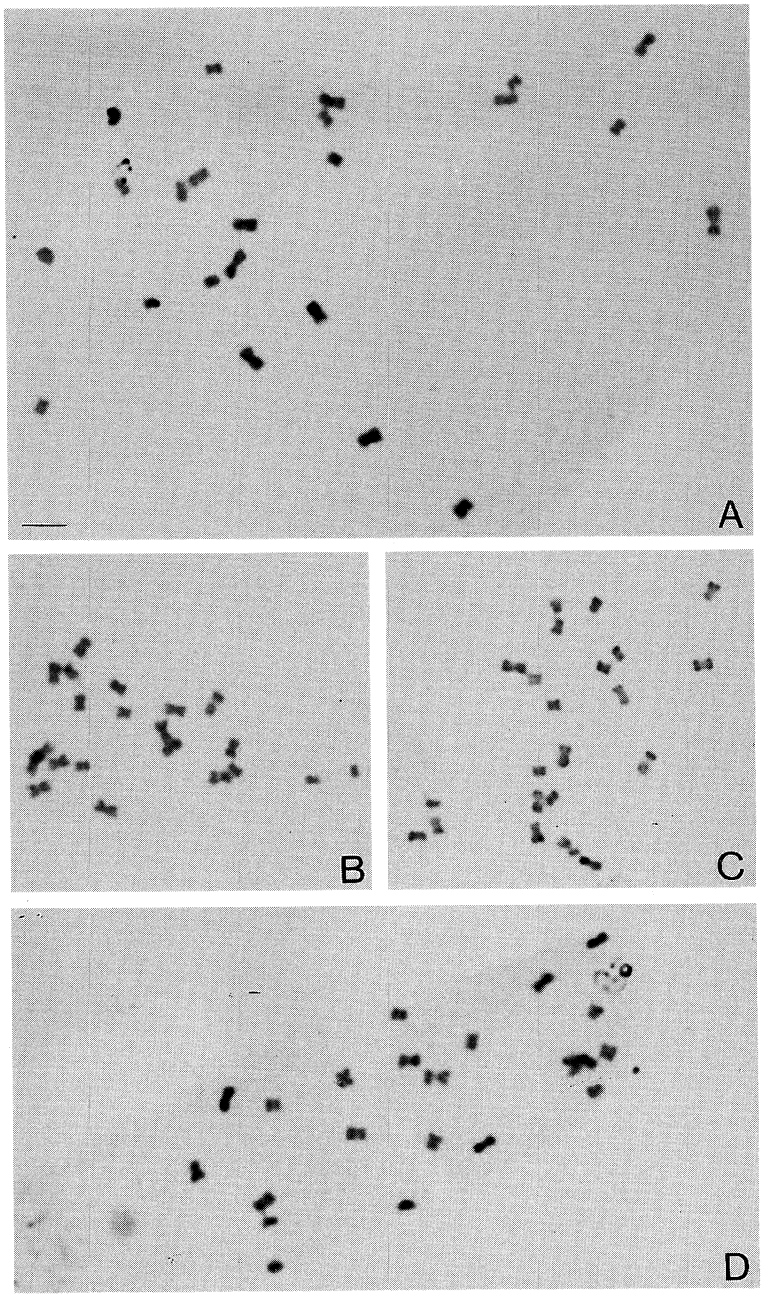

Fig. 1. Metaphases of the *Eucalyptus* species. A) *E. dunni*, B) *E. grandis*, C) *E. saligna*, D) Hybrid.

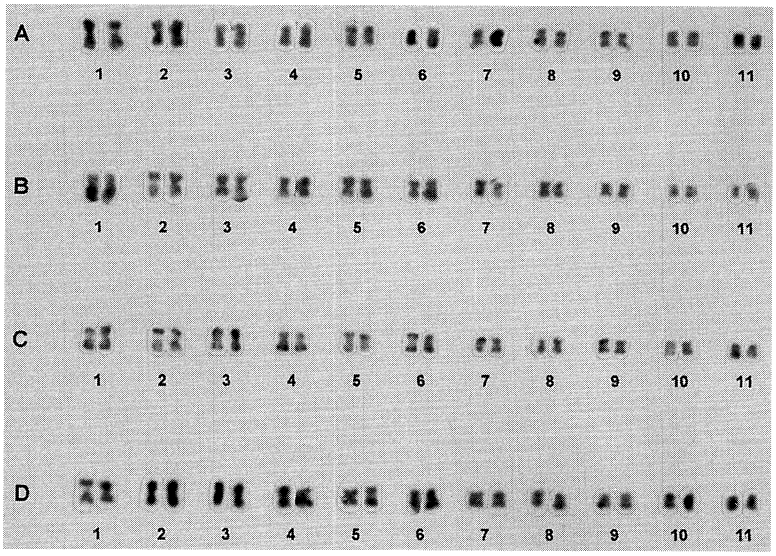

Fig. 2. Karyotype of the *Eucalyptus* species with $2n=22$ chromosomes. A) *E. dunni*, B) *E. grandis*, C) *E. saligna*, D) Hybrid.

ed in detail below.

Eucalyptus dunni

This species showed a karyotype formula of 2M+9m (Figs. 1–3, Tables 1, 2). The absolute chromosome length ranged from 0.67±0.11 to 1.39±0.01 μm and relative length ranged from 4.74±0.35 to 12.59±0.38%. The length of the haploid lot was 11.40 μm and the symmetry index (TF%) was 47.14±1.12. The intrachromosomal and interchromosomal asymmetry indices were 0.13±0.05 and 0.05, respectively (Table 3).

Eucalyptus grandis

E. grandis, which only presented m type chromosomes (Figs. 1–3, Tables 1, 2), showed an absolute chromosome length ranging from 0.75±0.00 to 1.35±0.02 μm. Relative chromosome length ranged from 6.64±0.18 to 11.86±0.18%. The TF% value obtained for this species was 46.89±0.27% and the length of the haploid set was 11.40 μm. The intrachromosomal and interchromosomal asymmetry indices were 0.12±0.01 and 0.03 (Table 3).

Eucalyptus saligna

This species presented a karyotype formula of 1M+9m+1sm, as shown in Figs. 1, 2 and 3 and Tables 1 and 2. The absolute chromosome number ranged from 0.68±0.04 to 1.39±0.01 μm and relative chromosome number from 5.96±0.29 to 12.13±0.17%. The TF value was 46.03±0.42% and the length of the haploid lot was 11.50 μm. The intrachromosomal and interchromosomal asymmetry indices were 0.15±0.01 and 0.02 (Table 3).

Interspecific natural hybrid of the genus Eucalyptus

The karyotype formula observed for this hybrid was 2M+8m+1sm (Figs. 1–3, Tables 1, 2), with chromosome length ranging from 0.75±0.04 to 1.38±0.02 μm. Relative chromosome length ranged from 6.42±0.25 to 11.90±0.37%. The TF value was 45.96±0.84% and the length of the haploid lot was 11.60 μm. The intrachromosomal and interchromosomal asymmetry indices were 0.15±0.03 and 0.03 (Table 3).

Discussion and conclusions

The 3 *Eucalyptus* species and the hybrid analyzed showed $2n=22$ chromosomes, in agreement with most authors who defined a number of $2n=22$ for the genus (Ruggeri 1960, 1961, Boyland and Kleining 1983, Haque 1984, Vijayakumar and Subramanian 1985, Matsumoto *et al.* 2000). Many studies carried out on the genus *Eucalyptus* are available, with most of them only reporting the chromosome number, while studies that karyomorphologically characterize the species of this genus are scarce. A karyomorphology study on the species of this genus was conducted by Matsumoto *et al.* (2000), who observed karyotypes which mostly consisted of medium (m) type chromosomes.

All *Eucalyptus* species described by Matsumoto *et al.* (2000) and those of the present study, as well as the natural hybrid, showed small chromosomes ranging in length from 0.67 to 1.39 μm. This finding disagrees with the results reported by Haque (1984), who found a chromosome size ranging from 2 to 6 μm for the species *E. torelian*, *E. citriodora*, *E. camaldulensis* and *E. tereticornis*. In a later study (Haque, 1984), this author reported the presence of metacentric and submetacentric chromosomes with one of the pairs containing satellites, while in the present study no satellite-containing chromosomes were observed.

The karyotype homogeneity of *Eucalyptus* species may be related to reduced derivation of the genus since, according to Stebbins

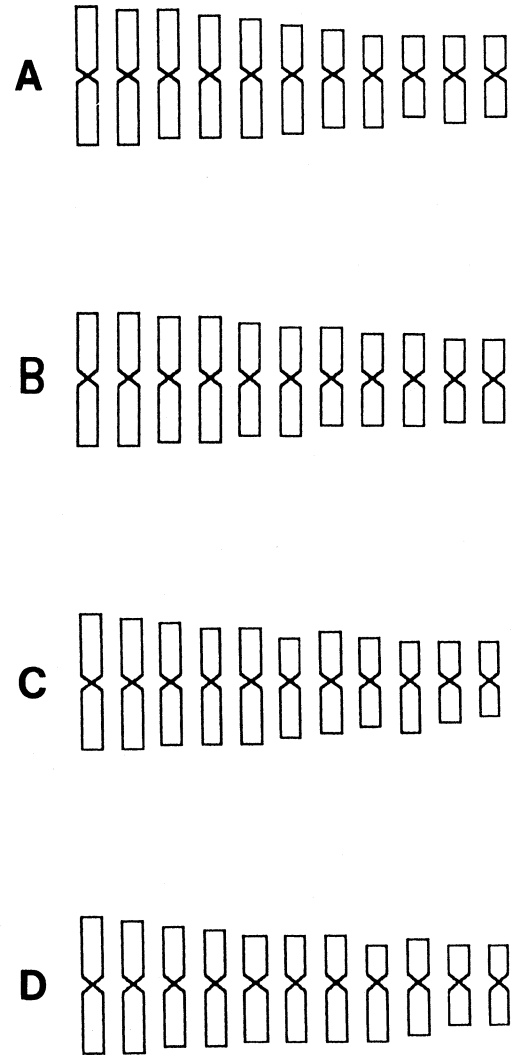

Fig. 3. Ideogram of the *Eucalyptus* species with $2n=22$ chromosomes. A) *E. dunni*, B) *E. grandis*, C) *E. saligna*, D) Hybrid.

Table 2. Chromosome type of the *Eucalyptus* species and the hybrid studied

Chromosome pair	*E. dunni*	*E. grandis*	*E. saligna*	Hybrid
1	M	m	M	M
2	m	m	m	m
3	M	m	m	M
4	m	m	m	m
5	m	m	m	m
6	m	m	m	m
7	m	m	m	m
8	m	m	m	m
9	m	m	sm	sm
10	m	m	m	m
11	m	m	m	m

Table 3. Intrachromosomal (A1) and interchromosomal (A2) karyotype asymmetry indices obtained for the *Eucalyptus* species and the natural hybrid

Species	A1	A2
E. dunni	0.13±0.05	0.05
E. grandis	0.12±0.01	0.03
E. saligna	0.15±0.01	0.02
Hybrid	0.15±0.03	0.03

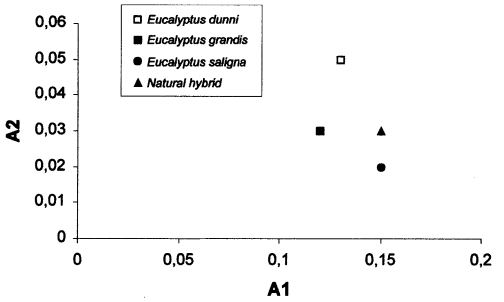

Fig. 4. Distribution of the A1 and A2 asymmetry indexes in the *Eucalyptus* species and the natural hybrid according to Zarco (1986).

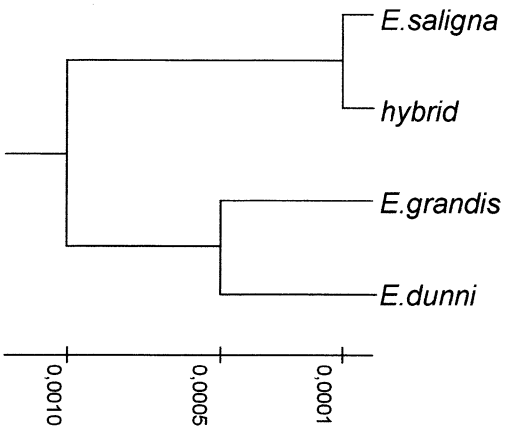

Fig. 5. Dendrogram of the *Eucalyptus* species and the natural hybrid.

(1971), symmetrical karyotypes are indicative of a primitive characteristic. This chromosome homogeneity may offer advantages to the genus in terms of interspecific hybridization mechanisms, thus producing fertile hybrids (Venkatesh and Sharma 1977, Haque 1984). The present results demonstrate that the interspecific natural F_1 hybrid was karyotypically similar to the other species studied.

Karyomorphological analysis of *E. dunni*, *E. grandis*, *E. saligna* and the hybrid revealed that only *E. saligna* and the hybrid presented sm type chromosomes, in both cases characterizing the morphology of pair 9 (Table 2).

The hybrid also showed homology in terms of chromosome morphology of pairs 1 and 3 with *E. dunni*, with the chromosome type being characterized as medium *strictu sensu* (M) in both cases (Table 2).

Analysis of intrachromosomal and interchromosomal karyotype asymmetry according to Zarco (1986) revealed a close association between *E. dunni* and *E. grandis* (Table 3, Fig. 4). The hybrid showed closer proximity to *E. saligna*, indicating a closer association of the hybrid with this species than with the other 2 (Fig. 5).

According to the karyotype classification of Stebbins (1971) with respect to the ratio between the length of the largest chromosome and the length of the smallest chromosome, *E. dunni* and *E. saligna* were classified into category 1B, while *E. grandis* and the interspecific natural hybrid were classified as 1A.

Based on the karyotype asymmetry data obtained by the method of Zarco (1986), together with the chromosome morphology results, we suggest that *E. saligna* is one of the parental species involved in the production of the interspecific hybrid, although the data are not conclusive. The determination of the other parental species involved in the production of the hybrid based only on cytogenetic data is therefore difficult.

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