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Chromosome numbers of the 59 species of Eucalyptus L'Herit. (Myrtaceae)

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Abstract — The paper presents the results of the chromosomal counting of 59 species of genus *Eucalyptus* among which 55 are newly examined. All these species possess $2n = 22$ including the four species for which the numbers $2n = 20$, 24 and 28 have been reported. The number $2n = 24$ previously raported in 9 species appears to result from a break of the chromosome first pair very often observed in the *Eucalyptus* in the metaphase and especially premetaphase stage.

Although some contradictory cases of aneuploid have been reported in the literature, the large homogeneity of the chromosome number 2n = 22, now known for 135 (33.5%) distinct species, makes the genus *Eucalyptus* to be considered as a vast caryologic continuum who probably adopts a process of evolution based fundamentally on chromosome alterations.

Key words: Chromosome, cytogenetics, *Eucalyptus*, *Myrtaceae*.

INTRODUCTION

The *Eucalyptus*, a widespread genus of the *Myrtaceae* family, covers more than 50 % of the Australian territory (Pryor 1978 and Boden 1964) and also spread considerably in many countries of the world (F.A.O,1982). The taxonomy of this genus is complex and requires continuous and varied studies (Pryor 1978 and Johnson 1976). The difficulties are imposed by the considerable intraspecific variability and from the frequent interspecific hybridization favored by the mode of allogame reproduction of the genus (Pryor 1978 and Boden 1964) and from the raised number of species and synonyms (Pryor and Johnson 1971). The contribution of cytogenetics to the solution of the *Eucalyptus* taxonomic problem is still very limited because the chromosomes of this plant group are of small size (1.2 to 2.5 μm according to Atchison 1947) and their staining and their scattering are done with difficulty as for fruit trees (Salesses 1967). In this domain, the main available publications (Suguira1936; Atchison 1947; Darlington and Wyli 1955; Ruggeri 1960a; 1960b; 1960c; 1960d; 1960e and 1961; Moussel 1965; Bolkhoskik *et al.* 1969; Rye 1979; Haque 1984; Vijayakumar and Sybramanian 1985; Matsumoto *et al.* 2000) have permitted the knowledge of the chromosomal number $2n$ and / or n in about 80 species belonging in their large majority to the subgenus *Syphyomurtus* of Pryor and Johnson (1971). The analysis of these data suggests that the genus *Eucalyptus* is concerned with an interspecific and intraspecific variation of the diploid chromosomal number 2n = 20, 22, 24 and 28.

On the basis of $2n = 22$ and $2n = 24$ observed, Ruggeri (1960d; 1960e) suggested two basic numbers for the genus *Eucalyptus*: $x = 11$ and $x = 12$.

The present investigation purpose is to determine the number of mitotic chromosomes of 59 species of genus *Eucalyptus*. For some species previously published with $2n = 20$, 24 or 28, two to three sources have been reexamined to confirm or invalidate the presence of the intraspecific and interspecific variability in the genus *Eucalyptus*.

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MATERIALS AND METHODS

Seed samples of all species of *Eucalyptus* examined were germinated in Petri dishes with filter paper moistened with tap water at 27°C. Roots were collected preferably when they were about 1-4 mm long, beyond they turn brown, harden and contain very little or not metaphasic plates. The pretreatment procedure of TLASKAL (1980) was used with modifications. The germinated seeds were placed in an aqueous mixture of 700 ppm of cycloheximide (Sigma) and 2500 ppm of 8-hydroxyquinoline (Sigma) in presence of an aquarium type aerator, at room temperature for 3 hours. After washing in tap water for 15 minutes, the seeds provided with their roots were fixed in 3:1 (v/v) absolute ethyl alcohol-acetic acid at 4°C for at least 24 hours. Roots were hydrolyzed in 1 N HCl at 60°C for 5 minutes and stained with Schiff's reagent for 1 hour. Root tips were squashed on slides in a drop of iron alum haematoxylin prepared following the methodology described by HENDERSON and READ (1968). This combined staining method gives better results. For each species, the chromosomal number was counted in 75 to 120 metaphase plates extrated from 15 to 20 different roots.

RESULTS AND DISCUSSIONS

Chromosomal numbering results done show that the mitotic cells of all species examined possess whatever their geographical locality and their taxonomic position $2n = 22$ (table 1, Fig.1). The basic number is $x = 11$ corresponding almost all the *Myrtaceae* family (BOLKHOSKIK *et al.* 1969; PRYOR and JOHNSON 1971 and RYE 1979). However, the number $4n = 44$ has been counted in about 5-12 % of cells of very rare individuals of *E. lansdowneana and E. ptychocarpa* (Fig. 2). This situation has also been observed by VIJAYAKUMAR and SUBRAMANIAN (1985) in E. *citriodora*. The chromosomes of the cells with $4n = 44$ appear clearly to be of more reduced size than those of the cells with $2n = 22$ as noticed by JANAKI AMMAL and KHOSLA (1969) in E. *citriodora* artificial tetraploid. The plants (or cells) with $4n = 44$ of the genus *Eucalyptus* are probably less or not viable. The absence of the polyploidy for the genus *Eucalyptus* would be due to the size of the cambial cells, too small to contain a larger chromosome number (JANAKI AMMAL and KHOSLA 1969). According to DARLINGTON (1937), the mechanical difficulty in the chromosomal separation in cambial cells constitutes an barrier to polyploidy in trees. MEHRA (1972) sustains the hypothesis of implication of physiological factors that oppose the proliferation of the polyploidy. It is known that the artificial tetraploids obtained as well for the genus *Eucalyptus* (JANAKI AMMAL and KHOSLA 1969; KAPOOR and SHARMA 1985) that *Pinus* (MEHRA 1972) reveal a slow growth and a weak vigor. This perturbation can be interpreted as an indicator of the natural species inability of the Eucalyptus and similar genus to support the doubling of their chromosome complement.

The numbers 2n = 20, 24 and 28 reported respectively for E. *citriodora* and E. *globulus* (SUGUIRA 1936), E. *flocktoniae* (RUGGERI 1960c) and E. *erythronema* (RUGGERI 1960d) and E. *citriodora* and E. *globulus* (HARRISON 1934) have not been confirmed in any of the different sources investigated (table 1). We think that the number 2n = 24 also signalled in seven other species (E. *redunca* by ATCHISON 1947; E. *corynocalyx* by RUGGERI 1960d and ATCHISON 1947; E. *pyriformis*, E. *forrestiana*, E. *bicolor*, E. *huberiana* and E. *cordierie* by RUGGERI 1960e) result probably from a situation that we have often met with the *Eucalyptus*, the dislocation of the chromosome first pair arms at the level of the centromere. The two arms of the same chromosome sometimes remain connected by a long heterochromatin region, similar to the one observed by RYE (1979) in E. *conglobata.* The bonding of the arms of the two longer chromosomes seems to be very fragile in the metaphase and especially premetaphase stage. After the squashing, each of the two chromosomes often splits into two unequal parts (short and long arm), giving thus the appearance of a supplementary pair that leads to an counting a incorrect. Examples of these fractures are given in fig. 3.

The stability of the chromosomal number limits unfortunately the interest of this study for the taxonomy of the genus *Eucalyptus*. Nevertheless, this constancy reveals the strong degree of closeness of the species of the different subgenera and explain why so many hybrids occur in nature (126 have been cited by PRYOR and JOHNSON 1971), and why fertile artificial hybrids are easily obtained. According to ATCHISON (1947) and MATSUMOTO *et al.* (2000) this homogeneity of the chromosome number also shows that the genus *Eucalyptus* remains a natural group with an evolution founded more on the reorganization of genes than on the alteration of the chromosome number. The *Eucalyptus* can be considered as belonging to the numerous more primitive genus as *Pinus*, *Juglans* and *Michelia* in which the polyploidy is unknown (JANAKI AMMAL and KHOSLA 1969).

CONCLUSION

The results of the present study and the data of the literature raise to 135 (33.5 %) the distinct

Table 1 — Origins of the *Eucalyptus* species examined and chromosomal number 2n observed.

Species	Seed lot no.	Organization	Locality [(a)]	2n[(b)]
E. *amplifolia* Naudin	73/52/10419	FTI	Buladelah NSW Australia,	22*
E *amygdalina* Labill.	73/52 /7022	FTI	North East Branxholm Tasmania Australia	22*
E. *argillacea* W.V. Fitzg.	Prov.3.1037	TFTC	Australia	22*
E. *bleeseri* Blakely	Prov.3.1164	TFTC	Australia	22*
E. *blaxomei* Maiden	Prov.1.291	TFTC	Australia	22*
E. *brassiana*	81/3332 N	TFTC	Capeyork Peninsula Coden Qld. Australia	22*
E. *calycogona* Turcz.	DA1/86/9855	CSIRO	9.5 km From Kondinin Western Australia	22*
E. *cameronii* Blakely & Mckie	Prov.1.161	TFTC	Australia	22*
E. *citriodora* Hook (prov. 1)	8O/2887 N	TFTC	S F 54 Monte Qld. Australia	22
E. *citriodora* Hook (prov. 2)	73/52 /10155	FTI	Parkes NSW Australia	22
E. *cloeziana* F. Muell.	81/3514 N	TFTC	S F 28 Monto Qld. Australia	22*
E. *contracta*	Prov.1. 591	TFTC	Louandjili Congo	22*
E. *crebra* F. Muell.	81/3364 N	TFTC	Australia	22*
E. *cullenii* Cambag.	Prov.2. 834	TFTC	Australia	22*
E. *decorticans* Maiden	Prav.1.415	TFTC	25 Km North West Chinchilla Qld. Australia	22*
E. *deglupta* Blume	80/3288 N	TFTC	Bulolo Rock Pile New Guinea	22*
E. *delegatensis* R.T.Bak	81/3313 N	TFTC	35 Km North Maydena Tasmania Australia	22*
E. *dichromophloia* F.Muell.	Prov.5.1452	TFTC	Porth Hedland Western Australia	22*
E. *erythrocorys* F. Muell.	82/3761 N	TFTC	Australia	22*
E. *erythronema* Turez ssp. *Erythronema*	DA1/86/11270	CSIRO	Kulin Townsite Western Australia	22
E. *ferrugianea* Schauer	Prov.8.1358	TFTC	80 Km West Timber Creek Northern Australia	22*
E. *flocktoniae* Maiden (prov. 1)	73/52 / 7978	FTI	Western Australia	22*
E. *flocktoniae* Maiden (prov. 2)	Dal/86/10417	CSIRO	Condobolin NSW Australia	22*
E. *flocktoniae* Maiden (prov. 3)	A46	FRED	Morocco	22*
E. *foelscheana* F. Muell.	Prov.1.1090	TFTC	3 Km South East Mataraka Northern Australia	22*
E. *globoidea* Blakely	Prov.1. 204	TFTC	11 Km North Wodgoolga NSW Australia	22*
E. *globulus* Labill. ssp. *globulus* (prov. 1)	73/52/5586	FTI	Huan Valley Tasmania Australia	22
E. *globulus* Labill. ssp. *maidenü* (prov. 2)	80/3008 N	TFTC	Lake Coen Gary Highway Western Australia	22
E. *griffithsii* Maiden	DA1/86/9187	CSIRO	6 Km West Kalgoorlie Western Australia	22*
E. *jacobsiana* Blakely	Prov.2. 1296	TFTC	Goyder River pite Gove Northern Australia	22*
E. *jensenii* Maiden	Prov.1. 1191	TFTC	Gulalug Northern Australia	22*
E. *lansdowneana* F. Muell.	DA1/86/12235	CSIRO	30 km Frm Chilpuddiet South Australia	22*
E. *latifolia* F. Muell.	Prov.2.1279	TFTC	35.5 km East Gone Butman North Australia	22*
E. *lesouefii* Maiden	DA1/86/9887	CSIRO	15.3 km South Norsman Western Australia	22*
E. *macrorhyncha* F. Muell. ssp. *cannonii*	DA1/86/10805	CSIRO	3 Km Frm Capertee NSW Australia	22*
E. *mannensis*	Prov.1.1550	TFTC	34.5 Km West Overlandes Western Australia	22*
E. *megacarpa* F. Muell.	DA1/86/12265	CSIRO	East Mundijong Western Australia	22*
E. *megacornuta* C.A.Garden	DA1/86/10400	CSIRO	Finley District NSW Australia	22*
E. *moluccana* Roxb.	Prov.2. 708	TCTF	S F 268 South W. Ingharn Qld. Aust.	22*
E. *microcorys* F. Muell.	73/52/ 6917	FTI	Coffs Harbour NSW Australia	22*
E. *microneura* Maiden & Blakely	Prov.1. 830	TFTC	Mont surprise Qld. Australia	22*
E. *nesophila* Blakely	Prov.3.1173	TFTC	Merville Island North Australia	22*
E. *odontocarpa* F. Muell.	85/ 4901N	TFTC	Billiluna Tasmania Western Australia	22*
E. *oligantha,* Schauer	Prov.1.1309	TFTC	Arnhem Land Réserve North Australia	22*
E. *peltata* Benth.	80/3093 N	TFTC	Mutuan Downs Qld. Australia	22*
E. *pilularis* Sm.	82/3767 N	TFTC	Fraser Island Qld. Australia	22*
E. *planchoniana* F. Muell.	Prov.1.218	TFTC	North Woolgoolga NSW Australia	22*
E. *polycarpa* F. Muell.	Prov.7.1328	TFTC	Drimie Head Melville Gove Australia	22*
E. *preissiana* Schauer	DA1/86/6342	CSIRO	Ongerup area Western Australia	22*
E. *ptychocarpa* F. Muell.	Prov.5.1271	TFTC	102 Km South Maningrida North Australia.	22*
E. *punctata* Dc.	Prov.1.177	TFTC	Graffon Forestry District NSW Australia	22*
E. *setosa* Schauer	Prov.1.1488	TFTC	Australia	22*
E. *resinifera* Sm.	80 / 3097 N	TFTC	South. Ravenshoe Qld. Autralia	22
E. *shirleyi* Maiden	DA1/86/10264	CSIRO	6.4 Km Frm Herberton Qld. Australia	22*
E. *signata*	Prov.2. 170	TFTC	Australia	22*
E. *stoatei* C. A. Gardner	DA1/86/12157	CSIRO	West Wyalong NSW Australia	22*
E. *stricklandii* Maiden	DA1/86/9927	CSIRO	56.6 Km South E. Coolgardie Western Australia	22*
E. *tenuipes* Maiden & Blakely	Prov.1.330	TFTC	Australia	22*
E. *terminalis* F. Muell.	Prov.1.1502	TFTC	Australia	22*
E. *tetrodonta* F. Muell.	Prov.7.1236	TFTC	Arutrem Land North Australia	22*
E. *trachyphloia* F. Muell.	Prov.1.384	TFTC	Australia	22*
E. *umbra* R.T.Bak.	Prov.1.319	TFTC	Australia	22*
E. *youngiana*	DA1/86/12237	CSIRO	17 Km North Watson South Australia	22*

(a): Qld.: Queensland, NSW: New South Wales, CSIRO: Commonwealth Scientific and Industrial Research Organization (Australia), TFTC: Tropical Forest Technique Center (France), FRED: Forestry Research Experimentation Division (Morocco), FTI: Forest Technologic Institute (Algeria). (b): 22*: firstly reported, 22: already reported.

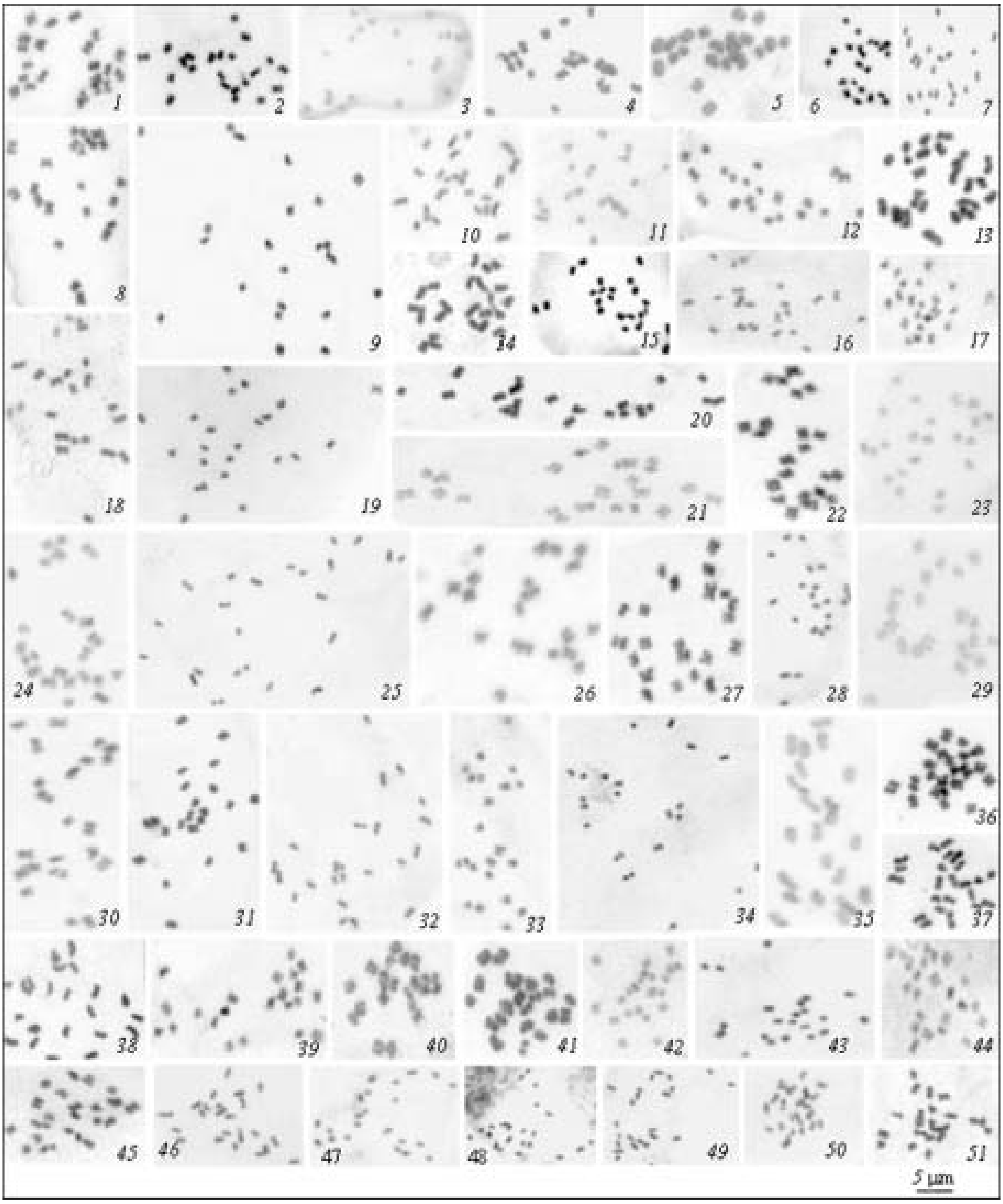

Fig. 1 — Metaphases in root apex of *Eucalyptus* species with $2n$ = 22 chromosomes: 1) E. *delegatensis*, 2) E. *argillacea*, 3) E. *blesseri, 4*) E. *calycogona*, 5) E. *cameronii*, 6) E. *amygdalina*, 7) E. *globulus*, 8) E. *decorticans*, 9) E. *bloxomei*, 10) E. *punctata*, 11) E. *cloeziana*, 12) E. *crebra*, 13) E. *globoidea*, 14) E. *contracta*, 15) E. *jensenii*, 16) E. *ferrugianea*, 17) E. *microcorys*, 18) E. *foelscheana,* 19) E. *erythronema*, 20) E. *microneura*, 21) E. *planchoniana*, 22) E. *jacobsiana*, 23) E. *peltata,* 24) E. *latifolia*, 25) E. *mannensis*, 26) E. *setosa*, 27) E. *tenuipes,* 28) E. *machrorhyncha*, 29) E. *preissiana,* 30) E. *oligantha*, 31) E. *shirleyi*, 32) E. *stoatii*, 33) E. *lesouefii*, 34) E. *umbra,* 35) E. *megacarpa*, 36) E. *grifthsii*, 37) E. *megacornuta*, 38) E. *flocktoniae*, 39) E. *tetrodonta,* 40) E. *polycarpa*, 41) E. *nesophila*, 42) E. *youngiana*, 43) E. *trachyphloia*, 44) E. *terminalis*, 45)) E. *dichromophloia*, 46) E. *erythrocorys*, 47) E. *brassiana*, 48) E. *citriodora,* 49) E. s*ignata,* 50) E. *landsdowneana*, 51) E. *odontocarpa*.

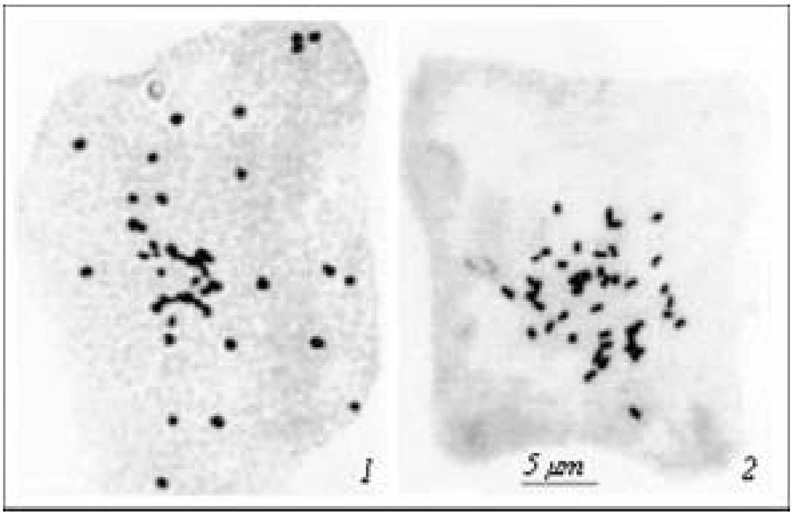

Fig. 2 — Metaphases in root apex of *Eucalyptus* species with 4n = 44 chromosomes in 5 - 12 % of cells: 1) E. *lansdowniana*, 2) E. *ptychocarpa*.

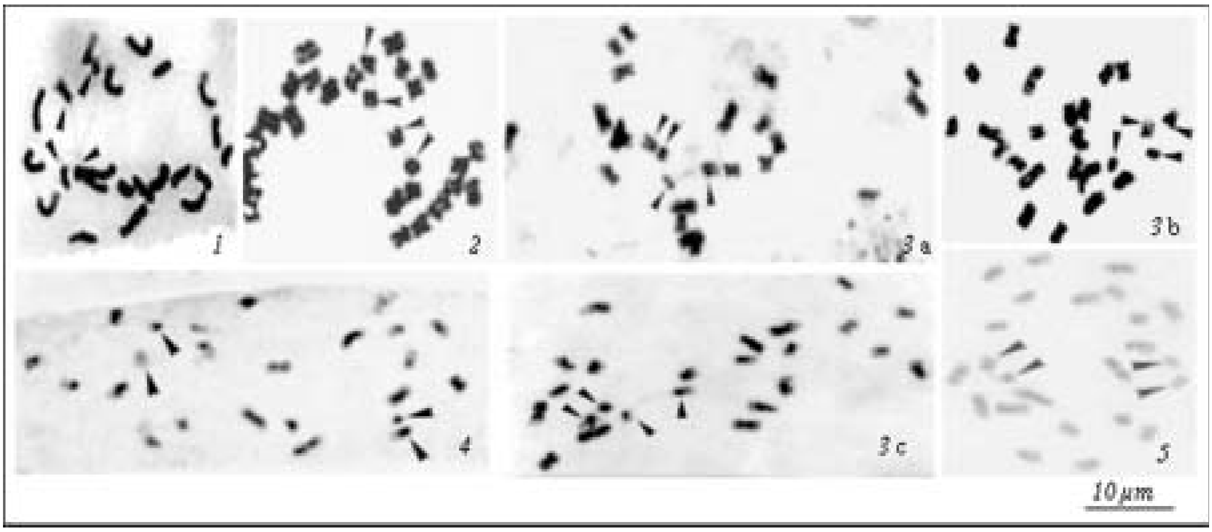

Fig. 3 — Metaphases in root apex of *Eucalyptus* species, showing the accidental dislocations of arms (arrows) of chromosomes of the first pair. The number of chromosomes appears $2n = 23\text{-}24$. The separated arms sometimes remain connected by long heterochromatic trial: 1) E. *globulus*, 2) E. *megacornuta*, 3 a, b, c) E. *citriodora*, 4) E. *resinifera* 5) E. *dichromophloia*.

species total of which the number of chromosomes $2n$ or n is now known. All 59 examined species invariably count 2n = 22. The reexamination of 4 species presumed with 2n = 20, 24 or 28 don't confirm these results. In spite of its ecological and morphological diversity, the genus *Eucalyptus* remains a natural group with a strong intraspecific and interspecific constancy of the chromosome number and keep an unique basic number x = 11. The use of the chromosome number as a useful criterion in the taxonomy of the *Eucalyptus* seems to be less probable. However, the absence of variability of the number of chromosome doesn't necessarily mean that the cytogenetics is without interest for the systematic of the *Eucalyptus*. A discrimination between species groups on the basis of the variability of the chromosome morphology and structure is not excluded. Karyotype comparison studies are in progress for the analysis of resemblances or the dissimilarities of species.

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