Chromosome numbers in two African *Acacia* species

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Summary. Chromosome numbers were surveyed in populations of *Acacia karroo* Hayne and *A. tortilis* (Forssk.) Hayne sampled across their wide geographical range. Only one cytotype, with a chromosome number 2n = 52, was found in populations of *A. karroo* and *A. tortilis* subspecies *tortilis*, *spirocarpa* and *heteracantha*. Both 2n = 52 and 2n = 104 were found in populations of *A. tortilis* subspecies *raddiana*. It was concluded that most common morphological varieties of *A. karroo* and *A. tortilis* subspecies *tortilis*, *spirocarpa* and *heteracantha* are tetraploids, but the possibility of isolated cases of other cytotypes cannot be excluded given that naturally sterile trees have been documented previously in *A. karroo*. The cytotypes of *A. tortilis* are more variable. Clarification will require more detailed analysis.

INTRODUCTION

About 129 species of indigenous acacias are known in Africa (Ross 1979). Of these, only 18 species are widespread, whereas the remainder are more local (Coe & Beentje 1991). Most of the species form part of arid and semi-arid tropical and sub-tropical vegetation. *Acacia karroo* Hayne is the most widespread acacia in southern Africa (Ross 1979). It is distributed from latitude 12°S to 34°S, at the southern tip of Africa (Barnes 1992). Within that area, it ranges from the eastern to the western coast, and is absent only from sites which are very arid, cold and humid, or at high altitudes. *A. tortilis* (Forssk.) Hayne occurs throughout the hot tropical and sub-tropical regions of eastern and southern Africa, the Sudano-Sahelian region, north Africa, and the Middle East (Ross 1979).

Diurnal temperatures in these ecological zones range from 5–50°C, and annual rainfall from 40–1200 mm (Fagg 1991). Both species are intolerant of frost but insensitive to soil type and fertility. Growing as they do in these marginal climatic conditions, the acacias are of great economic importance. They are sources of forage, fuelwood, timber, medicine, fencing material, tannin, dyes and cordage; *A. karroo* is second only to *A. senegal* as a source of sweet gum (Watt & Breyer-Brandwijk 1962). The species are nitrogen-fixing and are useful for soil protection and microclimatic regulation. However, there is immense phenotypic variation within both species; this affects their productivity and utility.

Phenotypic variation of *Acacia karroo* has been reported in several publications (Brenan 1970, Ross 1971a & b, 1973, 1975, 1979, Robertse *et al.* 1981; Von Breitenbach 1989). In some of these, the authors have suggested the establishment of infra-specific taxonomic categories of varieties, subspecies, and even species separate from the "normal *karroo*" (Brenan 1970), a term which is indicative of the ambiguity within the taxon. Detailed investigation of this using

Accepted for publication April 1993.

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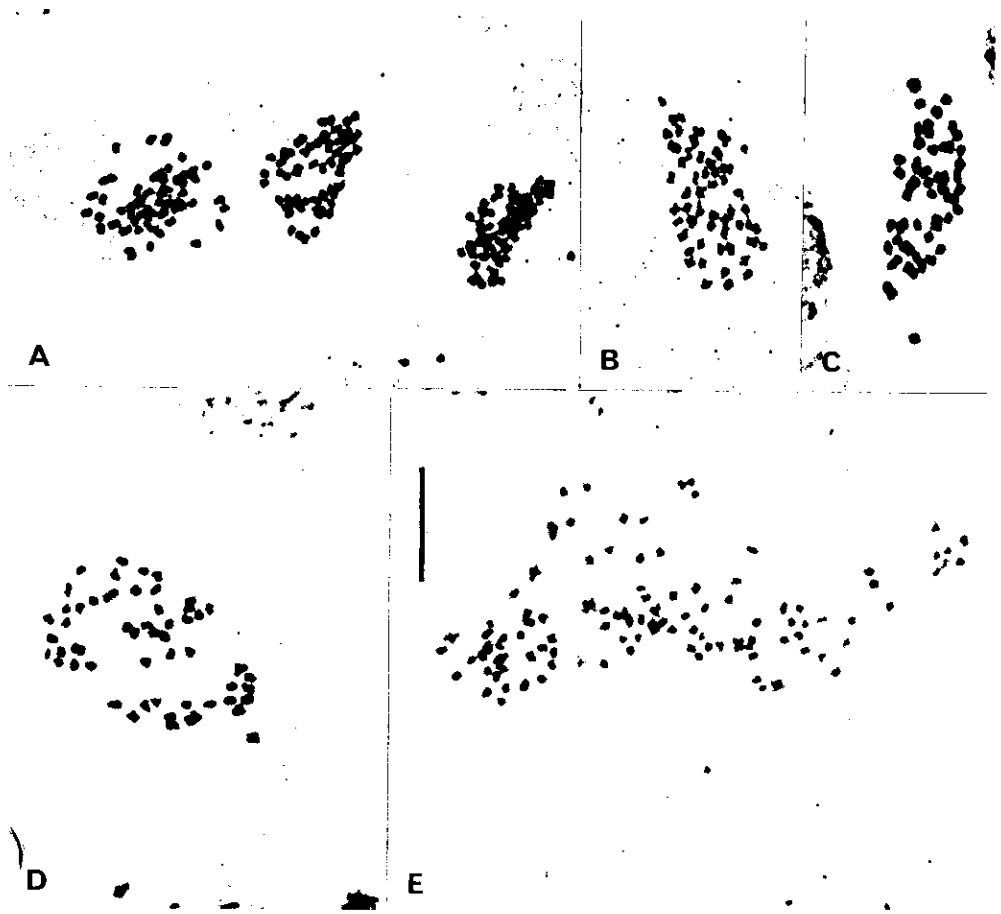

FIG. 1. Somatic chromosomes of *Acacia* species. **A** *A. karroo* (9214), adjacent dividing cells; **B** *A. karroo* (9214), 2n = 52; **C** *A. karroo* (9210), 2n = 52; **D** *A. tortilis* ssp. *spirocarpa* (9177), 2n = 52; **E** *A. tortilis* ssp. *raddiana* (9165), 2n = 104. Scale bar = 10 μm.

morphological features has compounded the situation as new varieties come to light (Robbertse *et al.* 1981), and suggests high genetic differentiation within the taxon. Similarly, *A. tortilis* is widely distributed (Ross 1979) and is morphologically variable across its range, a factor which led to recognition by Brenan (1983) of four subspecies: *tortilis*, *spirocarpa* (A. Rich.) Brenan, *heteracantha* (Burchell) Brenan and *raddiana* (Savi) Brenan.

To understand the extent to which the observed phenotypic variation reflects high genetic diversity, work was initiated to clarify the allozyme polymorphism within and among populations of the species. According to Crawford (1985, 1989), congeneric species are often (but not always) divergent at the genes specifying soluble enzymes. Thus, from the allozymic data, it can be ascertained whether two different morphological types represent different gene pools. However, interpretation of allozyme data depends on knowledge of ploidy level, and thus requires some basic understanding of the cytotypes within a taxon. Recent

TABLE 1. Seed sources and chromosome counts for the populations of *A. karroo* and *A. tortilis* used in the study

Origin				Alt.	Jodrell cyt. accession	2n	subsp.
A. karroo							N/A
Zambia	Lusaka	15°24'S	28°18'E	1280m	929	52	"
Zimbabwe	Umgusa Valley	20°10'S	28°43'E	600m	9210	52	"
S. Africa	Queenstown	31°52'S	26°52'E	1077m	9211	52	"
S. Africa	Hluhluwe	28°03'S	32°03'E	500m	9212	52	"
S. Africa	Manzingwenya	27°15'S	32°46'E	100m	9213	52	"
S. Africa	Hartbeespoort	25°44'S	27°52'E	1326m	9214	52	"
S. Africa	Tugela Mouth	29°20'S	31°16'E	60m	9215	52	"
S. Africa	Vanrhynsdorp	31°35'S	18°43'E	122m	9216	52	"
Malawi	Zomba	15°36'S	35°10'E	1030m	9210–1	52	"
Malawi	Dedza	14°19'S	34°16'E	1300m	9210–2	52	"
S. Africa	Babanango	28°23'S	31°17'E	1300m	9212–1	52	"
S. Africa	Sardinia Bay	33°52'S	25°12'E	58m	9215–1	52	"
A. tortilis							subsp.
Israel	Negev	30°47'N	35°12'E	100m	9172	52	*tortilis*
Israel	Ein-Gedi	31°28'N	35°23'E	-400m	9168	104	*raddiana*
Senegal	Rao	15°56'N	16°23'W	8m	9165	104	*raddiana*
Niger	Filingue	14°30'N	03°17'W	360m	9163	104	*raddiana*
Kenya	Lamu	02°17'S	40°54'E	10m	9171	52	*raddiana*
Kenya	Namukuse	03°33'N	35°55'E	480m	9176	52	*spirocarpa*
Kenya	Marigat	00°28'N	35°58'E	1060m	9177	52	*spirocarpa*
Zimbabwe	Shinga Pan	20°15'S	32°19'E	500m	9179	52	*spirocarpa*
Zimbabwe	Wengesi	19°31'S	32°35'E	550m	9182	52	*heteracantha*
Botswana	Lake Ngami	20°32'S	22°39'E	1000m	9181	52	*heteracantha*

cytological studies, for example, on *Heuchera grossulariifolia* (Wolf *et al.* 1990), *Polystichum talamancanum* (Barington 1990) and *Cystopteris tennesseensis* (Haufler *et al.* 1990) confirm that prior knowledge of cytotype distribution within the populations under study is essential to the interpretation of complex electrophoretic patterns that arise with increasing ploidy level.

The chromosome number of *A. karroo* under its original name of *A. horrida* (L.) Willd., was initially reported by Grimpu (1929, cited by Atchinson 1948, see Darlington & Wylie 1955) as 2n = 52, locating the species among the polyploids of the genus. Atchinson (1948) reported the presence of tetraploid (2n = 52) and octoploid (2n = 104) cytotypes within the *A. tortilis* complex. It is important to note that true *A. horrida* (L.) Willd. occurs only in eastern Africa and Asia (Lock 1989), and is not sympatric with *A. karroo*. Subsequently, the chromosome numbers of *A. karroo* and *A. tortilis* were confirmed by Vassal (1974) and Harmant *et al.* (1975).

In some *Acacia* species, chromosome number varies among the subspecies, as noted above for *A. tortilis* ssp. *heteracantha*, *tortilis*, *spirocarpa* and *raddiana*. Hybrid swarms can also deviate from the normal chromosome number: e.g., the triploid 2n = 39 reported for *A. laeta* by Khan (1951) and later confirmed by Elamin (1976). Although sterile trees of *A. karroo* have been observed in nature (Ross 1971c), their chromosome numbers have not been reported, and they were not investigated in the present study, as seeds were used to produce roots for chromosome assay. No cytological survey to determine the distribution of cytotypes

among the existing ecotypes and proposed varieties has been undertaken for *A. karroo*.

The aims of this study was to ascertain whether there are other cytotypes among the ecotypes of *A. karroo*, and to determine the distribution of cytotypes within populations of the two species, so as to be able to interpret any complexity of allozyme data caused by variability in ploidy level.

Materials and methods

Seeds of *A. karroo* and *A. tortilis* were collected from the various populations listed in Table 1, and maintained in the Oxford Forestry Institute. Seeds from some sub-populations of *A. karroo* had consistently distinctive morphological features including size and thickness of testa, which influenced germination period and growth rate. These observations were not recorded for *A. tortilis*. Ten randomly selected seeds from each population of the two species were germinated in petri dishes at a temperature of about 30°C. The larger seeds of *A. karroo* took longer (3–4 days) to germinate, and their root growth to the required length was more rapid than that from small seeds, which germinated within 24 hours, but were not ready until the third or the fourth day.

The rapidly growing roots were severed when they were about 2–3 cm long and pretreated in saturated aqueous paradichlorobenzene (PDB) at 4°C or in 0.002 M 8-hydroxyquinoline at room temperature (18°C) for 4–7 hours. Pretreated roots were then fixed in freshly prepared acetic ethanol (1:3 parts v/v) for at least one hour, or until required. Roots were hydrolysed in Normal hydrochloric acid at 60°C for 8–12 minutes, then stained in Feulgen (Schiff's reagent) at room temperature in the dark for at least one hour. The stained portion was then chopped into smaller pieces under a microscope. A drop of 2% aceto-orcein was added to aid staining, definition, and spreading of individual cells. Squashing was done under a clean coverslip overlaid with chromatography paper to remove excess stain. Slides were temporarily ringed with rubber solution to stop desiccation before being made permanent with carbon dioxide under pressure (Bowen 1956, cited by Johnson & Taylor 1989) and euparal. Slides were frozen, then dehydrated in absolute ethanol before euparal was applied. Mounted slides were retained in an incubator at a temperature of about 43°C for 2 weeks for the euparal to harden.

Chromosomes were counted and photographs taken on a Zeiss Photomicroscope III under a ×100 oil immersion objective. Permanent slides made from the present survey and negatives of the photographs are stored in The Jodrell Laboratory, Royal Botanic Gardens, Kew.

Results and discussion

The most easily countable chromosomes were obtained by pretreatment with paradichlorobenzene for 4 hours and a hydrolysis time of 8 minutes for *A. tortilis*, and corresponding periods of 7 hours and 12 minutes, respectively, for *A. karroo*. Difficulties in staining root tips of other acacias with Feulgen or aceto-carmine have been reported by Khan (1951), who attributed the problem to tannin content. Other problems noted included the woodiness of slightly older seedlings,

and gumminess which made the penetration of Feulgen and the spreading of individual cells difficult. However, with tender roots, a satisfactory stain and spread could be obtained. The growth rate of the roots after germination directly influenced the mitotic index of the material. Rapidly growing material had a greater number of dividing cells, giving a better chance of achieving good results (Fig. 1A).

The survey revealed that all populations of *A. karroo* studied had only one cytotype with 2n = 52 chromosomes (Table 1, Fig. 1A–C). *A. tortilis* subspecies *spirocarpa*, *tortilis*, and *heteracantha* had the same chromosome number (2n = 52), whereas *A. tortilis* subspecies *raddiana* included both tetraploid (2n = 52) and octoploid (2n = 104) cytotypes (Fig. 1E). The octoploid cytotypes were from Senegal, Niger and Israel, while the tetraploid was from Kenya. Chromosomes of the two species were too small to allow observation of most variations in karyotypes, but in a well squashed cell, with every chromosome separated, some chromosomes in *A. karroo* appeared larger than others (sizes not measured) and could easily confuse estimates of the total number Variation in chromosome size within a nucleus of some *Acacia* species has been documented by Khan (1951) on allopolyploids of *A. arabica* and *A. farnesiana*, with chromosome lengths ranging from 0·9–1·55 μm and 1·0–1·7 μm, respectively. Both species had 52 chromosomes.

Despite the morphological variability of *A. karroo* (Ross 1979), the species has retained a single genome. Hybrids may perhaps occur occasionally in areas of introgression with other *Acacia* species, for example with *A. tenuispina* (Robbertse *et al.* 1981), but such cases should be sampled specifically. Since *A. karroo* is a polyploid, if it hybridizes with other *Acacia* species with similar genomes, the hybrids formed will also have the same complement of chromosomes and identification will not be possible without a thorough study. Brenan (1983), reporting on *A. tortilis*, noted that hybridization even at subspecies level can cause taxonomic uncertainties unless sufficient evidence is studied. Until a detailed examination of karyotypes can be undertaken in these areas of introgression and diversity, it would be difficult to apportion chromosomes to their origin, i.e. the progenitors of polyploids. With such detailed studies, however, one can detect whether the polyploid was formed by doubling of the genome of the same, or a congeneric, species. Such a detailed study of karyotypes could also help to elucidate the nature of infertile *A. karroo*, and its progenitors, since it has been stated that *A. laeta*, a hybrid between *A. senegal* and *A. mellifera*, is a triploid (2n = 39), but remains fertile (Khan 1951; Elamin 1976). Confirmation that all ecotypes sampled are polyploids is useful in allozyme analysis, which in turn will reveal the nature of polyploidy of *A. karroo* (Oballa in prep.), i.e. whether it is an autotetraploid, allotetraploid, or segmental tetraploid. Olng'otie (1992) found, using isoenzyme techniques, that all sampled populations of *A. tortilis* were autopolyploids.

ACKNOWLEDGEMENTS

We would like to thank Dr. J. Burley, Director of Oxford Forestry Institute (OFI), Drs. R. Barnes, P. Kanowski and L. Lockhart for the technical advice and the inter-institutional and financial arrangements to study cytology at Kew, Prof.

M. Bennett, Keeper of the Jodrell Laboratory, Dr. P. Brandham, Head of Cytogenetics, and Ms. M. Johnson of the Jodrell Laboratory, Kew, for training. Sincere gratitude to C. Fagg and D. Boshier of OFI, and Caroline Keown and Jon West of the Jodrell Laboratory, for the crucial assistance, without which initial work would not have been easy.

We are grateful to the Government of the Republic of Kenya for kind permission and assistance to conduct this research work, which was supported by grants from the U.K. Overseas Development Administration (ODA), through the British Council.

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