CARYOLOGIA Vol. 58, no. 1: 78-82, 2005

Karyological observations on *Artemisia alba* Turra (Asteraceae)

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Abstract — *Artemisia alba* Turra from Calabria is karyologically studied ($2n = 18$). From the analysis of literature it results that this species shows three cytotypes, with a rather distinct geographical distribution: 1) diploids with $2n = 18$, previously identified as *A. alba* subsp. *chitachensis* Maire (name which is here typified) or *A. alba* subsp. *glabrescens* (Willk.) Valdés-Bermejo. This diploid cytotype, known from Spain, Morocco and Bosnia-Hercegovina, occurs also in S Italy (Timpa di S. Lorenzo, Calabria). 2) Tetraploids with $2n = 36$, generally identified with the typus subspecies. This cytotype appears the most widespread in northern part of the species range. 3) hexaploids with $2n = 54$, known only from Abruzzo and Apulia. This cytotype seems of more recent origin, and is here considered of probable hybridogen origin between the $2n = 36$ and $2n = 18$ cytotypes. This unit can possibly be identified with *A. columnae* Ten. (= *A. camphorata* var. *garganica* Ten.). These latter names are lectotypified. We tested also the correlation of the ploidy level with the size of pollen-grains.

Key words: *Artemisia,* cytogeography, pollen-grains, taxonomy, typification.

INTRODUCTION

According to Tutin (1976) and Pignatti (1982), *Artemisia alba* Turra is an Euro-Mediterranean species, extraordinarily variable in shape and size of the leaves, indumentum, smell and habit. Both authors emphasize that the *A. alba* complex deserves further investigation on its variability. Owing to the polymorphism of this species, many specific and infraspecific taxa with doubtful taxonomic value were described during the years.

The *A. alba* complex was the object of several karyological studies. Diploid cytotypes are known from Teruel, Spain (Valdés-Bermejo 1985), identified as *A. alba* subsp. *glabrescens* (Willk.) Valdés-Bermejo; from Middle Atlas, Morocco (Quezel 1957; Ouyahya & Viano 1981; 1988), identified as *A. alba* subsp. *chitachensis* Maire, retained endemic to N Africa; from Bosnia-Hercegovina (Aquaro *et al*. 2004). Tetraploid cytotypes $2n = 36$, are reported for N-E Spain (Sobrarbre) and S-W France (Lambinon 1986; Vallès & Torrel 1995; Vallès Xirau & Siljak-Yakovlev 1997); Italy, Sicily (Madonie, Quacella; Raimondo *et al*. 1983) and Tuscany (Posocco 1968; Cesca 1972); Hungary (Bakšay 1958); Bulgaria (Kuzmanov & Ancev 1973; Kuzmanov *et al*. 1986) and finally for material of uncertain origin (Kawatani & Ohno 1964). Hexaploid cytotypes $2n = 54$ are known only for Abruzzo and Apulia, C-S Italy (Capineri *et al*. 1978).

Consequently, our paper aims to critically summarize all these informations, including a new report from the only Calabrian (S Italy) locality of this species. Finally, we tested the correlation between ploidy level and size of pollen-grains and its suitability discriminating some of the already described taxonomic units (such as *A. alba* subsp. *chitachensis* Maire from Morocco, *A. columnae* Ten. from Abruzzo, *A. camphorata* var. *garganica* Ten. from Apulia).

MATERIALS AND METHODS

Plant material. – Living plants of *A. alba* were collected in Calabria, foothills of Timpa di S. Lorenzo, ca. 850 m a.s.l., 17/IV/2002, *Peruzzi*, *Gargano* et *Cesca* (cult. Hort. Bot. Calabria University, accession number 237). Voucher specimens from the same locality of the plants used for karyological analyses are kept in CLU (see over).

Chromosome analysis. – Squash preparations were made from root tips of plants collected *in situ*, according to the following schedule: pretreatment in 0,5% colchicine solution for 4 hours;

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Carnoy fixing for at least 1 hour; hydrolysis in HCl 1 N for 7 minutes at 60°C; staining with leuco-basic fuchsin for 3 hours. Karyotype formulas and terminology are according to LEVAN *et al.* (1964). At least five plates were measured in order to build the idiogram.

Pollen. – 50 pollen-grains for each of eight different specimens (representing all the three cytotypes and typus material of *A. alba* subsp. *chitachensis*, *A. columnae* and *A. camphorata* var. *garganica*) were collected from the *exsiccata* listed in Appendix 1. Subsequently, the pollen was directly observed by Light Microscopy from slides closed with Euparal and measured from microphotographs. We did not taken into account a recent study (CARAMIELLO *et al.* 1995), effected on acetolized pollen-grains of *A. alba* from Piedmont (N Italy), because of the different methodology followed, which possibly can affect the size of pollen-grains.

RESULTS

The chromosome complement of the studied population was with $2n = 18 + 0\text{-}1B$ (Figs. 1A-1B); Chromosome size ranges between 4.5 and 6.5 µm. Karyotype formula can be expressed as follows: $2n = 6m + 4M + 2m + 2sm + 2m + 2st + 0\text{-}1B$ (Fig. 2). A map showing the known ploidy levels in *A. alba* is presented in Fig. 3.

Any significant relation between pollen-grains and ploidy levels was found (Table 1), but for a light tendency of the tetraploid cytotype to have pollen-grains bigger than others. All the cytotypes appeared with almost the same shape and size. However, the typus of *A. columnae* Ten. shows values close to those of our Calabrian population. *A. camphorata* var. *garganica* is somewhat intermediate between the hexaploid population from Abruzzo and the tetraploid one from Sicily. Finally, the typus of *A. alba* subsp. *chitachensis* is close to the Spanish diploid population (*A. alba* subsp. *glabrescens*).

DISCUSSION

Diploid plants, known before only from Morocco, Spain and Bosnia-Hercegovina, occur also in S Italy, with possible presence of B-chromosomes. This latter feature was already evidenced in *A. alba* by POSOCCO (1968), but in tetraploid plants from Tuscany (C Italy).

By examining the known karyological data, summarized in Fig. 3, it seems that the diploids,

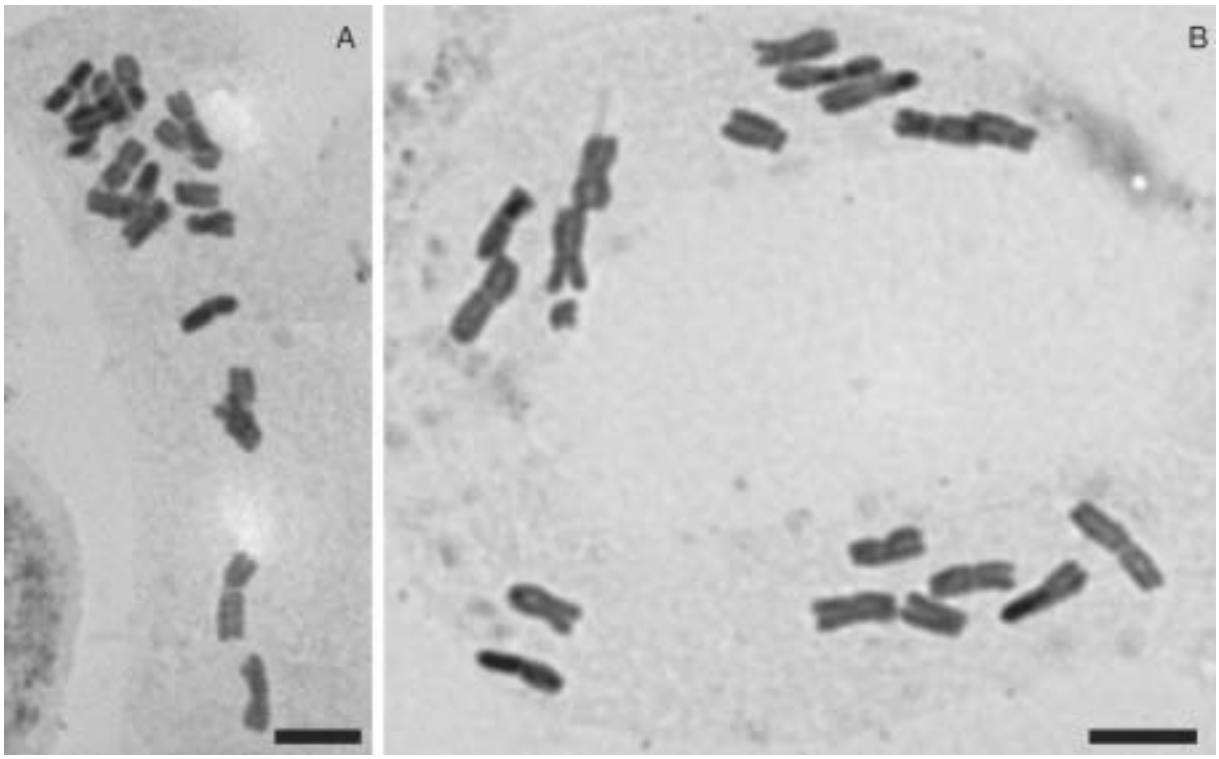

Fig. 1 — *A. alba* from Timpa di S. Lorenzo, Calabria (S Italy), $2n = 18$ (A); $2n = 18 + 1B$ (B). Scale bars = 5µm.

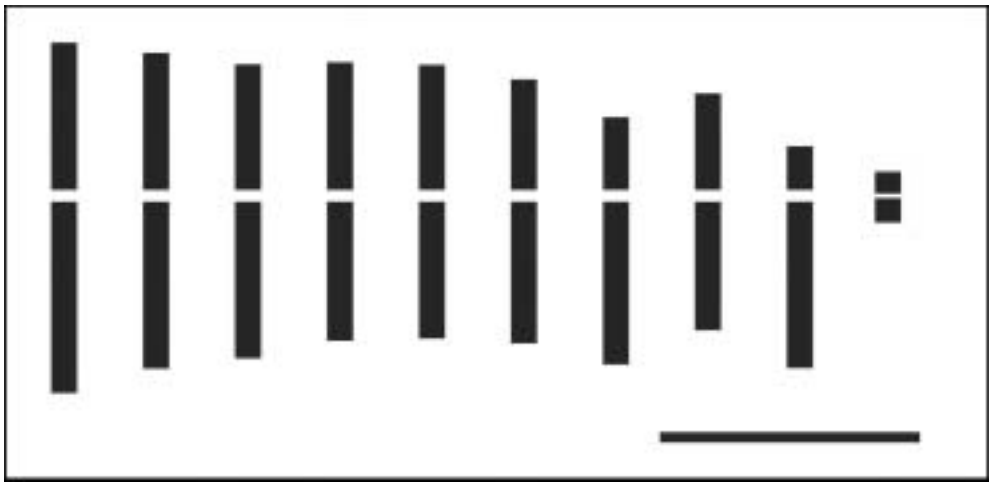

Fig. 2 — Haploid idiogram of *A. alba* from Timpa di S. Lorenzo, Calabria (S Italy). Scale bars = 5µm.

Fig. 3 — Range of *A. alba* Turra (from MEUSEL & JÄGER, 1992, modified). Localities of karyological studies (from literature and the present study) are marked by the indication of the ploidy level. For what concerns Hungary and Bulgaria, the localities are only indicative, because we were not able to find the exact places of $2n = 36$ counts.

assumed here as the most primitives, have a relic value, being separated among them and mainly confined to the most southern part of the range of *A. alba*. The tetraploids appear more widespread, especially in the northern portion of the range of this species. They are probably derived by one or more independent autopolyploidy phenomena, acquiring so characteristics which made they able

Table 1 — Size of pollen-grains in *A. alba* complex from five different provenances of the three known cytotypes and from the types of *A. alba* subsp. *chitachensis*, *A. columnae* and *A. camphorata* var. *garganica.* Measurement are made by polar axis (P) and equatorial axis (E) and expressed in µm.

		10-90 percentiles, with extreme values in brackets	Mean	s	Moda	Median
2*x*						
Spain, Teruel	P	(33,5) 34,1 - 42,0 (48,5)	38,1	3,7	40,3	37,4
	E	(26,3) 29,3 - 39,2 (41,3)	33,7	4	31,3	33
S Italy, Calabria	P	(20,4) 28,5 - 34,9 (36,5)	30,7	4,1	29,5	30,5
	E	(20,5) 24,5 - 30,28 (38,2)	27,7	2,9	26	27,7
4*x*						
Spain, Sobrarbre	P	(38,3) 45,3 - 53,1 (55,5)	49,5	3,5	49,5	49,5
	E	(24,0) 28,0 - 39,5 (41,3)	32,9	4,3	33,5	32,3
Sicily, Madonie	P	(34,25) 35,3 - 41,8 (43)	38,1	2,7	36	37
	E	(25,8) 29,3 - 37,7 (40)	33	3,6	31	32,6
6*x*						
C Italy, Lucoli	P	(31,7) 33,8 - 45,8 (49,5)	38,8	4,4	35,7	38
	E	(22) 23,2 - 33,5 (37,5)	29,1	2,7	28,5	29
C Italy, typus of *A. columnae* Ten.	P	(26,3) 27,0 - 33,5 (35,1)	30,2	2,3	30	30
	E	(18,6) 23,7 - 30,1 (35,5)	26,6	2,8	25	26,3
S Italy, typus of *A. camphorata* var. *garganica*	P	(30,0) 32,4 - 36,8 (38,8)	34,8	2,5	34	34,8
	E	(25,5) 28,5 - 42,1 (49,3)	34,6	7,2	34,3	34,3
Morocco, iso-typus of *A. alba* subsp. *chitachensis* Maire.	P	(29,8) 31,2 - 35,3 (41,8)	33,5	2,5	34	33,3
	E	(24,8) 26,2 - 34,0 (35,8)	30,4	3	28,3	30,4

to colonize temperate territories. The hexaploids, instead, occur only in C-S Italy and are directly bordering with the $2n = 18$ and $2n = 36$ cytotypes. They possibly derived from recent "allopolyploidy" phenomena among the tetraploids, come down along the Apennine, and the diploids, already present as relics in the southern extremity of the Italian peninsula.

For what concerns the analysis of pollen-grain size, in the case of *A. alba* group, the theory that in the polyploids the pollen-grain size is generally bigger than in the diploids seems to be slightly confirmed only for the tetraploid cytotype, while the hexaploids completely overlap with the diploids. All data, well distributed within the range of the species, always show P values > E values, as those reported by Caramiello *et al.* (1995) for plants from Piedmont.

By a taxonomical point of view, diploids can be identified with *A. alba* subsp. *chitachensis* Maire, Bull. Soc. Hist. Nat. Afr. Nord, 19: 55-56 (1928) [Lectotypus (here designated): *Artemisia chitachensis* Coss., Soc. Dauphinoise, n° 3996 (P!, Isotypes in FI!)], or with *A. alba* subsp. *glabrescens*; while tetraploids would represent *A. alba* s.s. The taxonomic identification of the hexaploids is instead more difficult: Capineri *et al.* (1978) have counted $2n = 54$ chromosomes in plants coming from Lucoli (Abruzzo, C Italy) and Manfredonia (Apulia, S Italy). Tenore (1831) described two taxa, presently included in *A. alba* s.l., just for Abruzzo [*A. columnae* Ten., Syll. Pl. Fl. Neapol.: 422 (1831) – Lectotypus (here designated): Assergi[o], s.d., *Tenore* (NAP!)] and for S. Giovanni Rotondo, Gargano, about 15 Km from Manfredonia [*A. camphorata* var. *garganica* Ten., Syll. Pl. Fl. Neapol.: 421 (1831) – Lectotypus (here designated): ex Apulia, s.d., *Gussone* (NAP!, Herb. Tenore, sub *A. crithmifolia*, revised as *A. camphorata* var. *garganica* by Tenore)]. Therefore, it seems logical that one of these two names can be applied to the hexaploid unit. Under our present data, however, it is not possible to draw any definitive taxonomic conclusion.

Acknowledgents — We would like to thank the National Park of the Pollino, that partially funded this study; kindly thanks are due also to: Prof. G. G. Aymonin, curator of the Herbarium of Paris (P); Dr. Annalisa Santangelo, curator of Herbarium of Naples (NAP); Dr. Chiara Nepi, curator of Herbarium of Florence (FI), for the precious help and disposability; Prof. Giovanni D'Amato, University of Rome (RO), which kindly provided capitula of the hexaploid *A. alba*.

Appendix 1 - Specimina visa selecta. – **Morocco**: Djebel Bouachfol, montagne du territoire des Aït Chitachen, prov. de Demnat (Maroc), 5/VII/1882 *Ibrahim* (FI, sub *Artemisia chitachensis* Coss., n° 3996, 4 duplicates, Isotypes of *A. alba* subsp. *chitachensis*); **Spain:** Villar del Cobo (Hispania, prov. Teruel), UTM 30T XK 1490, alt. 1470 m.s.m., in lapidosis calcareis, 21/IX/1979, *Antùnez, Lòpez et Valdés-Bermejo* (FI, sub *A. alba* subsp. *glabrescens*); L'ainsa del Sobrarbre (Espagne, prov. d'Huesca), coord. UTM: 31T BH 63.00, bord de

la route vers Boltaña à 1-2 Km de l'Ainsa, alt. 570 m, talus ensoillé tré chaud en été et fumé par infiltrations, soulone très protégée et d'aspect méditerranéen (olivier, pin d'Alep, romarin...), 15/IX/1977, *Montserrat* (FI); **Italy, Abruzzo**: Assergi, s.d., *Tenore* (NAP, lectotypus of *A. columnae*); Lucoli, L'Aquila, 2*n* = 54, X/1979, *s.c.* (RO); **Apulia**: ex Apulia, s.d., *Gussone* (NAP, sub *A. chritmifolia*, lectotypus of *A. camphorata* var. *garganica*); **Calabria**: Timpa di S. Lorenzo (S. Lorenzo Bellizzi, Cosenza), 24/IX/1993, *Bernardo* (CLU, n. 3069); **Sicily**: Quacella, Vallone Madonna degli Angeli (Madonie, Sicilia), 27/V/1996, *Bernardo* et *Passalacqua* (CLU, n. 3065).

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