



LOW BASE NUMBERS AND DYSPOIDY IN ANNUAL *HELICHRYSUM* MILL. (ASTERACEAE: GNAPHALIEAE)

MERCÈ GALBANY-CASALS^{1*}, ALFONSO SUSANNA¹, AND JULIÁN MOLERO BRIONES²

¹*Institut Botànic de Barcelona (CSIC-ICUB),
Psg. Migdia s/n, E-08038 Barcelona, Spain*

²*Departament de Productes Naturals, Biologia Vegetal i Edafologia,
Universitat de Barcelona, Avda. Joan XIII s/n, 08028 Barcelona, Spain*

Received June 22, 2009; revision accepted October 15, 2009

We report one new base number for *Helichrysum*, $x = 5$, found in the South African species *H. indicum* ($2n = 10$), and additional evidence for the recently documented $x = 4$ found in several South African species: *H. litorale* ($2n = 8$), *H. spiralepis* ($2n = 8$) and *H. zwartbergense* ($2n = 8$). Dysploidy is shown as an additional evolutionary trend within the genus, apart from polyploidy which is most extensive and documented. The association of dysploidy processes with the acquisition of an annual life cycle and adaptation to aridity is discussed for these species and other plant groups.

Key words: *Helichrysum* evolution, karyology, karyotype symmetry, polyploidy.

INTRODUCTION

The genus *Helichrysum* Mill. (Asteraceae, Gnaphalieae) comprises ~500 (Hilliard, 1983) to ~600 (Anderberg, 1991; Bayer et al., 2007) species, distributed mainly on the African continent and Madagascar, but also with ~45 of them distributed in the Mediterranean basin, Macaronesia and Asia.

With regard to life cycle, the genus includes species ranging from annual plants to trees, although most are subshrubs or shrubs, or perennial herbs with basal leaf rosettes. Annual herbs are rare and only about 6% of the ~245 species from Southern Africa are annual (Hilliard, 1983). One example is *Helichrysum indicum* (L.) Grierson, an endemic to the SW Cape in South Africa, classified in group 15 in Hilliard's (1983) treatment of southern African species, and which occurs on sandy soils in open patches of shrubby communities. A group of species interesting in terms of life cycle are members of group 14 (Hilliard, 1983). Hilliard (1983) expressed her uncertainty about most of these species' life cycle: while some were considered to be exclusively annuals, others, for example *Helichrysum litorale* H. Bol., *H. spiralepis* Hilliard & B. L. Burtt. and *H. zwartbergense* H. Bol., are probably perennials

because they have woody roots although their stems are annual. Annual and perennial cycles could appear in different individuals of the same species, depending on the particular micro-environmental and annual weather conditions.

The chromosome number most commonly found in *Helichrysum* is $2n = 28$, mainly in Mediterranean, Macaronesian and Asiatic species, but in some African species as well. Some records of $2n = 14$ for African species and $2n = 56$ for some East Mediterranean and Asiatic species are also known (Galbany-Casals and Romo, 2008). We know of only two records of $2n = 42$ in the literature (Febles, 1989; Galbany-Casals and Romo, 2008). These data indicate a predominant base number of $x = 7$, as Namur and Verlaque (1976) first suggested. The existence of four different ploidy levels based on $x = 7$ shows polyploidy as the most significant evolutionary trend of chromosome number within the genus, as discussed in a previous paper (Galbany-Casals and Romo, 2008). However, a new base number was recently documented for the genus, $x = 4$, found in *H. litorale*, one of the species of group 14 (Hilliard, 1983) cited above (Galbany-Casals and Romo, 2008), which indicated the additional presence of dysploidy in the chromosome number evolution of the genus.

*email: pallenis@yahoo.com

TABLE 1. Localities and vouchers of the studied species. $2n$ – somatic chromosome number

Taxon	Locality and voucher	$2n$	Comments
<i>H. litorale</i> H. Bol.	Republic of South Africa, Eastern Cape Province, Port Alfred, 5 Feb 2007, Arrabal, Galbany, Koekemoer & Romo 14500 (BC 867722)	8	Previous references: $2n = 8$ (Galbany-Casals and Romo, 2008)
<i>H. spiralepis</i> Hilliard & B. L. Burt.	Republic of South Africa, Eastern Cape Province, Katberg Pass, 4 Feb 2007, Arrabal, Galbany, Koekemoer & Romo 14479 (BC 867706)	8	First count
<i>H. zwartbergense</i> H. Bol.	Republic of South Africa, Western Cape Province, Swartberg Pass, 6 Feb 2007, Arrabal, Galbany, Koekemoer & Romo 14520 (BC 867739)	8	First count
<i>H. indicum</i> (L.) Grierson	Republic of South Africa, Western Cape Province, Worcester, Langeberg Mts., 10 Feb 2007, Arrabal, Galbany, Koekemoer & Romo 14547 (BC 867758)	10	First count
<i>H. globosum</i> Schultz-Bip.	Kenya, Mount Kenya, between Minto's Hut and Meru Lodge, 13 Sep 2006, Arrabal & Galbany s. n. (BC 867838)	14	Previous references: $2n = 14$ (Thulin, 1970; Hedberg and Hedberg, 1977)
<i>H. patulum</i> (L.) D. Don	Republic of South Africa, Western Cape Province, southern slopes of Swartberg Pass, 6 Feb 2007, Arrabal, Galbany, Koekemoer & Romo 14507 (BC 867729)	14	Previous references: $2n = 14$ (Galbany-Casals and Romo, 2008)
<i>H. setosum</i> Harv.	Tanzania, between Bulati and Empaakai crater, 5 Feb 2006, Arrabal & Galbany s. n. (BC 867824)	14	First count
<i>H. pendulum</i> (C. Presl) C. Presl	Spain, Balearic Islands, Majorca, Cingles d'es Racó, 8 May 1997, Sáez s. n. (BCN 6118)	28	Previous references: $2n = 28$ (D'Amato, 1971, sub. <i>H. rupestre</i> var. <i>stramineum</i> and sub. <i>H. rupestre</i> var. <i>pendulum</i> ; Cardona, 1976, sub. <i>H. fontanesii</i> ; Galbany-Casals and Romo, 2008)
<i>H. serotinum</i> (DC.) Boiss.	Spain, Tarragona, Ports de Beseit, road to Mt. Caro, 28 Jul 2001, Blanco, Galbany & Lanau s. n. (BCN 6120)	28	Previous references: $x = 14$ (Lorenzo-Andreu and García Sanz, 1950); $2n = 28$ (Galbany-Casals and Romo, 2008).
<i>H. plicatum</i> DC.	Turkey, Konya, between Çukuryurt pass and Gevne Valley, 25 km from Taşkent, 31 Jul 2002, Susanna 2276 et al. (BCN 25366)	56	Previous references: $2n = 56$ (Namur and Verlaque, 1976; Galbany-Casals and Romo, 2008)

Previously, Turner (1970) provided chromosome counts for several Australian *Helichrysum* species that showed variation of base numbers comprising $x = 8, 10, 11, 12$ and 14 . However, several phylogenetic works based on morphology (Anderberg, 1991) and on DNA sequences (Bayer et al., 2000; Galbany-Casals et al., 2004) lately suggested the need to exclude the Australian and New Zealand species from *Helichrysum*. Therefore, only $x = 7$ and $x = 4$ would remain as the known base numbers for the genus.

Nevertheless, chromosome number is known for only about 10–12% of the species of this large genus; the African species in particular are very poorly known in terms of karyology. To our knowledge, the karyotype of only one Western Mediterranean species, *H. crassifolium* (L.) D. Don, has been studied (Castro and Rosselló, 2007). In this paper we provide chromosome number and

karyotypes for a group of annual South African species, and compare them with the chromosome number and karyotypes of several perennial species representing chromosome variability within the genus.

MATERIALS AND METHODS

We focused on four South African *Helichrysum* species, in view of their particular life cycle characteristics: *H. litorale*, *H. spiralepis*, *H. zwartbergense* and *H. indicum*. Some additional species have been included as well for comparison purposes, with the aim of representing the maximum chromosome variability of perennial species within the genus. Three are African species: *H. globosum* Schultz-Bip., which is found quite widespread in several tropical African countries; *H. setosum* Harv.,

TABLE 2. Karyological data of the studied species. N – number of mitotic metaphases measured; $2n$ – somatic chromosome number; SD – standard deviation; chromosomal formulae according to Levan et al. (1964): m – metacentric; sm – submetacentric; st – subtelo-centric; L% and S%, according to Bazzichelli (1967); A_1 – intrachromosomal asymmetry index (Romero Zarco, 1986); A_2 – interchromosomal asymmetry index (Romero Zarco, 1986); Steb. – Stebbins symmetry category

Taxon	N	$2n$	Karyotype length (μm) \pm SD	Length range (μm)	Chromosomal formula	L%	S%	A_1	A_2	Steb.
<i>H. litorale</i>	5	8	31.14 \pm 4.12	5.98–9.16	2 sm + 6 st	77.73	22.27	0.69	0.50	4A
<i>H. spiralepis</i>	5	8	29.64 \pm 2.74	5.64–9.8	2 m + 2 sm + 4 st	77.15	22.85	0.65	0.51	3A
<i>H. zwarbergense</i>	5	8	23.56 \pm 2.32	5.12–6.65	2 sm + 6 st	75.34	24.66	0.66	0.49	4A
<i>H. indicum</i>	5	10	28.30 \pm 5.69	4.32–7.29	2 sm + 8 st	78.47	21.53	0.73	0.18	3A
<i>H. globosum</i>	4	14	34.61 \pm 7.04	3.95–6.29	6 m + 8 sm	64.60	35.40	0.45	0.14	2A
<i>H. patulum</i>	5	14	35.95 \pm 2.82	3.76–7.23	12 m + 2 sm	60.39	39.61	0.33	0.21	2A
<i>H. setosum</i>	6	14	29.64 \pm 3.95	3.47–5.40	10 m + 4 sm	60.66	39.34	0.35	0.14	2A
<i>H. pendulum</i>	8	28	21.10 \pm 1.76	1.10–2.08	24 m + 4 sm	57.21	42.79	0.25	0.18	2A
<i>H. serotinum</i>	7	28	20.18 \pm 2.10	1.09–2.22	24 m + 4 sm	58.43	41.57	0.28	0.22	2A

which grows in tropical Africa and Southern Africa and was classified in group 30 by Hilliard (1983); and *H. patulum* (L.) D. Don, which is endemic to the South and Southwest Cape in South Africa and belongs to Hilliard's group 18 (Hilliard 1983). Two more are Mediterranean species with $2n = 28$, *H. pendulum* (C. Presl) C. Presl and *H. serotinum* (DC.) Boiss., and finally *H. plicatum* DC., which grows in the Eastern Mediterranean area and Western Asia and has $2n = 56$ (Tab. 1).

Chromosome counts were made from somatic metaphases using squash technique. Seeds collected in the wild were used. Root tip meristems were obtained by germinating seeds on wet filter paper in Petri dishes at room temperature. Samples were pretreated with 0.05% colchicine for 2 h 15 min at room temperature. The material was fixed in 3:1 v/v absolute ethanol : glacial acetic acid for a minimum 24 h at 4°C. Meristems were hydrolyzed in 5N HCl for 50 min at room temperature. They were stained in 2% acetic orcein for a minimum 3 h at 4°C. Squashes were made in 45% acetic acid. Preparations were fixed by freezing with CO₂, ethanol-dehydrating, and mounting in Canada Balsam. Photographs were taken through a Zeiss Standard microscope with an Olympus C3030 camera.

For each studied species, 4–8 metaphases were selected for measurements in order to characterize their karyotype, except for *H. plicatum*, which was excluded from this part of the study because of technical difficulties in obtaining a sufficient number of good metaphases. The nomenclature used for describing chromosome morphology follows Levan et al. (1964). Idiograms were drawn based on mean measurements and arranged in order of decreasing size. For numerical characterization of the karyotypes the following parameters were calculated:

total karyotype length; chromosome length range; contribution of each arm of each chromosome to total length of karyotype according to Bazzichelli (1967) (L%, contribution of long arm; S%, contribution of short arm); asymmetry indices A_1 (intrachromosome) and A_2 (interchromosome) according to Romero Zarco (1986); and asymmetry categories according to Stebbins (1971).

RESULTS

Three base numbers are found within *Helichrysum*: $x = 4$, $x = 5$ and $x = 7$, the latter represented by diploids ($2n = 14$), tetraploids ($2n = 28$) and octoploids ($2n = 56$) (Fig. 1; Tab. 1). From Table 2, where chromosomal characteristics for each species are given, and Figure 2, where haploid idiograms are shown, two main groups can easily be distinguished by karyotype characteristics: one group is formed by *H. litorale*, *H. spiralepis*, *H. zwarbergense* ($x = 4$), and *H. indicum* ($x = 5$), which have rather asymmetrical karyotypes (Stebbins' categories 3A and 4A, the predominance of st chromosomes, and high A_1 index) composed of large chromosomes; the second group comprises species with $x = 7$, which generally present more symmetrical karyotypes (Stebbins' category 2A, the predominance of m chromosomes, and much lower A_1 index). In Figure 3, where species are plotted for the relationship between A_1 and A_2 Romero Zarco asymmetry indices (Romero Zarco, 1986), four groups are seen: the first including the two Mediterranean species; the second including the African species with $2n = 14$, which are very close to the Mediterranean species; the third including the South African species with $2n = 8$; and the fourth, containing only *H. indicum*, with $2n = 10$.

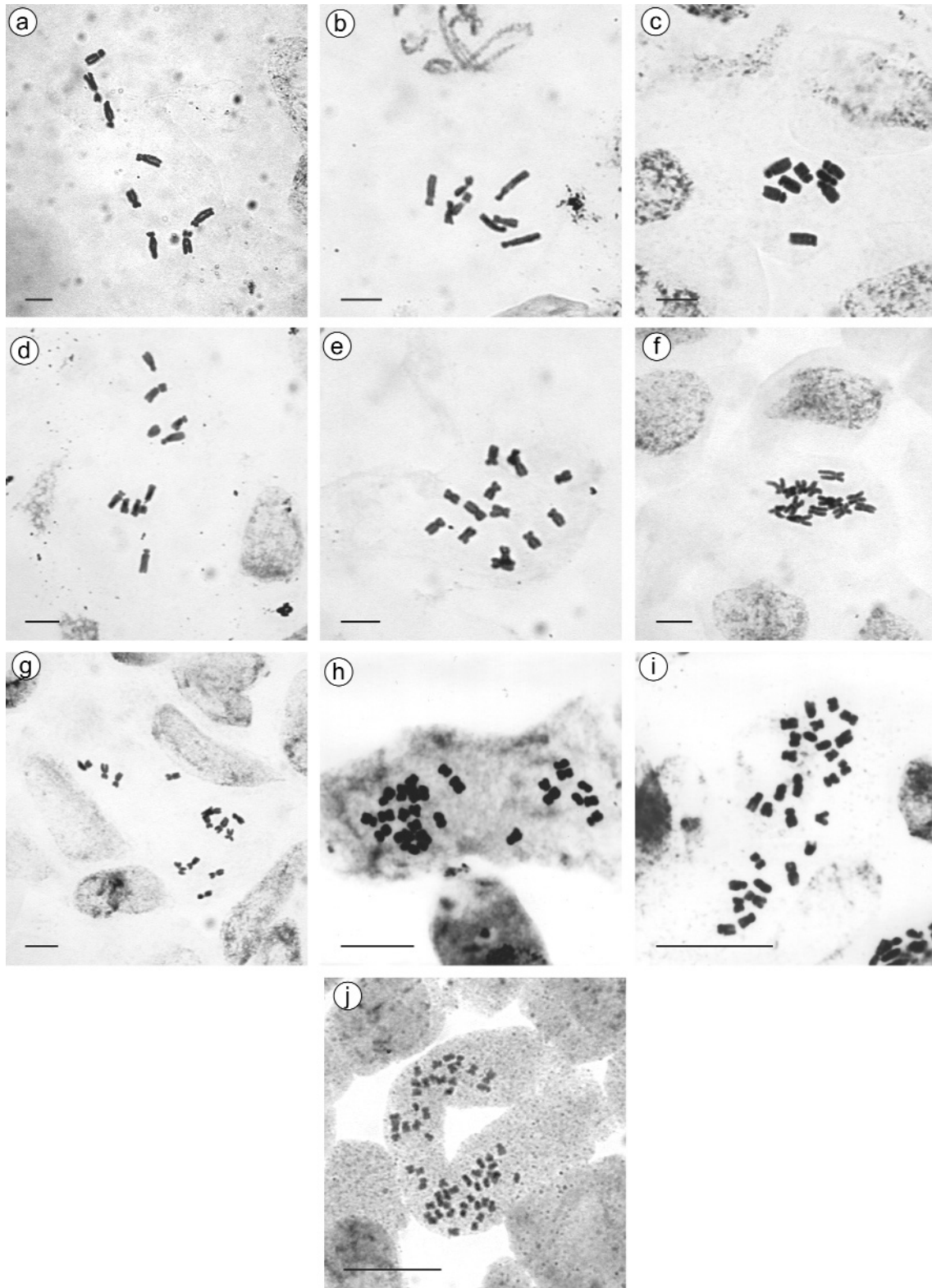


Fig. 1. Mitotic metaphases. **(a)** *H. litorale* ($2n = 8$), **(b)** *H. spiralepis* ($2n = 8$), **(c)** *H. zwartbergense* ($2n = 8$), **(d)** *H. indicum* ($2n = 10$), **(e)** *H. globosum* ($2n = 14$), **(f)** *H. patulum* ($2n = 14$), **(g)** *H. setosum* ($2n = 14$), **(h)** *H. pendulum* ($2n = 28$), **(i)** *H. serotinum* ($2n = 28$), **(j)** *H. plicatum* ($2n = 56$). Bar = 10 μm .

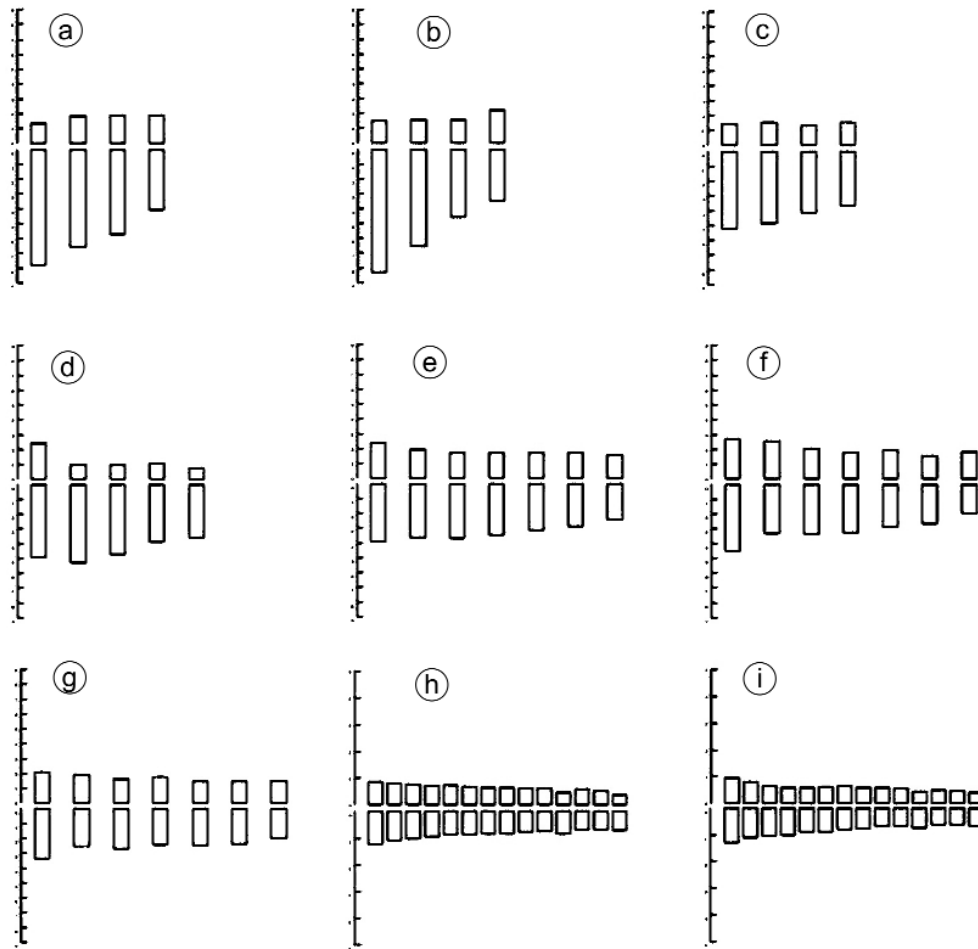


Fig. 2. Haploid idiograms of the studied species. Chromosome number and chromosomal formula are indicated for each one: **(a)** *H. litorale* ($2n = 8$; 2 sm + 6 st), **(b)** *H. spiralepis* ($2n = 8$; 2 m + 2 sm + 4 st), **(c)** *H. zwartbergense* ($2n = 8$; 2 sm + 6 st), **(d)** *H. indicum* ($2n = 10$; 2 sm + 8 st), **(e)** *H. globosum* ($2n = 14$; 6 m + 8 sm), **(f)** *H. patulum* ($2n = 14$; 12 m + 2 sm), **(g)** *H. setosum* ($2n = 14$; 10 m + 4 sm), **(h)** *H. pendulum* ($2n = 28$; 24 m + 4 sm), **(i)** *H. serotinum* ($2n = 28$; 24 m + 4 sm). Bars graduated in μm .

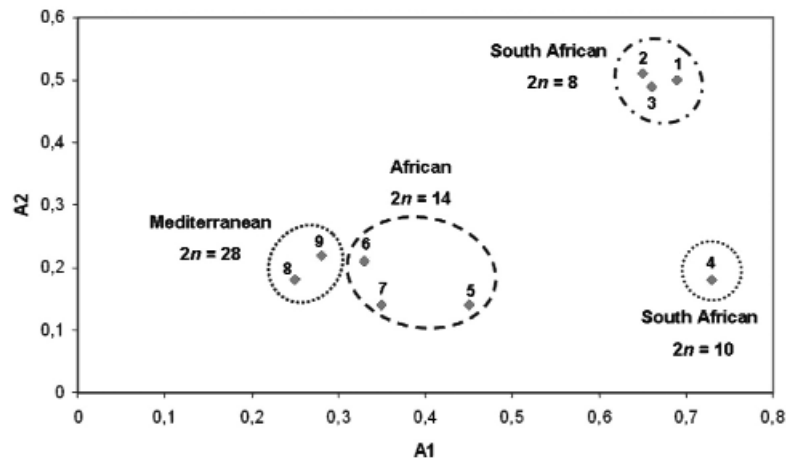


Fig. 3. Scatter plot of the Romero Zarco asymmetry indices. Values of A1 and A2 are summarized in Table 2. Species codes: 1. *Helichrysum litorale*; 2. *H. spiralepis*; 3. *H. zwartbergense*; 4. *H. indicum*; 5. *H. globosum*; 6. *H. patulum*; 7. *H. setosum*; 8. *H. pendulum*; 9. *H. serotinum*.

DISCUSSION

Our results provide evidence of a new base number in the genus *Helichrysum*, $x = 5$. Moreover, base number $x = 4$ is documented for two additional species, after the first record of this number for *H. litorale* in Galbany-Casals and Romo (2008). This finding slightly extends the small list of genera with such reduced base numbers within the whole tribe Gnaphalieae. Up to now it has seemed that the only documented cases are two species of the genus *Leysera* with $2n = 8$ [*L. gnaphalodes* (Nordenstam, 1967) and *L. tenella* (Bremer, 1978)], one species of *Nestlera*, *N. biennis* with $2n = 10$ (Bremer, 1976), two species of *Rhynchopsidium* also with $2n = 10$ [*R. pumilum* and *R. sessiliflorum* (Bremer, 1976)], *Pogonolepis stricta* with $2n = 8$ or $2n = 10$ (Short, 1986; Watanabe et al., 1999), *Trichanthodium skirrophorum* with $2n = 8$ (Short, 1990; Watanabe et al., 1999) and *Epitriche demissus* with $2n = 10$ (Watanabe et al., 2007). Some species within the tribe have an even lower chromosome number, $2n = 6$: *Podolepis capillaris* (Konishi et al., 2000), *Trichanthodium baracchianum*, *T. exilis* (Watanabe et al., 1999), *T. scarletianum* (Short, 1990; Watanabe et al., 1999) and *Sondottia connata* (Watanabe et al., 1999).

The finding of these two base numbers adds evidence for the importance of dysploidy as an evolutionary trend in the karyological evolution of the genus, apart from extensive and well documented polyploidy. Within the Gnaphalieae there are also several examples of infrageneric dysploidy series in Australian genera: *Angianthus* ($n = 13, 12, 6$), *Asteridea* ($n = 9, 7$), *Millotia* ($n = 13, 11, 10, 8$) and *Pogonolepis* ($n = 6, 5, 4$), among others (Watanabe et al., 1999). The differences in karyotype formulae (Tab. 2) and asymmetry indices (Fig. 3) suggest that dysploidy in *Helichrysum* is associated with deep structural changes in chromosomes. However, total genome size, interpreted from total karyotype length (Tab. 2), does not show notable differences between species with $2n = 14$ and species with $2n = 8$ and 10 , although there is an evident decrease in chromosome length, correlated with an increase in chromosome number and ploidy level (Tab. 2). Insufficient sampling may account for the gap between $2n = 14$ and $2n = 10$ and $2n = 8$; cytotypes or species of *Helichrysum* with intermediate numbers ($2n = 12$) belonging to the same or else to other African clades could exist.

It is generally believed that an annual life cycle can increase the overall evolutionary dynamics of a particular genome. It is also thought that more and faster evolutionary divergence enables annual plants to penetrate less favorable and more unstable habitats (Ehrendorfer, 1970). This evolutionary

divergence can be represented by increased variability of chromosome number within a group of related plants (Nagl and Ehrendorfer, 1974). In particular, descending dysploidy is considered an active evolutionary mechanism in plants, especially in the Asteraceae (Garnatje et al., 2004, and references within). Garnatje et al. (2004) hypothesized adaptation to xeric habitats as a trigger of processes leading towards annuality and descending dysploidy in the *Xeranthemum* group (Cardueae), where perennial mountain species of *Amphoricarpos* have $x = 14$ while annual xerophytes of the genera *Chardinia*, *Siebera* and *Xeranthemum* show a complex dysploid series from $x = 12$ to $x = 5$. Subtribe Centaureinae (Cardueae) also shows a complex descending dysploid chromosome series ranging from $x = 16$ to $x = 7$ (Garcia-Jacas et al., 1996), in which species adapted to more extreme habitats show lower chromosome numbers, correlated with other secondary adaptations that suggest a similar course of evolution (Garcia-Jacas and Susanna, 2009). Watanabe et al. (1999) also found a relationship between low chromosome numbers, annual habits and dry habitats in the Australian Gnaphalieae genera *Pogonolepis*, *Sondottia* and *Trichanthodium*. Finally, Selvi and Bigazzi (2002) also hypothesized descending dysploidy associated with a tendency to shorten the life cycle in *Nonea* (Boraginaceae) as an adaptation to arid habitats.

In our study, the annual life cycle or at least annuality of the aerial part of the plant in *H. litorale*, *H. zwartbergense* and *H. spiralepis* would agree with the observed low chromosome numbers. Moreover, with the exception of *H. spiralepis* and *H. lineatum*, the species of group 14 (Hilliard, 1983) are, in general, distributed in the Cape, some of them also in Namibia, and they are adapted to arid environments; the same is true of *H. indicum*, endemic to the SW Cape. However, until more chromosome number data are available and the molecular phylogeny of this huge genus is worked out comprehensively, we will not know whether the low chromosome numbers found in *Helichrysum* are due to descending, ascending or mixed (both ascending and descending starting from $x = 5$) dysploidy.

CONCLUDING REMARKS

Although polyploidy is known to play a very important role in *Helichrysum* evolution (Galbany-Casals and Romo, 2008), karyological evolution within the genus is shown here to be more complex than has been thought, since it becomes evident that dysploidy is also a significant force which seems to be correlated with an annual habit and adaptation to

arid habitats. The extent of this phenomenon in *Helichrysum* is unknown because the chromosome data for this large and not satisfactorily delimited genus is sparse. We present our data as a starting point and a call for further karyological work focused on African *Helichrysum* species in the coming years.

ACKNOWLEDGEMENTS

We thank Sebastian Arrabal, Marinda Koekemoer and Ángel Romo for their assistance with field collections, Julie Ready for her help with identifications, Sara López and Núria Garcia-Jacas for technical assistance, two anonymous reviewers for helpful comments on the manuscript, and Samuel Pyke for English revision. This work was financed in part by the Spanish government (CGL2006-01765/BOS) and Generalitat de Catalunya ("Ajuts a grups consolidats" 2005/SGR/00344).

REFERENCES

- ANDERBERG AA. 1991. Taxonomy and phylogeny of the tribe *Gnaphalieae* (Asteraceae). *Opera Botanica* 104: 1–195.
- BAYER RJ, PUTTOCK CF, and KELCHNER SA. 2000. Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. *American Journal of Botany* 87: 259–272.
- BAYER RJ, BREITWIESER I, WARD J, and PUTTOCK CF. 2007. Tribe Gnaphalieae (Cass.) Lecoq & Juillet (1831). In: Kadereit JW and Jeffrey C [eds.], *The Families and Genera of Vascular Plants*, vol. 8. *Asterales*, 246–284. Springer Verlag, Berlin, Heidelberg, Springer.
- BAZZICHELLI G. 1967. Studi del ciclo del *Leucanthemum atratum* (Jacq.) DC. sensu amplo. *Annali di Botanica*. Rome 29: 385–391.
- BREMER K. 1976. The genus *Relhania* (Compositae). *Opera Botanica* 40: 1–86.
- BREMER K. 1978. The genus *Leysera* (Compositae). *Botaniska Notiser* 131: 369–383.
- CARDONA MA. 1976. Contribución al estudio citotaxonomico de la flora de Baleares. IV. *Lagascalia* 6: 265–274.
- CASTRO M, and ROSSELLO JA. 2007. Karyological observations on plant taxa endemic to the Balearic Islands. *Botanical Journal of the Linnean Society* 153: 463–476.
- D'AMATO G. 1971. In Numeri cromosomici per la Flora Italiana. *Informatore Botanico Italiano* 3: 124–157.
- EHRENDORFER F. 1970. Evolutionary patterns and strategies in seed plants. *Taxon* 19: 185–195.
- FEBLES R. 1989. Estudios en la flora Macaronésica: algunos números de cromosomas VI. *Botánica Macaronésica* 17: 57–71.
- GALBANY-CASALS M, and ROMO A. 2008. Polyploidy and new chromosome counts in *Helichrysum* Mill. (Asteraceae, Gnaphalieae). *Botanical Journal of the Linnean Society* 158: 511–521.
- GALBANY-CASALS M, GARCIA-JACAS N, SUSANNA S, SÁEZ L, and BENEDÍ C. 2004. Phylogenetic relationships in the Mediterranean *Helichrysum* (Asteraceae, Gnaphalieae) based on nuclear rDNA ITS sequence data. *Australian Systematic Botany* 17: 241–253.
- GARCIA-JACAS N and SUSANNA A. 2009. Cardueae. In: Funk VA, Susanna A, Stuessy T, and Bayer RJ [eds.], *Systematics, Evolution, and Biogeography of the Compositae*, 293–313. International Association of Plant Taxonomists, Vienna.
- GARCIA-JACAS N, SUSANNA A, and ILARISAN R. 1996. Aneuploidy in the Centaureinae: is $n = 7$ the end of the series? *Taxon* 45: 39–42.
- GARNATJE T, VALLÈS J, VILATERSANA R, GARCIA-JACAS N, SUSANNA A, and SILJAK-YAKOVLEV S. 2004. Molecular cytogenetics of *Xeranthemum* L. and related genera (Asteraceae, Cardueae). *Plant Biology* 6: 140–146.
- HEDBERG I, and HEDBERG O. 1977. Chromosome numbers of afroalpine and afromontane angiosperms. *Botaniska Notiser* 130: 1–24.
- HILLIARD OM. 1983. Asteraceae (Compositae) – Inuleae subtribe Gnaphaliinae (first part). In: Leistner OA [ed.], *Flora of Southern Africa*, vol. 33, 61–310. Department of Agriculture, Pretoria.
- KONISHI N, WATANABE K, and KOSUGE K. 2000. Molecular systematics of Australian *Podolepis* (Asteraceae: Gnaphalieae): Evidence from DNA sequences of the nuclear ITS region and the chloroplast *mat K* gene. *Australian Systematic Botany* 13: 709–727.
- LEVAN A, FREDGA K, and SANDBERG A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220.
- LORENZO-ANDREU A, and GARCÍA SANZ P. 1950. Cromosomas somáticos de plantas espontáneas en la estepa de Aragón. II. *Anales de la Estación Experimental Aula Dei* 2: 12–63.
- NAGL W, and EHRENDORFER F. 1974. DNA content, heterochromatin, mitotic index and growth in perennial and annual Anthemidea (Asteraceae). *Plant Systematics and Evolution* 123: 35–54.
- NAMUR C, and VERLAQUE R. 1976. Contribution à l'étude biogéographique du genre *Helichrysum* Miller. *Annales de l'Université de Provence – Biologie & Écologie Méditerranéenne* 3 (2): 17–22.
- NORDENSTAM B. 1967. Chromosome numbers in South African Compositae. *Aquilo Ser. Botanica* 6: 219–227.
- ROMERO ZARCO C. 1986. A new method for estimating karyotype asymmetry. *Taxon* 35: 526–530.
- SELVI F, and BIGAZZI M. 2002. Chromosome studies in Turkish species of *Nonea* (Boraginaceae): the role of polyploidy and descending dysploidy in the evolution of the genus. *Edinburgh Journal of Botany* 59 (3): 405–420.
- SHORT PS. 1986. A revision of *Pogonolepis* Steetz (Compositae: Inuleae: Gnaphaliinae). *Muelleria* 5: 237–253.
- SHORT PS. 1990. A revision of *Trichanthodium* Sond. and F. Muell. ex Sond. (Asteraceae: Inuleae: Gnaphaliinae). *Muelleria* 7: 213–224.
- STEBBINS GL. 1971. *Chromosomal Evolution in Higher Plants*. Edward Arnold, London.
- THULIN M. 1970. Chromosome numbers of some vascular plants from East Africa. *Botaniska Notiser* 123: 488–494.

- TURNER BL. 1970. Chromosome numbers in the Compositae. XII. Australian species. *American Journal of Botany* 57 (4): 382–389.
- WATANABE K, SHORT PS, DENDA T, KONISHI N, ITO M, and KOSUGE K. 1999. Chromosome numbers and karyotypes in the Australian Gnaphalieae and Plucheeae (Asteraceae). *Australian Systematic Botany* 12: 781–802.
- WATANABE K, YAHARA T, HASHIMOTO G, NAGATANI Y, SOEJIMA A, KAWAHARA T, and NAKAZAWA M. 2007. Chromosome numbers and karyotypes in Asteraceae. *Annals of the Missouri Botanical Garden* 94: 644–655.