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A cytotaxonomic study of three endemic orchids of the Canary Islands

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This paper reports somatic chromosome numbers for three orchid taxa endemic to the Canary Islands (*Habenaria tridactylites*, *Himantoglossum metlesicsianum* and *Orchis canariensis*), about whose cytogenetics very little is known. Chromosome numbers for the western Mediterranean and Atlantic monotypic genus *Gennaria* are also reported. The chromosome number of *Himantoglossum metlesicsianum* (2n = 36) is reported for the first time. New chromosome numbers were found for *Habenaria tridactylites* (2n = 34) and *Orchis canariensis* (2n = 84), confirming the tetraploidy of the latter. Chromosome numbers are reported also for *Gennaria diphylla* from North Africa (Tunisia) and the Iberian Peninsula (Portugal) (2n = 34 in both). Some taxonomic and phylogenetic comments are made on these species.

Key words: chromosome numbers, cytology, endemic orchids, *Gennaria diphylla*, *Habenaria tridactylites*, *Himantoglossum metlesicsianum*, *Orchis canariensis*, phyletic relationships

Introduction

Chromosome counts provide indispensable information on genetic discontinuities within and among species, and they contribute to our understanding of phylogenetic relationships at all taxonomic levels (e.g. Semple *et al*. 1989).

The family Orchidaceae shows a high diversity of chromosome numbers (e.g. Jones 1974, Tanaka & Kamemoto 1984, Brandham 1999). Few authors have specifically attempted a karyological study of the Orchidaceae in the Canary Islands or of the endemic orchid species (e.g. Hautzinger 1978, Dalgaard 1991). Thus, the available karyological data are scattered. The importance of karyological data to support the interpretation and discussion of these studies has been widely acknowledged (Pridgeon *et al*. 1997, Bateman *et al*. 2003). Thus, the main aim of this work is to provide karyological information of three endemic orchids from the Canary Islands, viz. *Habenaria tridactylites*, *Himantoglossum metlesicsianum* and *Orchis canariensis*, and the western Mediterranean and Atlantic monotypic genus *Gennaria* (*G. diphylla*), in order to achieve a better understanding of these taxa. The results are discussed with respect to the phylogeny of the groups.

Material and methods

We examined four species of orchids: three from the Canary Islands and one from Tunisia and Portugal. Karyological observations were based on the material collected between 2002 and 2004 from natural populations in various localities. A representative voucher specimen from each population was collected and deposited at SALA. On occasion, and when populations were very small, no material was collected; rather, photographic proof of existence was deposited in the Photographic Library of the Department of Botany, University of Salamanca.

Chromosome counts were usually made using ovaries sampled at a very early stage of development. Young flower buds were fixed in absolute ethanol–glacial acetic acid (3:1), sometimes modified to 6:1. Fixed material was stored at 4 °C until staining with 2% acetic orcein. Mounting involved squashing in 45% acetic acid. The karyological techniques used in this study were described in detail in Bernardos *et al*. (2003).

At least three metaphases were drawn for each population (including 3–5 individuals) using a Kaiser camera lucida (Kaiser, Germany). A Nikon Eclipse 50i microscope connected to a Nikon Coolpix 5400 digital camera was used to take the microphotographs. Drawings and microphotographs were deposited in the Department of Botany of the University of Salamanca.

Concerning nomenclature and delimitation of taxa, we follow Delforge (2005).

Results and discussion

The main results are given in Table 1, in which the chromosome numbers of the six studied populations are shown. There the species appear in alphabetical order, together with the diploid number, locality, collectors, herbarium and number of voucher.

Of the four studied species, the chromosome number of *Himantoglossum metlesicsianum* is here reported for the first time (2n = 36), new chromosome numbers were found for *Habenaria tridactylites* (2n = 34) and *Orchis canariensis*

Table 1. Material studied and chromosome numbers found.

Species/Sample	2n	Localities, collectors	Voucher
Gennaria diphylla			
GDI 03/02	34	Tunisia, SW El Haouaria, 50 m, *Bernardos et al. s.n.*, 4 Mar. 2002	SALA 109012
GDI 11/03	34	Portugal, Estremadura, Serra da Arrábida, 130 m, *Amich & Bernardos s.n.*, 29 Feb. 2003	SLIDE 9765
Habenaria tridactylites			
HTR 12/04	34	Canary Islands, Tenerife, El Bailadero, 325 m, *Bernardos et al. s.n.*, 28 Jan. 2004	SALA 108468
HTR 15/04	34	Canary Islands, Tenerife, Valle de Masca, 685 m, *Bernardos et al. s.n.*, 30 Jan. 2004	SLIDE 10122
Himantoglossum metlesicsianum			
HME 27/04	36	Canary Islands, Tenerife, Chío, 1035 m, *Bernardos et al. s.n.*, 30 Jan. 2004	SALA 108466
Orchis canariensis			
OCA 21/04	84	Canary Islands, Tenerife, La Orotava, 1210 m, *Bernardos et al. s.n.*, 29 Jan. 2004	SALA 108465

(2n = 84), and 2n = 34 is reported for the North African and Iberian *Gennaria diphylla* material.

Habenaria tridactylites Lindley

Though hardly any karyological data is available on 115 species of the 600 or so that form the genus *Habenaria*, it would appear to show great karyological diversity. Chromosome numbers ranging from 2n = 28 to 2n = 168 have been reported (Pridgeon *et al*. 2001, Felix & Guerra 2005). In the literature, the most common somatic number for the genus is 2n = 42 (for example *see* Fedorov 1974, Felix & Guerra 2005), with n = 21 in approximately 69% of the species analysed and n = 14 in another 3.5% (Felix & Guerra 2005). Brandham (1999) indicates that several basic chromosome numbers exist in this complex genus.

For *H. tridactylites*, only the number 2n = 42 is known (Dalgaard 1991). In an earlier paper in which n = 21 (Mehra & Vij 1970) was reported, the experimental material was actually another species from the eastern Himalayas. In the present work we observed the number 2n = 34 (Fig. 1A–B). This does not conform with that reported by Dalgaard (1991) nor with the predominant somatic number of representatives of the genus from the Americas and Asia, 2n = 42 (Yokota 1990, Felix & Guerra 1998).

Gennaria diphylla (Link) Parl.

Several somatic chromosome numbers have been reported for *Gennaria*, including 2n = 34 (Dolcher & Dolcher 1961, Scrugli 1978), 2n = 36 (Dalgaard 1991) and 2n = 40 (Sundermann & von der Bank 1977). In the present work, the number 2n = 34 was obtained in material from Tunisia (sample GDI 03/02, Fig. 1C) and Portugal (sample GDI 11/03, Fig. 1D).

Thus, in *Gennaria diphylla* and *Habenaria tridactylites*, the only two representatives of the subtribe Habenariinae in the Mediterranean Basin and Macaronesian Islands, descending ploidy must have occurred (x = 17) with respect to the palaeopolyploid basic number of chromosomes in the family Orchidaceae, x = 21 (Greilhuber & Ehrendorfer 1975). The number x = 21 is also the commonest in the subtribe Habenariinae (Felix & Guerra 2005).

Orchis canariensis Lindley

In this group (*sensu* Delforge 2005), the somatic chromosome number of 2n = 42 has been commonly reported, as for several species of the Iberian Peninsula (*O. langei*, *O. mascula*, *O. olbiensis*, *O. tenera*; Löve & Kjellqvist 1973, Silvestre 1987, Bernardos *et al*. 2004) and other parts of Europe and the Mediterranean Basin (Del Prete 1978, Averyanov *et al*. 1985, Balayer 1986). However, tetraploids are also known, with 2n = 84 for *O. patens* (Pridgeon *et al*. 2001) and *O. olbiensis* (Bernardos *et al*. 2002). The tetraploidy of the latter would seem to support its independence from *O. mascula* at the species level (*see* Bateman *et al*. 2003), despite some deviating views (*see* Aedo 2005).

Hautzinger (1978) reported 2n = 80 for *Orchis canariensis* although Pridgeon *et al*. (2001) indicated that the number should presumably be 2n = 84. The present work confirms a somatic chromosome number of 2n = 84 (sample OCA 21/04, Fig. 1E). This supports a close relationship with the tetraploid *O. patens*, originally shown at the level of ITS sequences (Aceto *et al*. 1999, Bateman *et al*. 2003). These data suggest that *O. canariensis* arose through fragmentation of an ancient species whose distribution stretched from the central Mediterranean Basin (Italy) to the Canary Islands and Maghreb. It has, however, long been isolated in the western confines of its range. Its sister species, *O. patens*, is currently found in Liguria and the Tellian Atlas of Tunisia and Algeria, while the third tetraploid of the group, *O. olbiensis*, is found only in the western Mediterranean (Aedo 2005). *Orchis spitzelii* and *O. cazorlensis*, both diploid taxa with 2n = 40 and 2n = 42 respectively (Delforge 2005), are closely related to this group of polyploid taxa.

Himantoglossum metlesicsianum (Teschner) P. Delforge

Himantoglossum has recently been carefully examined and shown to have 36 chromosomes (Mazzola *et al*. 1982, Cauwet-Marc & Balayer 1986, Bianco *et al*. 1987, Capineri & Rossi 1987,

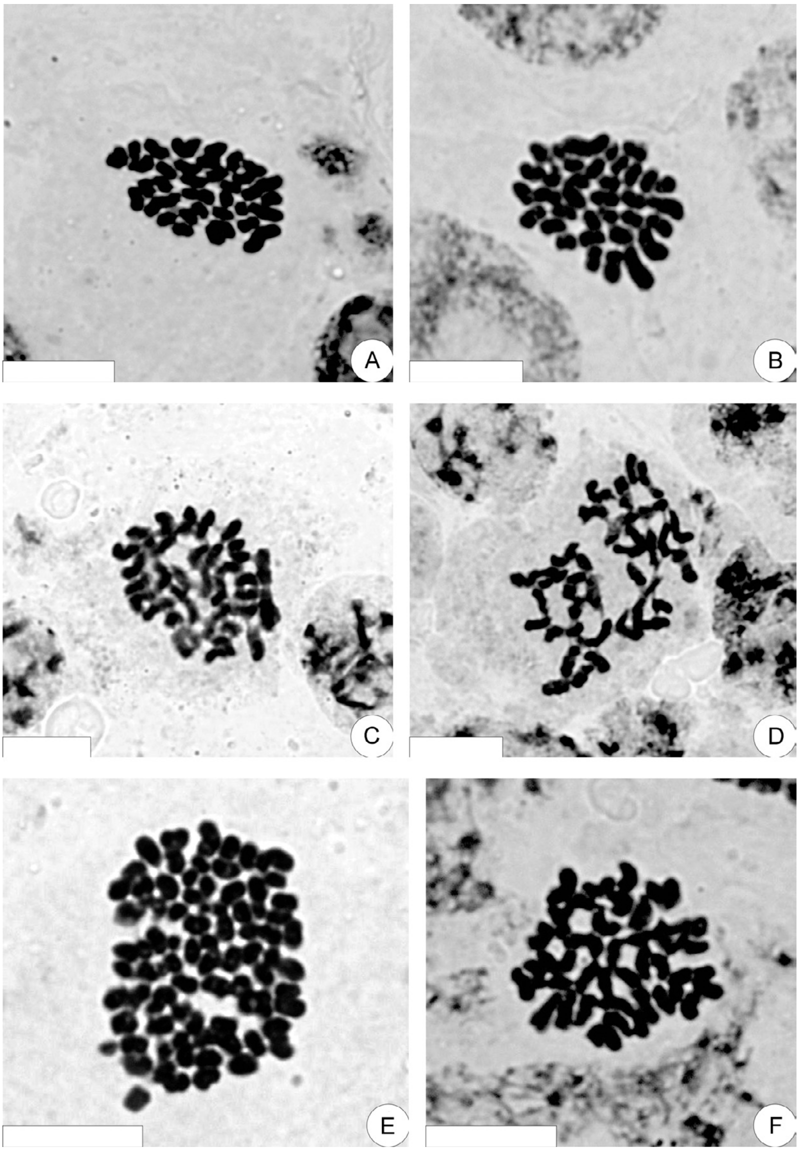

Fig. 1. Microphotographs of chromosomes. Scale bars = 10 µm. — **A**: *Habenaria tridactylites*, mitotic metaphase, 2n = 34 (sample HTR 12/04). — **B**: *H. tridactylites*, mitotic metaphase, 2n = 34 (sample HTR 15/04). — **C**: *Gennaria diphylla*, mitotic metaphase, 2n = 34 (sample GDI 03/02). — **D**: *G. diphylla*, mitotic metaphase, 2n = 34 (sample GDI 11/03). — **E**: *Orchis canariensis*, mitotic metaphase, 2n = 84 (sample OCA 21/04). — **F**: *Himantoglossum metlesicsianum*, mitotic metaphase, 2n = 36 (sample HME 27/04).

D'Emerico *et al*. 1990, Bernardos & Amich 2002, Bernardos *et al*. 2004, 2005). Aneuploids with 38 chromosomes are also known (Bernardos *et al*. 2004), and on occasion an additional B chromosome has been reported (Capineri & Rossi 1987, D'Emerico *et al*. 1992). The chromosome number of 2n = 30 for *H. comperianum* (Ströhlein & Sundermann 1972) appears to be an error (Delforge 1999). The frequent count of 2n = 36 for this genus suggests the existence of a basic number x = 6, giving records of 4x = 24, 6x = 36.

No prior karyological data was available on *H. metlesicsianum*. This work is the first to report a somatic chromosome number of 2n = 36 (sample HME 27/04, Fig. 1F). Thus, it has the same chromosome complement as the rest of the Mediterranean members of the genus. The strong genetic divergence of this Canary Island endemism (Bateman *et al*. 2003) with respect to *H. robertianum*, is not clearly reflected at the morphological level, nor in the number of somatic chromosomes.

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References

Aceto, S., Caputo, P., Cozzolino, S., Gaudio, L. & Moretti, A. 1999: Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. — *Mol. Phyl. Evol.* 13: 67–76.

Aedo, C. 2005: *Orchis* L. — In: Aedo, C. & Herrero, A. (eds.), *Flora Iberica, plantas vasculares de la Península Ibérica e Islas Baleares*, vol. 21: 114–146. Real Jardín Botánico, C.S.I.C., Madrid.

Averyanov, L.V., Medvedeva, N. A. & Serov, V. P. 1985: Chromosome numbers in the representatives of the family Orchidaceae from the Caucasus. — *Bot. Zh.* 70: 999–1000.

Balayer, M. 1986: Diagnoses de quelques taxons infraspécifiques d'Orchidaceae reconnus en Languedoc et Rousillon. — *Bull. Soc. Bot. France* 133: 279–283.

Bateman, R. M., Hollingsworth, P. M., Preston, J., Yi-Bo, L., Pridgeon, A. M. & Chase, M. W. 2003: Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). — *Bot. J. Linn. Soc.* 142: 1–40.

Bernardos, S. & Amich, F. 2002: Karyological, taxonomic and chorological notes on the Orchidaceae of the Central-Western Iberian Peninsula. — *Belg. J. Bot.* 135: 76–87.

Bernardos, S., Amich, F. & Gallego, F. 2003: Karyological and taxonomic notes on *Ophrys* L. (Orchidoideae, Orchidaceae) from the Iberian Peninsula. — *Bot. J. Linn. Soc.* 142: 395–406.

Bernardos, S., García-Barriuso, M. & Amich, F. 2005: New chromosome counts in Iberian orchids. — *J. Eur. Orchid.* 37: 639–650.

Bernardos, S., Tyteca, D. & Amich, F. 2004: Cytotaxonomic study of some taxa of the subtribe Orchidinae (Orchidoideae, Orchidaceae) from the Iberian Peninsula. — *Isr. J. Pl. Sci.* 52: 161–170.

Bernardos, S., Amich, F., Gallego, F. & Crespí, A. L. 2002: Contributions to the knowledge on the orchid flora of Northern Portugal. — *J. Eur. Orchid.* 34: 35–50.

Bianco, P., Medagli, P., D'Emerico, S. & Ruggiero, L. 1987: Numeri cromosomici per la Flora Italiana, 1139–1155. — *Inform. Bot. Ital.* 19: 322–332.

Brandham, P. 1999: Cytogenetics. — In: Pridgeon, A. M., Cribb, P. J., Chase, M. W. & Rasmussen, F. N. (eds.), *Genera Orchidacearum*, vol 1: 67–80. Oxford Univ. Press, Oxford.

Capineri, R. & Rossi, W. 1987: Numeri cromosomici per la Flora Italiana, 1127–1135. — *Inform. Bot. Ital.* 19: 314–318.

Cauwet-Marc, A. M. & Balayer, M. 1986: Les orchidées du bassin méditerranéen. Contribution à l'étude caryologique des espèces des Pyrénées orientales (France) et contrées limitrophes. II. Tribu des Ophrydeae. Lind. pro parte. — *Bull. Soc. Bot. France* 133: 265–277.

Dalgaard, V. 1991: Chromosome studies in flowering plants from Macaronesia II. — *Willdenowia* 20: 139–152.

Del Prete, C. 1978: Contributi alla conoscenza della Orchidaceae d'Italia VI. Tavole cromosomichi delle Orchidaceae italiane con alcune considerazioni citosistematiche sui generi *Ophrys*, *Orchis* e *Serapias*. — *Inform. Bot. Ital.* 10: 379–389.

Delforge, P. 1999: Contribution taxonomique et nomenclaturale au genre *Himantoglossum* (Orchidaceae). — *Nat. Belges* 80: 387–408.

Delforge, P. 2005: *Guide des Orchidées d'Europe, d'Afrique du Nord et du Proche-Orient*, 3ème ed. — Delachaux et Niestlé S.A., Lausanne-París.

D'Emerico, S., Bianco, P. & Medagli, P. 1992: Karyological studies on Orchidaceae. Tribe Ophrydeae, subtribe Serapiadinae. — *Caryologia* 45: 301–311.

D'Emerico, S., Bianco, P., Medagli, P. & Ruggiero, L. 1990: Karyological studies of some taxa of the genera *Himantoglossum*, *Orchis*, *Serapias* and *Spiranthes* (Orchidaceae) from Apuly (Italy). — *Caryologia* 43: 267–276.

Dolcher, T. & Dolcher, A. 1961: Sulla cariologia de *Gennaria diphylla* (Link) Parl. (*Coeloglossum diphyllum* Fiori). — *Nuovo Giorn. Bot. Ital.* 68: 59–63.

Fedorov, A. 1974: *Chromosome numbers in flowering plants*. — O. Koeltz Sci. Publ., Koenigstein.

Felix, L. P. & Guerra, M. 1998: Cytological studies on species of *Habenaria* Willd. (Orchidaceae-Orchidoideae) occurring in the Northeast of Brazil. — *Lindleyana* 13: 224–230.

Felix, L. P. & Guerra, M. 2005: Basic chromosome numbers of terrestrial orchids. — *Pl. Syst. Evol.* 254: 131–148.

Greilhuber, J. & Ehrendorfer, F. 1975: Chromosome number and evolution in *Ophrys* (Orchidaceae). — *Pl. Syst. Evol.* 124: 125–138.

Hautzinger, L. 1978: Genus '*Orchis*' L. (Orchidaceae): Sectio '*Robustocalcare*' Hautzinger. — *Ann. Natur. Mus. Wien* 81: 31–3.

Jones, K. 1974: Cytology and the study of orchids. — In: Withner, C. L. (ed.), *The orchids. Scientific studies*: 383–389. John Wiley & Sons, New York.

Löve, A. & Kjellqvist, E. 1973: Cytotaxonomy of Spanish plants II. Monocotyledons. — *Lagascalia* 3: 142–182.

Mazzola, P., Miceli, G. & Not, R. 1982: Numeri cromosomici per la Flora Italiana, 926–936. — *Inform. Bot. Ital.* 14: 275–279.

Mehra, P. N. & Vij, S. P. 1970: IOPB chromosome numbers reports XXV. — *Taxon* 19: 102–113.

Pridgeon, A. M., Cribb, P. J., Chase, M. W. & Rasmussen, F. N. (eds.), 2001: *Genera Orchidacearum*, vol. 2. — Oxford Univ. Press, Oxford.

Pridgeon, A. M., Bateman, R. M., Cox, A. V., Hapeman, J. R. & Chase, M. W. 1997: Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis sensu lato*. — *Lindleyana* 12: 89–109.

Scrugli, A. 1978: Numeri cromosomici per la Flora Italiana, 526–533. — *Inform. Bot. Ital.* 10: 414–421.

Semple, J. C., Chmielewski, J. G. & Windham, M. D. 1989: Chromosome number determinations in fam. Compositae, tribe Astereae. III. Additional counts and comments on generic limits and ancestral base numbers. — *Rhodora* 91: 296–314.

Silvestre, S. 1987: Orchidaceae. — In: Valdés, B., Talavera, S. & Fernández-Galiano, E. (eds.), *Flora vascular de Andalucía occidental*: 495–519. Ketres Editora, Barcelona.

Ströhlein, H. & Sundermann, H. 1972: Chromosomenzählungen bei Erdorchideen. — *Jahresb. Natur. Ver. Wuppertal* 25: 75–80.

Sundermann, H. & von der Bank, C. 1977: Neue Beiträge zur Zytotaxonomie der Erdorchideen (*Ophrys*, *Orchis*, *Neotinea*, *Steveniella* und *Gennaria*). — *Orchidee* 28: 146–147.

Tanaka, R. & Kamemoto, H. 1984: Chromosomes in orchids: counting and numbers. — In: Arditti, J. (ed.), *Orchid biology. Reviews and perspectives*: 324–410. Cornell Univ. Press, Ithaca.

Yokota, M. 1990: Karyomorphological studies on *Habenaria*, Orchidaceae and allied genera from Japan. — *J. Sci. Hiroshima Univ.* 23: 53–161.