Cytogenetic studies in *Herbertia* Sw. (Iridaceae)

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Abstract — Conventional Feulgen staining, CMA/DAPI banding and FISH with 18-5.8-26S rDNA probe were used to determine chromosome numbers, karyotypes and physical position of GC or AT rich heterochromatin and rDNA sites in three South American species of *Herbertia*: *H. lahue* subsp. *amoena*, *H. quareimana*, and *H. darwinii*. The basic number of the genus is x=7, but the analyzed taxa have different ploidy levels. The chromosome number was 2n=14 for *H. darwinii* and 2n=28 for *H. quareimana*. The two populations studied of *H. lahue* subsp. *amoena* did result in: 2n=42 (Gualeguaychu) and 56 (Palma de Castillo). Chromosome size varied between 1.60 and 5 µm. The karyotypes did give with a total of 8 metacentric (*m*) + 6 submetacentric (*sm)* chromosomes for *H. darwinii* and 20 *m* + 8 *sm* chromosomes for *H. quareimana*. The two populations of *H. lahue* subsp. *amoena* showed with 26 *m* + 16 *sm* chromosomes and 36 *m* + 20 *sm* chromosomes. *H. darwinii* had a bimodal karyotype. *H. darwinii* showed one satellited pair whereas the remaining species two (always in short *m* chromosome arms). Moreover, the 2n=56 population of *H. lahue* subsp. *amoena* one satellite on the long arms. No $CMA^-/DAPI^+$ bands were detected in any species, but $CMA^+/DAPI^-$ bands associated with nucleolar organizing regions (NOR) were found in all cases. The locations of the 18-5.8-26S rDNA sites in all species coincided with $CMA^+/DAPI^-$ bands. Our data suggest that karyotypic diversification in *Herbertia* included changes in chromosome number, symmetry, and number and position of NORs regions.

Key Words: CMA/DAPI banding, FISH, *Herbertia*, karyotype, polyploidy, somatic chromosomes.

INTRODUCTION

Herbertia Sweet is genus of Iridaceae mainly growing in temperate South America with large, obovate outer tepals, and smaller oblanceolate inner tepals, staminal column narrow above, linear anthers, and bifid style (Goldblatt 1977). It is comprised by six South American species, *H. darwinii* Roitman and Castillo endemic to Argentina, and the remaining species growing from Uruguay to Chile; *H. crosae* Roitman and Castillo*, H. lahue* (Mol.) Goldbl.*, H. pulchella* Sw., *H. quareimana* Ravenna*, H. tigridioides* (Hick) Goldbl. (Goldblatt 1975, 1977, Ravenna 1968, 1989; Roitman and Castillo 2004, 2008) there is only one North American taxon: *H. lahue* subsp. *caerulea* (Herb.) Goldbl. (Goldblatt 1975). The genus belongs to tribe Tigridieae, subtribe Cipurinae, characterized by monosulcate pollen and basic chromosome number x=7 (Goldblatt 1982, 1990; Goldblatt and Henrich 1991).

The cytology of Iridaceae is relatively well known compared with other petaloid monocots (Kenton and Heywood 1984; Goldblatt and Takei 1997). However, cytogenetic studies in *Herbertia* are scarce, providing exclusively chromosome counts (*e.g.* Winge 1959; Goldblatt 1975, 1982; Kenton and Heywood 1984; Goldblatt and Takei 1997). There are not detailed karyotypic studies available either with conventional staining, banding, or fluorescent *in situ* hybridization.

The purposes of this study are to determine the chromosome numbers, to establish the number and physical location of 18-5.8-26S rDNA sites and $CMA^+/DAPI^-$ bands, and to analyze the karyotype in three South American *Herbertia* species, trying to understand the taxonomic and evolutionary relationships of the species investigated.

MATERIALS AND METHODS

Plant material - Vouchers were kept in the herbarium Gaspar Xuáres of the University of Buenos Aires (BAA):

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H. lahue subsp. *amoena.* ARGENTINA, Entre Ríos, Gualeyguachu, Roitman s.n.; URUGUAY, Artigas, Palma del Castillo, G. Roitman s.n.

H. quareimana. ARGENTINA, Corrientes, Monte Caseros, G. Roitman s.n.; URUGUAY, Artigas, Bella Unión, G. Roitman s.n.

H. darwinii. ARGENTINA, Corrientes, Bonpland, Roitman s.n.; Corrientes, Tres Cerros, G. Roitman s.n.

Specimens were planted in earthenware pots in an equal parts mixture of vermiculite and soil. Root tips from adventitious roots from bulbs were used. After pretreatment in a 2 mM solution of 8-hydroxyquinoline for 4 h, the roots were fixed in a 3:1 ethanol:glacial acetic acid mixture.

Feulgen staining - Root tips were hydrolyzed with HCl 5 N for 30 min at room temperature and then washed, stained with Schiff reagent for 2 h (JONG 1997), and squashed in a drop of 45% acetic acid. Permanent mounts were made using dry air. At least ten metaphases per species were photographed with a phase contrast optic Zeiss Axiophot microscope and a Leica DFC300FX camera. Photographs were used to take measurements of the following features for each chromosome pair: s (short arm), l (long arm), and c (total chromosome length); the length of the satellite was added to the respective chromosome arm. The arm ratio (r=l/s) was calculated and used to classify the chromosomes after LEVAN *et al.* (1964). Satellites were classified according to BATTAGLIA (1955). In addition, mean chromosome length (C), mean total haploid chromosome length of karyotype based on the mean lengths (tl), and mean arm ratio (R) were calculated. Idiograms were based on the mean values for each species. The chromosomes were arranged first into groups according to their increasing arm ratio and then according to the decreasing length within each group. Because of the close morphology and size of the chromosomes, homologues were difficult to match. Karyotype asymmetry was estimated using the asymmetry index of PASZCO (2001).

CMA/DAPI banding - Root tips were washed twice in distilled water (10 min each), digested with a 2% cellulase–20% pectinase solution (30 min), and squashed in a drop of 45% acetic acid. Only one root tip was used in each slide. After coverslip removal in liquid nitrogen, the slides were aged for three days, and stained according to the protocol to SCHWEIZER (1976).

Fluorescent in situ hybridization - The location and number of rDNA sites were determined by FISH using as probe the p*Ta*71 containing the 18-5.8-26S rDNA (GERLACH and BEDBROOK 1979) labeled with biotin-14-dATP (BioNick, Invitrogen Carlsbad, USA). The FISH protocol was according to SCHWARZACHER AND HESLOP-HARRISON (2000). Photomicrography was done with a fluorescent Zeiss Axiophot microscope and a Leica DFC300FX camera for image capture.

RESULTS

Karyotypes - Somatic chromosomes of six populations belonging to three species of *Herbertia* were analysed (Table 1). Four ploidy levels were found: diploid with $2n=2x=14$ (*H. darwinii*), tetraploid with and $2n=4x=28$ (*H. quareimana*), hexaploid with 2n=6x=42, and octoploid with 2n=8x=56 (in two populations of *H. lahue* subsp. *amoena* both were designated as cytotype 1 and 2, respectively). Representative metaphases of each species are presented in Fig. 1. Chromosome features and idiograms are shown in Table 1 and Fig. 2, respectively.

The karyotypes were composed of *m* and *sm* chromosomes, both in diploid and polyploid species (Figs. 1, 2). Mitotic chromosomes of *Herbertia* are medium to large sized (Table 1; Fig. 2). The average chromosome length varies from 2.6 to 2.9 µm; the shortest chromosome was nº 56 (1.43 µm) in a cell of *H. lahue* subsp. *amoena* cytotype 2 and the longest chromosome (4.82 µm) was nº 1 in a cell of *H. lahue* subsp. *amoena* cytotype 1.

Satellites were observed in *m* chromosomes in all taxa: two pairs in the polyploids *H. quareimana* and *H. lahue* subsp. *amoena*, and only one pair in the diploid *H. darwinii* (Fig. 1). All accessions presented satellites on the short arms, except cytotype 2 of *H. lahue* subsp. *amoena* that had one pair on the long arms (Fig. 2).

In *H. lahue* subsp. *amoena* and in *H. quareimana*, the chromosomes gradually decreased in size, whereas there were two clear chromosome sizes (large and medium) in *H. darwinii* (Fig. 2). The mean arm ratio was higher in diploids than in polyploids. The greatest mean arm ratio was found in *H. darwinii* and the lowest in *H. quareimana* (Table 1). The variation of the AI index was relatively small. *H. darwinii* had the most asymmetrical karyotype and *H. quareimana* the most symmetrical (Table 1).

Chromosome banding - The banding patterns always showed $CMA^+/DAPI^-$ constitutive heterochromatin associated with NOR (CG-rich), whereas, no $CMA^-/DAPI^+$ bands were detected in any species. The diploids had two $CMA^+/DAPI^-$ bands attached to the satellited chromosomes,

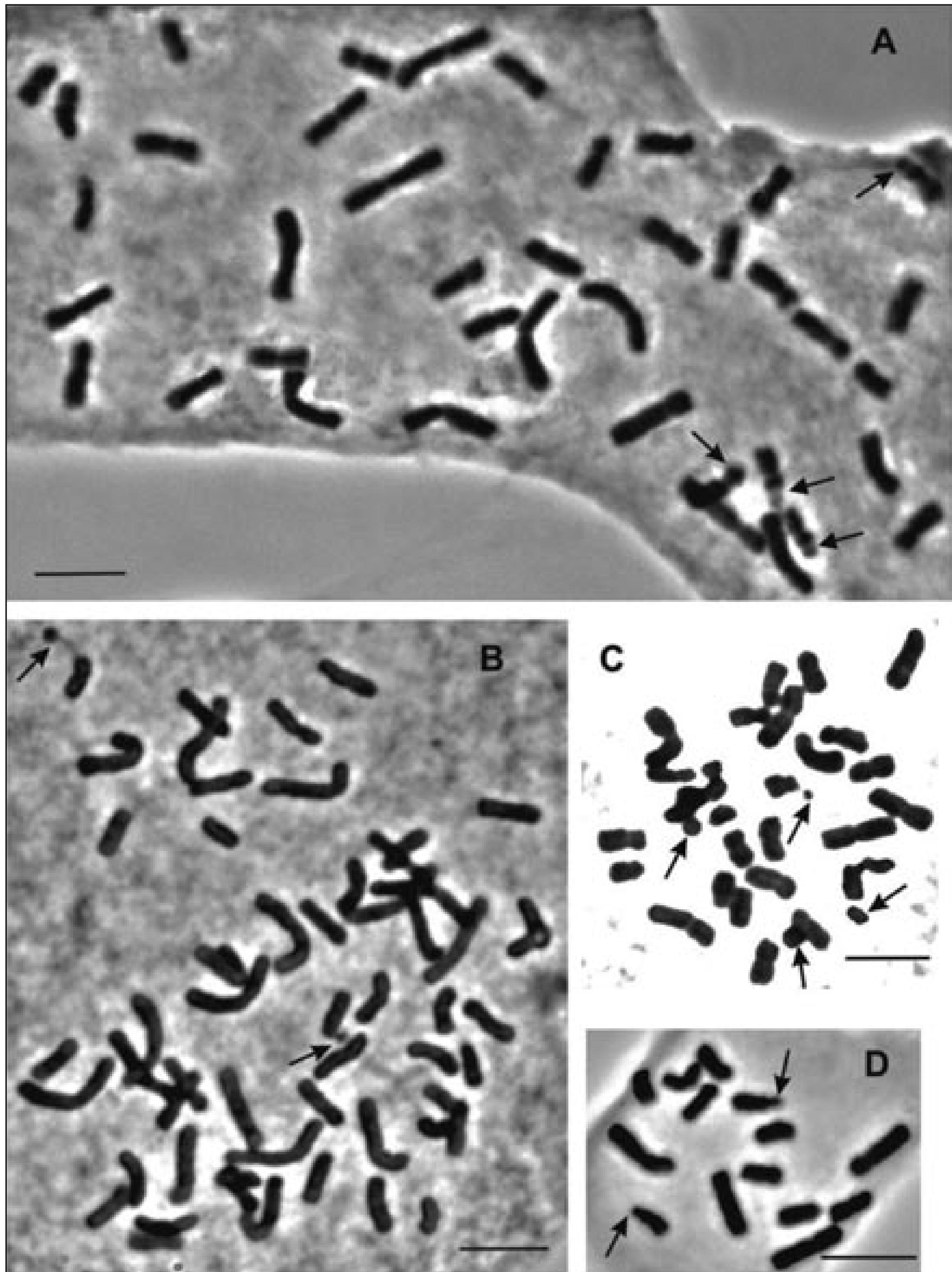

Fig. 1 — Mitotic metaphases of *Herbertia* species with Feulgen staining. A. *H. lahue* subsp. *amoena* cytotype 1 (2n=42). B. *H. lahue* subsp. *amoena* cytotype 2 (2n=56). C. *H. quareimana* (2n=28). D. *H. darwinii* (2n=14). Arrows point to satellites. Bars = 5 µm.

Table 1 — *Herbertia* taxa studied. Karyotype formulae; mean total haploid chromosome length (tl) ± s.d.; mean arm ratio (r); ratio between the largest and the smallest chromosomes in the complement (R); mean chromosome length (C); NOR-associated heterochromatin percentage (%HC NOR); and asymmetry index (AI).

Species	Karyotype composition	tl (μm)	r	R	C (μm)	% HC NOR	AI
H. lahue subsp. *amoena*							
Cytotype 1	26 *m* + 16 *sm*	60.4 ± 0.8	1.5	3	2.9	3.01	4.2
Cytotype 2	36 *m* + 20 *sm*	80.3 ± 0.9	1.6	3	2.9	2.12	4.6
H. quareimana	20 *m* + 8 *sm*	39.1 ± 0.74	1.5	2.5	2.8	3.99	3.7
H. darwinii	8 *m* + 6 *sm*	18.4 ± 0.76	1.7	2.5	2.6	4.01	5.2

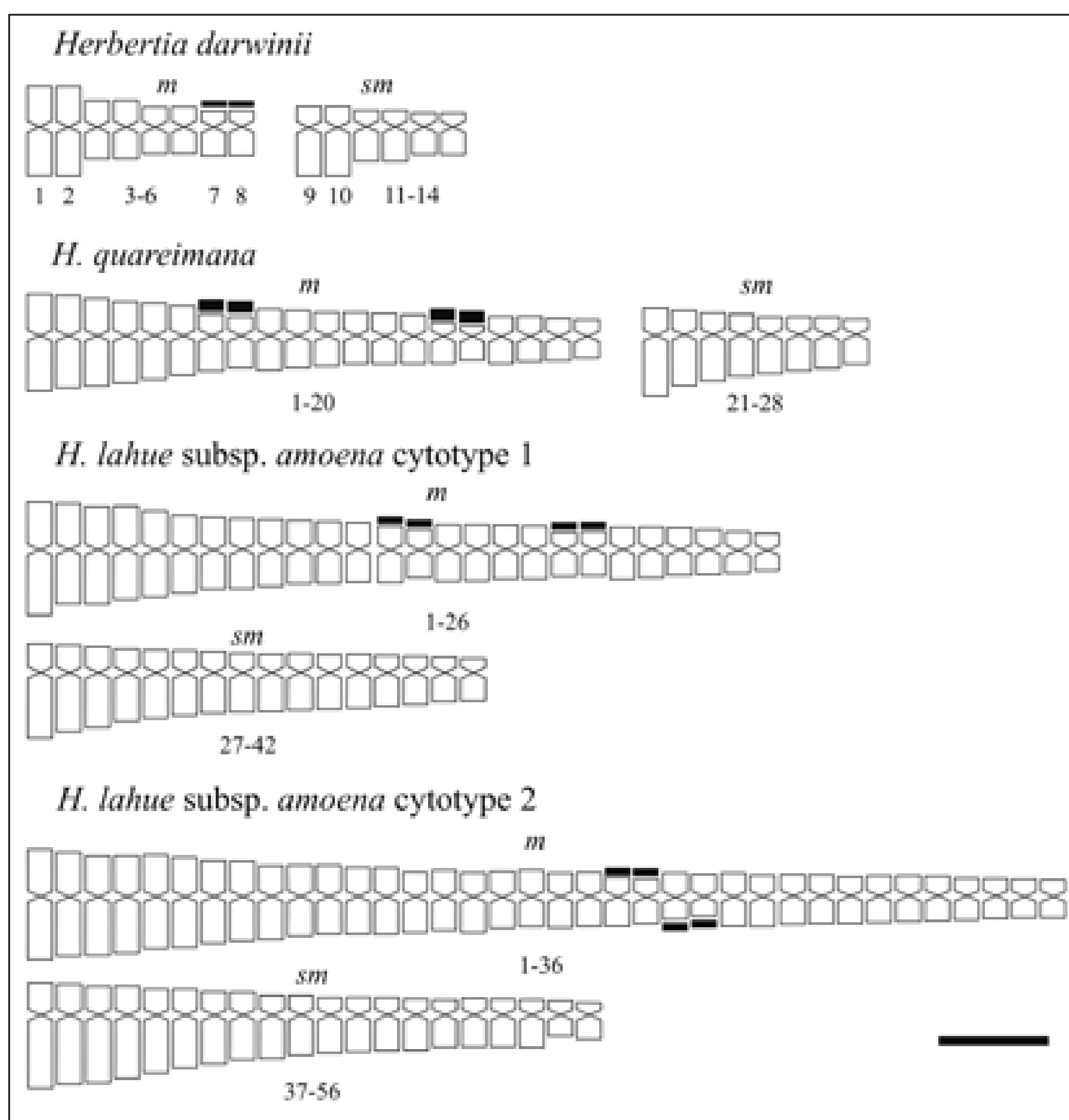

Fig. 2 — Idiograms of *Herbertia* species based on mean chromosome values showing heterochromatic fluorochrome banding paterns and 18-5.6-26S sites. Black squares indicate CMA+/DAPI- bands and 18S-5.6S-26S sites. Bars = 5 μm. All at the same scale.

and polyploids four bands independently of the ploidy level (Figs. 2).

Chromosomal mapping of the 18-5.8-26S rDNA by FISH - The locations of the 18-5.8-26S rDNA sites in all species studied coincided with CMA$^+$/DAPI$^-$ bands described above (Fig. 2). They were located on the secondary constrictions in a distal position.

DISCUSSION

The basic number x=7 is considered as a synapomorphy of tribe Tigridieae, along with the presence of bulbs and plicate leaves (GOLDBLATT 1982; 1990). Our results are consistent with this pattern. The chromosome number of *H. darwinii* is reported for the first time, whereas the tetraploid level of *H. quareimana* agrees with previous counts (KENTON and HEYWOOD 1984; GOLDBLATT and TAKEI 1997). For *H. lahue*, there is a polyploid series reported: diploid (WINGE 1959), tetraploid (WINGE 1959; KENTON and HEYWOOD 1984; GOLDBLATT and TAKEI 1997), hexaploid (KENTON and HEYWOOD 1984; GOLDBLATT and TAKEI 1997; this work), and octoploid populations in subsp. *amoena* (this work), and exclusively octoploid in subsp. *caerulea* (GOLDBLATT 1975). This multiplicity of chromosome numbers was also found in *H. pulchella* (2n=14, 28, 42; KENTON and HEYWOOD 1984; GOLDBLATT and TAKEI 1997).

The chromosomes of *Herbetia* are medium sized, a common feature in Iridoideae. The polyploids *H. quareimana* and *H. lahue* showed symmetrical karyotypes, whereas the diploid *H. darwinii* a bimodal karyotype coinciding with other members of the tribe (*e. g., Cypella* spp. , *Hesperoxiphion* spp.: KENTON *et al.* 1990; *Calydorea undulata*: DE TULLIO *et al.* 2008). Bimodal karyotypes occur in many plants and animals and represent a very specialized karyotypic form. In plants, bimodality frequently characterizes groups of genera and/or species (*e. g.*, KENTON *et al.* 1990; NARANJO *et al*. 1998). The existence of groups of taxa with close bimodal karyotypes is a result of karyotype orthoselection or karyotype conservation (WHITE 1973).

Fluorescent banding was applied in *Herbertia* for first time. Terminal CMA$^+$/DAPI$^-$ bands were observed, revealing GC-blocks in chromosome pairs associated with the NOR regions, a fact suggesting that they might be restricted to this location in the genus. The relationship between satellites and CMA$^+$/DAPI$^-$ bands was also registered in two other members of the tribe: *Eleutherine plicata* and *E. bulbosa* (ZAMAN *et al*. 1985; GUERRA 1988). Nevertheless, in these species CMA$^-$/DAPI$^+$ bands were found. CG-rich composition of NORs and NOR associated heterochromatin is the rule in plants as a whole (*e.g.,* SCHWEIZER 1979; GUERRA 1985; URDAMPILLETA *et al*. 2006).

The number of 18S-5.8S-26S rDNA sites was different in polyploid and diploid *Herbertia*, as reported in other monocots (*Rhynchospora*: VANZELA *et al*. 1998; Hordeum: TAKETA *et al*. 1999). The site number in the polyploids may be inferior to the expected according to the ploidy level, as found here for *H. lahue* and *H. quareimana* and for other plants (*Scilla*: VAUGHAN *et al*. 1993; *Sanguisorba*: MISHIMA 2002).

Polyploids tend to reduce the number of duplicate sites due to diploidization mechanism and gene silencing (LEITCH and BENNETT 1997). Translocations may be responsible for changing the position of the satellites, whereas duplications and deletions can cause differences in number, as might have happened in *H. lahue* and *H. quareimana*. From the evolutionary point of view, it is possible that one satellited pair was the ancestral condition and that additional pairs arose by duplications in more advanced taxa, as proposed to explain numerical and structural satellited pair variation in *Capsicum* (MOSCONE *et al*. 1995).

GOLDBLATT (1982) suggested that the basic karyotype in Tigridieae is composed of two long and five shorter chromosome pairs, the longest pair being *m* and the next longest *sm* or *st*. This trend agrees with our findings in *H. darwinii*, as well as with data obtained by KENTON and HEYWOOD (1984) in *H. pulchella*.

Our data and previous publications (WINGE 1959; KENTON and HEYWOOD 1984; GOLDBLATT and TAKEI 1997) suggest that karyotypic diversification in *Herbertia* included changes in chromosome number, symmetry, and number and position of NORs regions (GOLDBLATT 1982).

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