

# Evolutionary DNA variation in the genus *Hypochaeris*

M. CERBAH\*†, J. COULAUD†, S. C. BROWN‡ & S. SILJAK-YAKOVLEV†

†Laboratoire d'Evolution et Systématique, U.R.A. C.N.R.S. 2154, Université Paris XI, F-91405 Orsay cedex and

‡Institut des Sciences Végétales, Cytométrie, CNRS UP 40, 91198 Gif-sur-Yvette, France

The genome size and the base composition (GC%) of eight *Hypochaeris* species were determined by flow cytometry in order to establish the pattern of nuclear DNA variation within the genus. The species analysed showed an almost fivefold range of variation from 1.68 pg in *H. cretensis* to 8.10 pg in *H. uniflora*. This variation in DNA content is greater between taxonomic sections of *Hypochaeris* species than within a section. There was no correlation between 2C DNA content and GC% indicating that neither the GC fraction nor the AT fraction were preferentially associated with variation in genome size. Because there is little heterochromatin, these results show that it is interspersed repeated sequences that are most probably implicated in this variation. From phylogenetic analysis, it is likely that genome size has evolved by loss of DNA content in some lineages and by gain in one lineage from an ancestral genome which was probably similar to genomes of intermediate size in *Hypochaeris*.

**Keywords:** Asteraceae, DNA content, evolution, *Hypochaeris*, karyotypes, phylogeny, repeated sequences.

## Introduction

The genus *Hypochaeris* (Asteraceae) contains one species in Asia, about 10 species in Europe and the remaining 40 species in South America. This genus is considered by Stebbins *et al.* (1953) as having the most unusual geographical distribution of any in the tribe Lactuceae. Several hypotheses of karyotype and chromosome evolution within the genus have been suggested in earlier studies (Stebbins, 1971; Barghi *et al.*, 1989; Siljak-Yakovlev *et al.*, 1994; Cerbah *et al.*, 1995; Ruas *et al.*, 1995). European species display various chromosome numbers ( $2n=6, 8, 10, 12$ ) and symmetrical or asymmetrical karyotypes, whereas South American species display a single basic chromosome number ( $x=4$ ) and an asymmetrical bimodal karyotype. The karyological uniformity of South American species contrasts with a great diversity in their morphology and habitats.

Determination of nuclear DNA content is important in elucidating systematics and evolutionary relationships among species (Juan & Petitpierre, 1990; Srivastava & Lavania, 1991; Bharathan *et al.*, 1994). The

study of the genome size in closely related species can provide insight into the origin of DNA variation, but also into the direction of changes and the fraction of DNA involved in these changes (Bennett & Leitch, 1995). It is now established that the most important variation in DNA amount, apart from polyploidization and chromosome endoreplication, occurs in the repetitive fraction of the nuclear genome and that this variation can be under environmental selective pressure (Cullis, 1990). The genome can also rapidly change by the action of transposable elements which cause deletions or amplifications (Flavell, 1982). However, detailed knowledge about the mechanisms of change in DNA content and about factors determining the rates of amplification, deletions and rearrangements, remains lacking.

In the genus *Hypochaeris*, the variation observed in chromosome size (Mugnier & Siljak-Yakovlev, 1987; Barghi *et al.*, 1989; Siljak-Yakovlev *et al.*, 1994) suggests that there is significant variation in genome size. Detailed karyotypes of the species *H. oligocephala*, *H. achyrophorus* and *H. uniflora* are here constructed for the first time from morphometric statistical data. 2C values of four South American species (*H. chillensis* (H. B. K.) Hieron, *H. microcephala* (Sch. Bip.) Cabr. var. *albiflora* (O. K.) Cabr., *H. megapotamica* Cabr. and

\*Correspondence. E-mail: cerbah@esv.u-psud.fr

*H. pampasica* Cabr.) and two European species (*H. maculata* L. and *H. robertia* Fiori) have been already published (Cerbah *et al.*, 1995). It would be useful to know the variation within the whole genus. Hence, the genome size and base composition (GC%) of the studied species were determined by flow cytometry in order to establish the pattern of nuclear DNA variation among related species of *Hypochaeris*, as a contribution to the understanding of genome organization and evolution within this group.

## Materials and methods

Eight species of *Hypochaeris* were analysed for DNA amount and GC%. Origins of samples are given in Table 1. Plants were grown in the experimental greenhouse of the University of Paris XI, Orsay campus. In this selection of species, all the basic chromosome numbers known for the genus were represented.

The Feulgen method was used to establish karyotypes of the species *H. achyrophorus*, *H. oligocephala*, and *H. uniflora*. For this purpose, chromosome arms were measured on 10 metaphase plates from different individuals: chromosome types, centromeric indices and arm ratios were determined according to Levan *et al.* (1964). Idiograms were drawn using the mean values. Asymmetry indices [AsI = ( $\Sigma$  long arms/total chromosome length)  $\times$  100] were calculated according to Arano & Saito (1980).

Total DNA amount in nuclei was assessed by flow cytometry using *Petunia hybrida* (2C = 2.85 pg, 41% GC) as internal reference according to a technique adapted from Brown *et al.* (1991) and described in Cerbah *et al.* (1995). Total DNA content was determined by the ratio of the modal position of 2C stained nuclei of samples relative to those of *Petunia hybrida* cv. P $\times$ Pc6 (INRA, Dijon), or *Pisum sativum* cv. Long Express (Truffaut SA), using ethidium bromide (Sigma) at 30  $\mu$ g mL<sup>-1</sup>. Two other fluorochromes, AT-specific bisbenzimidazole Hoechst 33342 (Aldrich Chimie) at 5  $\mu$ g

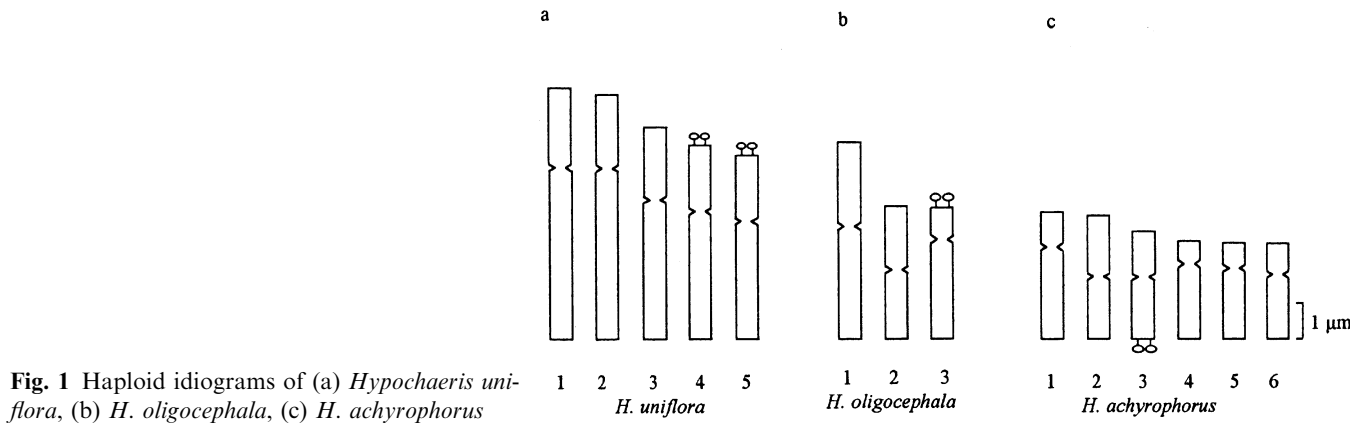
mL<sup>-1</sup> and GC-specific chromomycin A<sub>3</sub> (Serva) at 30  $\mu$ g mL<sup>-1</sup>, were also used to compute base ratios. Five individuals per species were assessed for each analysis, which was made on about 5000 nuclei using an EPICS V cytofluorometer (Coultronics France, Margency). The average coefficient of variation of the 2C *Hypochaeris* nuclei in histograms was about 3% for ethidium bromide, 5% for Hoechst 33342 and 6.5% for chromomycin A<sub>3</sub>. Base ratios were calculated according to the model of Godelle *et al.* (1993), which takes into account the number of fixation sites for fluorescence of each fluorochrome (on average a binding site for five A-T and three G-C base pairs, respectively, for Hoechst 33342 and chromomycin A<sub>3</sub>). Mean values of samples for total DNA amount and GC% were compared by ANOVA followed by a Tukey honest significant difference test for unequal *n*. 2C DNA content values for *P. hybrida* (2.85 pg) and *P. sativum* (8.37 pg) were taken from Marie & Brown (1993).

## Results

Morphometric chromosome data for the species *H. uniflora*, *H. oligocephala* and *H. achyrophorus* were used to construct idiograms (Fig. 1). The five chromosome pairs of *H. uniflora* were submetacentric, the two smallest pairs bearing a satellite on their short arm (Fig. 1a). According to the Asymmetry Index (67.7%) the karyotype of *H. uniflora* is symmetrical. *Hypochaeris oligocephala* had two metacentric chromosome pairs (1 and 2) and one subtelocentric pair (3) with a satellite on the short arm (Fig. 1b). This chromosomal region, including the satellite and its secondary constriction, is generally the chromosomal location of nuclear rDNA, also called the nucleolar organizer region (NOR). The karyotype of *H. achyrophorus* showed two submetacentric chromosomes pairs (1 and 6), two subtelocentric pairs (4 and 5) and two metacentric pairs (2 and 3). Chromosome pair 3 had a satellite on the long arm (Fig. 1c). This feature is reported here for the first time

**Table 1** Origins of *Hypochaeris* species samples studied

Species	Origin
<i>H. achyrophorus</i> L	Hanbury Botanical Garden, Genoa, Italy
<i>H. laevigata</i> (L.) Ces, P. et G.	Sicily, Mount Quacella 1850 m
<i>H. illyrica</i> K. Maly	Bosnia, Mount Vlasic 1850 m
<i>H. uniflora</i> Vill.	University Botanical Garden, Salzburg
<i>H. glabra</i> L.	Sicily, Mount Quacella 1850 m
<i>H. radicata</i> L.	Sicily, Mount Quacella 1850 m
<i>H. cretensis</i> (L.) Chaub. et Bory	Sicily, Mount Portella Mandarine, 1450 m
<i>H. oligocephala</i> (Swent. & Bramw.) Lack, Comb. nova	Conservatoire Botanique National de Brest, France

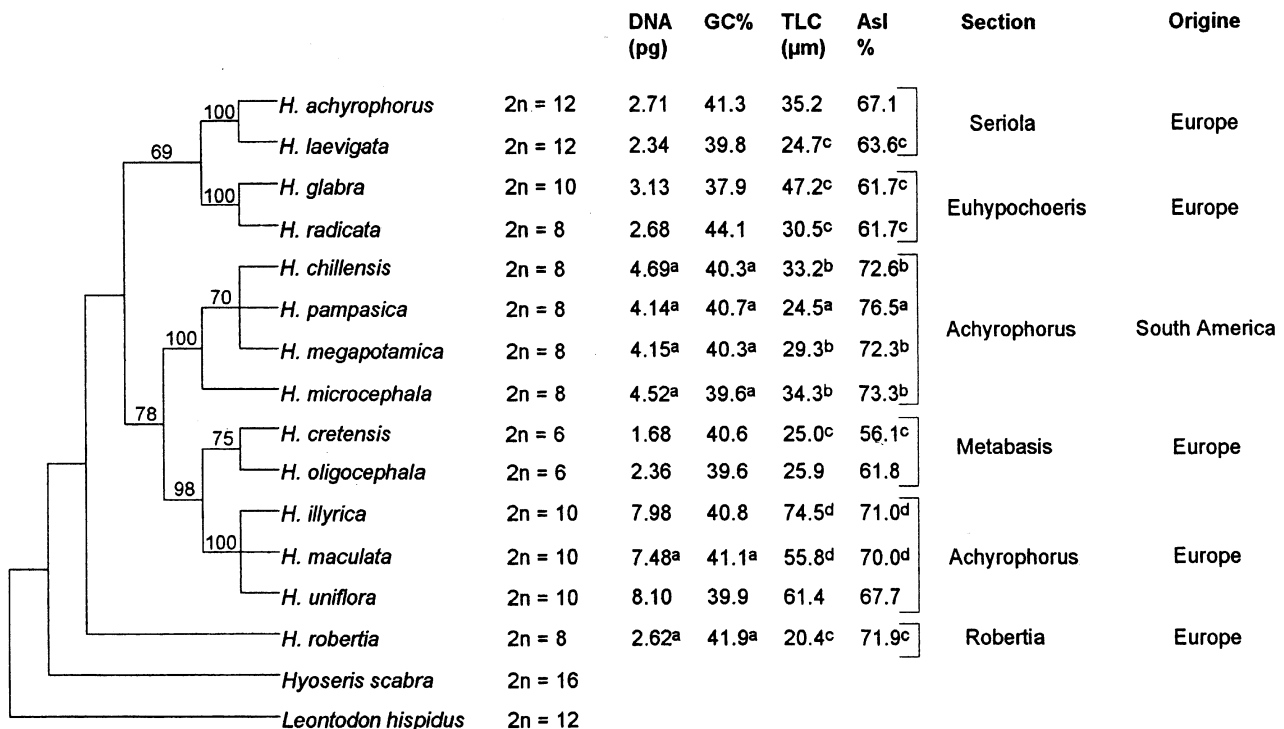


in the genus *Hypochaeris*, where species studied previously had satellites on the short arm of chromosomes.

Estimations of 2C DNA amounts and GC percentages are given in Fig. 2. Genome sizes of four South American species and two European species previously described (Cerbah *et al.*, 1995) are included in the same figure for comparison. The species analysed showed an almost fivefold range of variation from 1.68 pg in *H. cretensis* to 8.10 pg in *H. uniflora*. Comparison of results obtained by a Tukey-HSD-

unequal *n*-test showed that differences in DNA amounts between all *Hypochaeris* species were highly significant ( $P < 0.001$ ) except for comparisons between *H. maculata* and *H. illyrica* and between *H. robertia* and *H. radicata*.

The GC% ranged from 37.9% to 44.1%, a 6% variation between species. There was no correlation between DNA content and GC%, although the 2C DNA content was significantly positively correlated ( $r = 0.863$ ,  $P < 0.001$ ) with the total chromosome length (TCL).



**Fig. 2** Phylogenetic ITS consensus tree of 14 *Hypochaeris* species with the two outgroups *Leontodon hispidus* and *Hyoseris scabra* from Cerbah *et al.* (1998b) with bootstrap values indicated above corresponding nodes. Morphological taxonomic section according to Hoffman (1891). <sup>a</sup> Data published in Cerbah *et al.* (1995). <sup>b</sup> Data published in Siljak-Yakovlev *et al.* (1994). <sup>c</sup> Data published in Barghi *et al.* (1989). <sup>d</sup> Data published in Mugnier & Siljak-Yakovlev (1987).

From the results, it was particularly striking that species with the highest chromosome number  $2n=12$  (*H. achyrophorus* and *H. laevigata*) did not have the highest DNA content. However, species which showed the smallest chromosome number  $2n=6$  (*H. cretensis* and *H. oligocephala*) had among the smallest DNA contents.

## Discussion

Karyological comparisons of *H. uniflora* and *H. oligocephala* with their respective close relatives do not reveal distinctive features. *Hypochaeris oligocephala* had a similar karyotype to *H. cretensis*, but a higher asymmetry index (AsI). Chromosomes of *H. uniflora* were of similar morphology to those of its two relatives *H. maculata* and *H. illyrica* but its AsI was lower and its TCL intermediate.

*Hypochaeris achyrophorus* showed more differences from its relative *H. laevigata* ( $2n=12$ ). In *H. achyrophorus*, the TCL reflects the greater length of each chromosome pair and the chromosome pairs 4 and 5 were subtelocentric, increasing the AsI value (67.1%). This species showed only one chromosome pair bearing satellites (long arm of pair 3) instead of two pairs (short arms of pairs 2 and 3) in *H. laevigata* (Barghi *et al.*, 1989). These differences could be attributed to chromosomal rearrangements involving the NORs during evolution.

In order to investigate evolutionary trends of genome size in these related species, a molecular phylogeny based on Internal Transcribed Spacer (ITS) sequences of nuclear ribosomal DNA (Cerbah *et al.*, 1998b), was constructed. The phylogenetic consensus tree is shown in Fig. 2, where DNA contents, TLC, AsI, taxonomic sections and origin of species are added for comparison. Evolutionary trends previously proposed by Stebbins *et al.* (1953) for the genus could be summarized as follows: the original basic chromosome number of the genus *Hypochaeris* is  $x=5$  and a decrease of the basic chromosome number is associated with an increase of karyotype asymmetry, as generally observed in the tribe Lactuceae. The ITS phylogeny suggests an alternative hypothesis of both decrease and increase of chromosome number during evolution of the genus *Hypochaeris*, from an ancestral basic chromosome number of  $x=4$  (Cerbah *et al.*, 1998b). In the same way, the present results of genome size showed that both decrease and increase of DNA content have occurred during evolution. Because there is little heterochromatin in the genome of *Hypochaeris* species (Cerbah *et al.*, 1995, 1998a), we propose that genome size has evolved by variation in the amount of interspersed repeated sequences rather than by the amount of tandemly

repeated sequences. This is not the same in other Asteraceae such as *Crepis* (Godelle *et al.*, 1993) for which variation in the amount of heterochromatin between species is correlated with variation in DNA content.

*Hypochaeris robertia*, which had an intermediate value of 2C DNA (2.62 pg), has a position at the base of the phylogenetic tree (Fig. 2). We can consequently suppose that from a common ancestor with a genome size close to that of *H. robertia*, the genus has evolved by increasing the DNA amount in lineages of the South American and *H. maculata* groups, and decreasing the DNA amount in the lineage of *H. cretensis*.

Genome size variation in the genus *Hypochaeris* corresponded approximately to the grouping of species in morphological taxonomic sections. The genus was divided into five sections according to Hoffmann (1891). The section *Achyrophorus*, which includes all South American and some European species, showed the highest values of DNA amount in the genus (from 4.14 to 8.10 pg). The lowest values of 2C DNA have been found in the sections *Metabasis* (1.68 and 2.36 pg) and *Seriola* (2.34 pg). The sections *Euhypochaeris*, *Seriola*, and *Robertia* are represented by species having intermediate values of DNA content (from 2.62 to 3.13 pg). The variation of DNA content was 2.6-fold within species having a chromosome number of  $2n=10$ , and was 1.8-fold within species with  $2n=8$  chromosomes. Lastly, the variation was greater among taxonomic sections than within a section, except for the section *Achyrophorus*, which shows two classes of values, one for South American species and the other for European species (Fig. 2), showing that geographical species divergence was accompanied by changes in genome size.

The species *H. achyrophorus* ( $2n=12$ ) and *H. laevigata* ( $2n=12$ ) were suggested to be tetraploid (Stebbins *et al.*, 1953; Barghi *et al.*, 1989). However, Brullo *et al.* (1977) consider these species as diploids with a basic chromosome number of  $x=6$ , which is supported by the molecular phylogeny (Cerbah *et al.*, 1998b). Moreover, *H. oligocephala* ( $2n=6$ ; 2.36 pg) and *H. laevigata* ( $2n=12$ ; 2.34 pg) had very similar DNA contents. Nevertheless, because *H. achyrophorus* has a DNA content of about 1.6-fold that of *H. cretensis* ( $2n=6$ ), it could be that this species is derived by allotetraploidy. A loss of DNA following polyploid formation has been demonstrated in many studies (Raina *et al.*, 1994; Feldman *et al.*, 1997) but more experiments are needed to determine if this has occurred in *Hypochaeris*.

There was no overall significant correlation between 2C DNA content and GC%, indicating that neither the GC fraction nor the AT fraction was preferentially associated with variation of genome size. However, the

variation of DNA content between *H. cretensis* and *H. oligocephala* on the one hand, and *H. achyrophorus* and *H. laevigata* on the other, is associated with an increase in the GC fraction. In contrast, variation between *H. glabra* and *H. radicata* is associated with an increase in the AT fraction.

*Hypochaeris cretensis* and *H. oligocephala*, which showed the smallest DNA content, are very closely related to the group of *H. maculata*, which had the largest genome (Fig. 2). This implies that rapid size variation can arise in related species, as noticed by Cullis (1990).

Selection pressures such as environmental factors could have increased or decreased the DNA amounts. A decrease of DNA content, in the case of *H. cretensis* and *H. oligocephala*, associated with a specialization for a particular habitat, could have taken place during evolution. The natural ecological site for *H. oligocephala* is on cliffs in Tenerife (Lack, 1978) and for *H. cretensis*, on rocks (Brullo *et al.*, 1977). On the other hand, in the *H. maculata* group an increase of DNA content has occurred which could be associated with an adaptation to higher altitudes, as has been shown for *Zea mays* ssp. *mays* (Rayburn & Auger, 1990) and for the *Crepis praemorsa* complex of species (Godelle *et al.*, 1993). *Hypochaeris illyrica* and *H. uniflora* have significantly more DNA than *H. maculata* and are found at higher altitudes in mountain habitats.

Following these results, analysis of repeated interspersed DNA sequence families should provide a better understanding of genome evolution in *Hypochaeris* species.

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