*Botanical Journal of the Linnean Society*, 2007, **155**, 171–178. With 6 figures

# Chromosome studies in *Hippeastrum* (Amaryllidaceae): variation in genome size

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*Received April 2006; accepted for publication December 2006*

This paper presents the karyotype and DNA content of 12 diploid species of *Hippeastrum* from South America. The variation in genome size is compared with the karyotype and DNA content of *Amaryllis belladonna* from South Africa. The *Hippeastrum* species present a uniform and bimodal basic karyotype formula, but significant differences are found in the total chromosome volume (TCV) and nuclear DNA content. A positive correlation between the DNA content and TCV is also observed. The karyotype's constancy is a product of changes in DNA content occurring in the whole chromosome complement. The DNA addition to the long and short sets of chromosomes varies independently. In species with higher DNA contents, the short chromosomes add equal DNA amounts to both arms, maintaining their metacentric morphology, whereas the long chromosomes add DNA only to the short arm, increasing the chromosome symmetry. These data show that the evolutionary changes in DNA amount are proportional to chromosome length, maintaining the karyotypic uniformity. *A. belladonna* has a larger DNA content and possesses a karyotype different from that of *Hippeastrum* spp., supporting the distinction between the two genera and upholding the name *Amaryllis* for the South African entity against *Hippeastrum* for the South American genus.  *Botanical Journal of the Linnean Society*, 2007, **155**, 171–178.

ADDITIONAL KEYWORDS: bimodal karyotype – nuclear DNA content – orthoselection.

## INTRODUCTION

The genus *Hippeastrum* Herb. comprises more than 70 species in tropical and subtropical regions of South America. The karyotypes and chromosome numbers of many species have been studied previously (Naranjo & Andrada, 1975; Arroyo, 1982; Naranjo & Poggio, 1988; Brandham & Bhandol, 1997). The basic chromosome number is $x = 11$ and all species show a constant bimodal basic karyotype of four short [metacentric (m) or submetacentric (sm)] and seven long [four sm and three subterminal (st)] chromosomes. Polyploidy is the only conspicuous change in the karyotypic constitution, with triploids, tetraploids, and pentaploids being known (Naranjo, 1969; Naranjo & Andrada, 1975; Arroyo, 1982; Naranjo & Poggio, 1988; Brandham & Bhandol, 1997).

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 (Brandham, 1983; Kenton *et al*., 1990; Naranjo *et al*., 1998). The existence of groups of taxa with similar bimodal karyotypes is a result of karyotype orthoselection or karyotype conservation (White, 1973).

Recently, Bennett & Leitch (2005) emphasized the importance of genome size data in plant systematic and phylogenetic studies. Furthermore, it has been well documented in many taxa that variations in nuclear DNA amount are correlated with some karyological traits (Cerbah *et al*., 2001; Siljak-Yakovlev *et al*., 2003; Albach & Greilhuber, 2004; Garnatje *et al*., 2004).

In this paper, interspecific differences in the DNA amounts of 12 diploid South American species of *Hippeastrum* are documented, analysed, and discussed. The data are compared with the karyotype and DNA

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content of the monotypic species *Amaryllis belladonna* L. from South Africa.

## MATERIAL AND METHODS

Cytological studies were carried out on material cultivated at the Royal Botanic Gardens, Kew, with the exception of one accession of *H. argentinum* that was donated by A. T. Hunziker (ATH 18258). The sources of the material are listed in Table 1.

### Cytological analysis

For squash preparations, root tips were pretreated for 2.5 h in 0.002 M 8-hydroxyquinoline at 20 °C, fixed in 3 : 1 absolute ethanol : acetic acid, hydrolysed in 1 M HCl at 60 °C for 10 min, and stained in Feulgen. The total chromosome volume (TCV) was estimated using the formula $TCV = (\pi \times r^2 \times TCL) \times 2$ ($r$, average chromatid radius; TCL, total chromosome length). The average of the total centromeric index (TCI) was estimated using the formula TCI = (short arm/total length × 100)/$n$. The centromeric indices for short and long chromosomes ($CI_S$ and $CI_L$, respectively) were calculated separately. The nomenclature used for chromosome morphology is that of Levan, Fredga & Sandberg (1964). To estimate the karyotype asymmetry, two numerical parameters were used, following Romero Zarco (1986): $A_1$ [intrachromosomal asymmetry index = 1 – (short arm/long arm)/$n$] and $A_2$ [interchromosomal asymmetry index = standard deviation ($S$)/mean length ($X$)]. Both indices are independent of the number and size of the chromosomes. The determination of the karyotype parameters was carried out using a Mini Mop (Kontron) image analyser. Mean values for the karyotypes were calculated from measurements of a minimum of five scattered metaphase plates in each accession.

### Feulgen staining and cytophotometry

In each accession, 2C DNA content was measured in 20 telophase nuclei. Root tips were fixed in 3 : 1 absolute ethanol : acetic acid for 1–4 days. The staining method described in Tito, Poggio & Naranjo (1991) was used. The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 550 nm using the scanning method on a Vickers M85 microspectrophotometer. The DNA content per basic genome, expressed in picograms, was calculated using *Allium cepa* cv. 'Ailsa Craig' as a standard (2C = 33.55 pg; Bennett & Smith, 1976). The differences in DNA content were tested using an analysis of variance, and comparisons between means were performed using Scheffe's method (Scheffe, 1953).

## RESULTS AND DISCUSSION

The basic chromosome number $x = 11$ was found in the 12 studied species, all of which were diploid ($2n = 22$; Table 1). All the species analysed had a bimodal chromosome complement, based on four long sm, three long st, and four short m chromosomes in the haploid set (Table 1, Figs 1–5). Sometimes telocentrics (t) were present, or chromosome types intermediate between any of the above. All *Hippeastrum* species studied so far possess a nucleolar organizer region in a terminal position on the short arm of one st-t chromosome, forming a small satellite, as shown by positive staining with the silver technique by Naranjo & Poggio (1988). In four species, some chromosomal rearrangements were detected at low frequency, such as pericentric inversions and reciprocal translocations between long and short chromosomes (Fig. 5C, E, H, I). B chromosomes were found in two species, *H. morelianun* and *H. tucumanum* (Fig. 3). All of these data showed the presence of a conserved bimodal karyotype. In this case, selective mechanisms constraining changes in the karyotype could be active, suggesting that the existing karyotype may have some fundamental importance for genome organization and evolution of the genus.

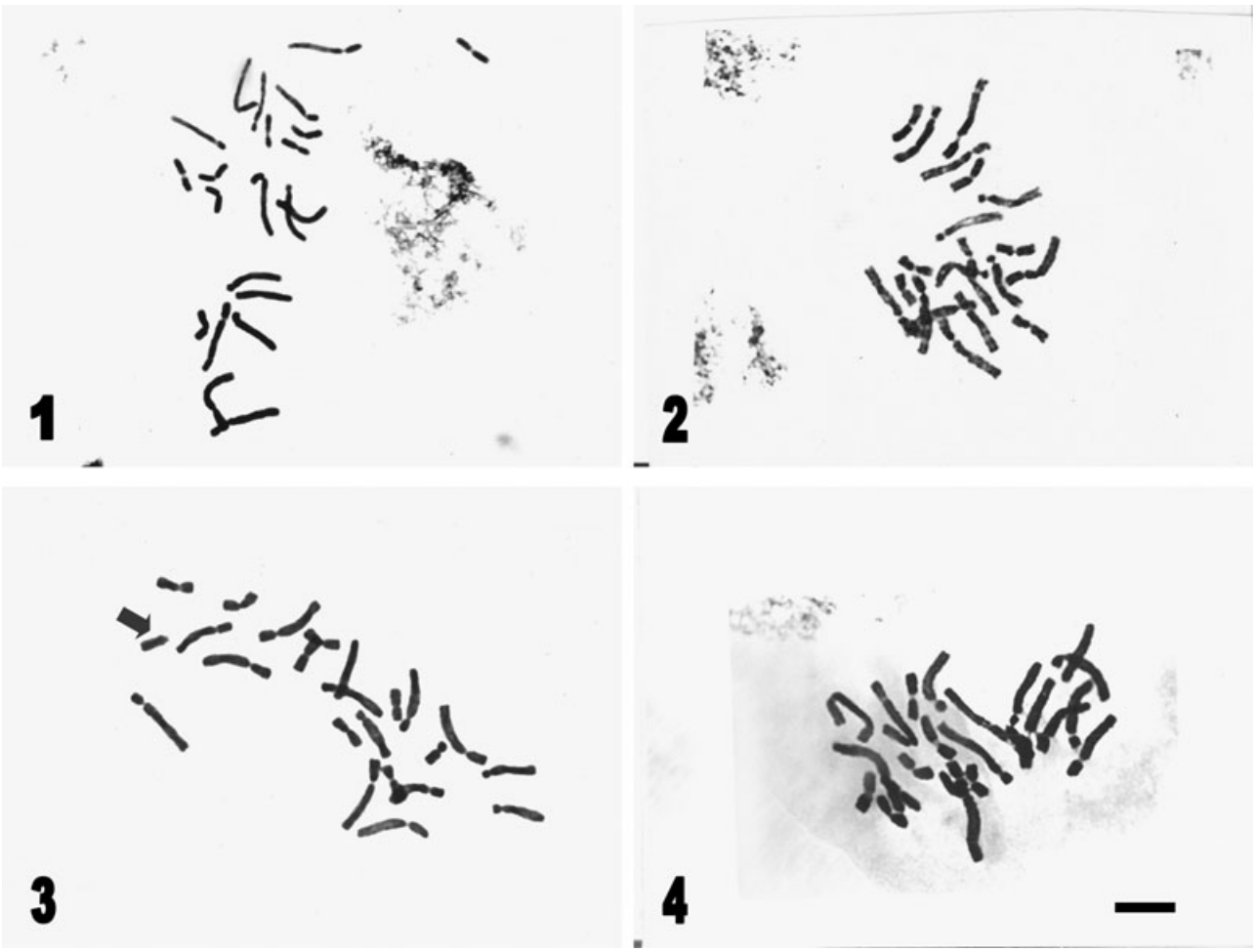
The total DNA content of the 12 studied species of *Hippeastrum* varied between 2C = 13.35 and 2C = 17.09 pg (Table 1). Significant differences in DNA amount of the basic genome were found in each species ($F = 63.67$, $P < 0.01$). A positive correlation between the DNA content and TCV was also found (Table 2). The distribution of DNA content between species was continuous, and no grouping of species with particular DNA contents was found (Tables 1, 2). These results were unexpected on the basis of the similar karyotype found in all species (Table 1, Figs 1–5). The significant increase in DNA amount without any significant karyotype change could be explained by a nonrandom distribution of these changes. Similar results have been found for South and North American species of *Lathyrus* (Klamt & Schifino-Wittman, 2000; Seijo & Fernández, 2003).

Rees (1984) proposed two strategies to explain the nonrandom distribution of DNA changes: (1) DNA addition proportional to chromosome size; or (2) addition of equal amounts of DNA to each chromosome. Both types of strategy are present in different sections of the genus *Vicia*. In the section *Vicia*, an increase in DNA content is achieved by the addition of equal increments to all the chromosomes of the complement, leading to a more symmetrical karyotype (Raina & Rees, 1983). By contrast, in section *Australis*, Naranjo *et al*. (1998) found that evolutionary changes in DNA amount were proportional to the relative length of each chromosome arm, maintaining

**Table 1.** Origin, karyotype formula, and DNA content in diploid species of *Hippeastrum* and *Amaryllis* (all $2n = 22$)

| Species | Origin | Kew accession | Basic karyotype formula | | | | | | | 2C DNA (pg) ± SE | DNA (pg) per basic genome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | m | m-sm | sm | sm-st | st | st-t | t | | |
| *Hippeastrum* Herb. | | | | | | | | | | | |
| *H. morelianum* (Lamaire) Traub | Brazil, Sao Paulo, Serra do Mar | 419-72-03853/1 | 4 | | 2 | 1 | 2 | | 2 | 26.69 ± 0.34 | 13.35 |
| | | 419-72-03853/2 | | | | | | | | 26.89 ± 0.27 | 13.45 |
| *H. correiense* (Bury) Worsley | Brazil, Sao Paulo | 419-72-03854 | 4 | | 2 | 2 | 1 | | 2 | 29.05 ± 0.25 | 14.52 |
| *H. parodii* Hunz et Cocucci* | Argentina, Corrientes, Tres Cerros | 400-76-03888 | 4 | | 4 | | 3 | | | 29.93 ± 0.30 | 14.96 |
| *H. rutilum* (Ker-Gawl.) Herb. | Brazil | 501-66-50111 | 4 | | 4 | | 3 | | | 30.20 ± 0.23 | 15.10 |
| *H. tucumanum* Holmberg | Argentina | 361-75-03430 | 4 | | 3 | 1 | 3 | | | 30.68 ± 0.16 | 15.34 |
| *H. psittacinum* (Ker-Gawl.) Herb. | Brazil | 088-60-08801 | 4 | | 3 | 1 | 3 | | | 31.33 ± 0.23 | 15.89 |
| *H. evansiae* (Traub & Nels.) Moore | Bolivia | 302-79-02858 | 4 | | 3 | 1 | 2 | 1 | | 31.92 ± 0.29 | 15.96 |
| *H. argentinum* (Pax) Hunz. | Argentina, Catamarca, Ambatos | ATH† 18258 | 4 | | 2 | 3 | | | 2 | 32.15 ± 0.18 | 16.07 |
| *H.* hybrid | | 344-79-03154 | 4 | | 3 | 1 | 3 | | | 32.40 ± 0.35 | 16.20 |
| *H. aulicum* (Ker-Gawl.) Herb. | Brazil, Santa Catarina | 434-79-04428 | | | | | | | | 33.12 ± 0.30 | 16.56 |
| *H. solandriflorum* Herb. | Argentina, Corrientes, Tres Arroyos | 301-79-02627 | 4 | | 4 | 1 | 2 | | | 33.35 ± 0.48 | 16.68 |
| *H. machupijchense* (Vargas) Hunt | Perú, Cuzco, Machupichu | 376-76-03600 | 4 | | 4 | | 3 | | | 34.18 ± 0.24 | 17.09 |
| *Amaryllis* L. | | | | | | | | | | | |
| *A. belladonna* L.* | South Africa | 000-69-34090 | 4 | 1 | 6 | | | | | 38.29 ± 0.19 | 19.14 |

*Data taken from Naranjo & Poggio (1988).
†A. T. Hunziker.




**Figures 1–4.** Mitotic metaphases in *Hippeastrum* species, all $2n = 22$. Fig. 1. *H. morelianum*. Fig. 2. *H. correiense*. Fig. 3. *H. tucumanum.* The arrow shows a B chromosome. Fig. 4. *H. evansiae*. Scale bar, 10 μm.

karyotype uniformity. An equivalent situation has been found in species of *Aloe*, which have different total genome sizes but retain bimodal karyotypes, with the longer and shorter chromosomes remaining in similar proportion between species (Brandham, 1983).

When TCI and $CI_L$ were measured (Table 2), positive relationships were obtained between TCI and DNA amount ($r = 0.798$) and $CI_L$ and DNA amount ($r = 0.977$) (Table 2). However, when $CI_S$ was analysed separately, it did not show any relationship with DNA amount (Table 2). This could mean that only the long chromosomes suffer changes in DNA amount, whereas the short chromosomes remain unchanged. If this were the case, the percentage volume of the short chromosomes ($CV_S$) would decrease when TCV increased. This is not the case, as the volume of the short chromosomes in relation to TCV is the same (or very similar) in all species studied (Table 2). To maintain $CI_S$ of the short chromosomes, whilst their volume and DNA content increase, the addition of DNA in proportion to each chromosome arm must occur. However, the more symmetrical CI of the longer chromosomes in species with a higher DNA content indicates that the addition of DNA occurs mainly in the short arms of all the long chromosomes.

In *Hippeastrum*, the addition of DNA to the long and short sets of chromosomes within each genome thus varies independently. The short chromosomes add equal DNA amounts to both arms, maintaining their metacentric morphology, whereas the long chromosomes add DNA only to the short arm, increasing their symmetry in the species with higher DNA amounts (Table 2).

In many cases, karyotype rearrangement may be implicit in the conservation of bimodality, reshuffling the new genome size into a more efficient organization. In the species of *Hippeastrum* studied previously, major structural chromosome changes have been reported rarely (Brandham & Bhandol, 1997)


**Figure 5.** Karyograms of *Hippeastrum* spp. A, *H. morelianun*. B, *H. correiense*. C, *H. parodii*. Arrow shows a pericentric inversion in a long chromosome. D, *H. rutilum*. E, *H. tucumanum.* Arrows indicate a reciprocal translocation between a short and a long chromosome. F, *H. psittacinum*. G, *H. evansiae*. H, *H. argentinum.* Arrows indicate a reciprocal translocation between a short and a long chromosome. I, *H.* hybrid. Arrow shows a pericentric inversion in a short chromosome. J, *H. solandriflorum*. Scale bar, 10 μm.

**Table 2.** Karyotype characteristics of diploid species of *Hippeastrum* and *Amaryllis*

| Species, $2n = 22$ | TCI | $CI_S$ | $CI_L$ | TCV ($\mu m^3$) | $CV_S$ (%) | 2C DNA (pg) ± SE | Asymmetry indices $A_1$ | Asymmetry indices $A_2$ |
|---|---|---|---|---|---|---|---|---|
| *Hippeastrum* | | | | | | | | |
| *H. morelianum* | 28.63 | 43.75 | 19.99 | 95.30 | 23.21 | 26.69 ± 0.34 | 0.55 | 0.32 |
| *H. correiense* | 31.07 | 45.58 | 22.78 | 104.20 | 24.44 | 29.05 ± 0.25 | 0.51 | 0.29 |
| *H. parodii** | 28.14 | 42.46 | 23.67 | 112.10 | 23.91 | 29.93 ± 0.30 | 0.52 | 0.29 |
| *H. tucumanum* | 31.35 | 43.20 | 24.89 | 112.20 | 24.90 | 30.68 ± 0.16 | 0.50 | 0.31 |
| *H. psittacinum* | 32.81 | 45.85 | 25.37 | 115.00 | 24.85 | 31.33 ± 0.23 | 0.48 | 0.32 |
| *H. evansiae* | 32.21 | 46.87 | 23.83 | 116.10 | 23.24 | 31.92 ± 0.29 | 0.47 | 0.32 |
| *H. solandriflorum* | 31.01 | 42.58 | 24.39 | 120.71 | 23.59 | 33.35 ± 0.48 | 0.51 | 0.31 |
| *H. machupijchense* | 30.24 | 42.42 | 26.17 | 121.79 | 23.65 | 34.18 ± 0.24 | 0.50 | 0.30 |
| *Amaryllis* | | | | | | | | |
| *A. belladonna** | 35.03 | 46.54 | 30.54 | 133.20 | 28.15 | 38.29 ± 0.19 | 0.43 | 0.21 |

$A_1$, intrachromosomal asymmetry index; $A_2$, interchromosomal asymmetry index; $CI_L$, average of centromeric indices of long chromosomes; $CI_S$, average of centromeric indices of short chromosomes; $CV_S$, volume of short chromosomes as percentage of volume of all chromosomes; TCI, total centromeric index; TCV, total chromosome volume.
*Data taken from Naranjo & Poggio (1988).

and then only as heterozygotes, as also reported here (Fig. 5). This suggests that they do not play any significant role in the chromosome evolution of the genus.

Brandham & Bhandol (1997) studied the karyotype of several species of *Hippeastrum* and *A. belladonna*, all from Bolivia, and found that both genera present a karyotype the same as that described here for *Hippeastrum* species. However, Naranjo & Poggio (1988) found important karyotypic differences between accessions of *A. belladonna* from South Africa and South America.

South African *A. belladonna* ($2n = 22$) has a DNA content larger than that of *Hippeastrum* spp. (2C = 38.29 pg) and possesses a different karyotype (4m + 1 m-sm + 6sm) (Naranjo & Poggio, 1988). Moreover, the nuclear organizer region (NOR) is in the short arm of an m-sm chromosome, forming a linear satellite, as demonstrated by Ag-NOR banding (Naranjo & Poggio, 1988). In Figure 6, the asymmetry of the karyotype, indicated by the arm ratio ($A_1$) and length ($A_2$), is plotted against the total DNA content. Amongst the *Hippeastrum* taxa, the species with a higher DNA content show karyotypes that are more symmetrical than those of species with a lower DNA amount. It can be seen that *A. belladonna*, with the highest DNA amount, occupies an isolated position when compared with the *Hippeastrum* species. This is a result of its more symmetrical karyotype (Naranjo & Poggio, 1988). These important karyotypic differences and several morphological and anatomical characteristics (Arroyo & Cutler, 1984) support the distinctiveness of the two genera, and uphold the name *Amaryllis* for the South African entity against *Hippeastrum* for the South American genus, as discussed by Naranjo & Poggio (1988).

In summary, *Hippeastrum* shows a karyotypic constancy, as all species have the same bimodal karyotype. However, there are significant interspecific differences in nuclear DNA amount. These are the result of an increase in DNA in the short arms of the long chromosomes and in both arms of the short chromosomes, the latter in proportion to the length of the arms, maintaining the uniformity of chromosome morphology. The results obtained in this work suggest that there is an internal homeostasis that selects for a particular type of genome organization in which the bimodality is achieved through a particular distribution of DNA.

## ACKNOWLEDGEMENTS

We thank Professor Dr Ovidio Nuñez for critical revision of the manuscript, Professor S. M. Bennett for making available facilities for DNA amount determination at the Royal Botanic Gardens, Kew, UK, K. Jones and Dr P. E. Brandham for the manuscript improvement, and Mr Diego Fink (Carrera del Investigador Científico, CONICET) for image technical assistance. The study was supported by grants from CONICET and Secretaría de Ciencia, Tecnología e Innovación Productiva de la República Argentina. The authors belong to the Carrera del Investigador Científico de CONICET.

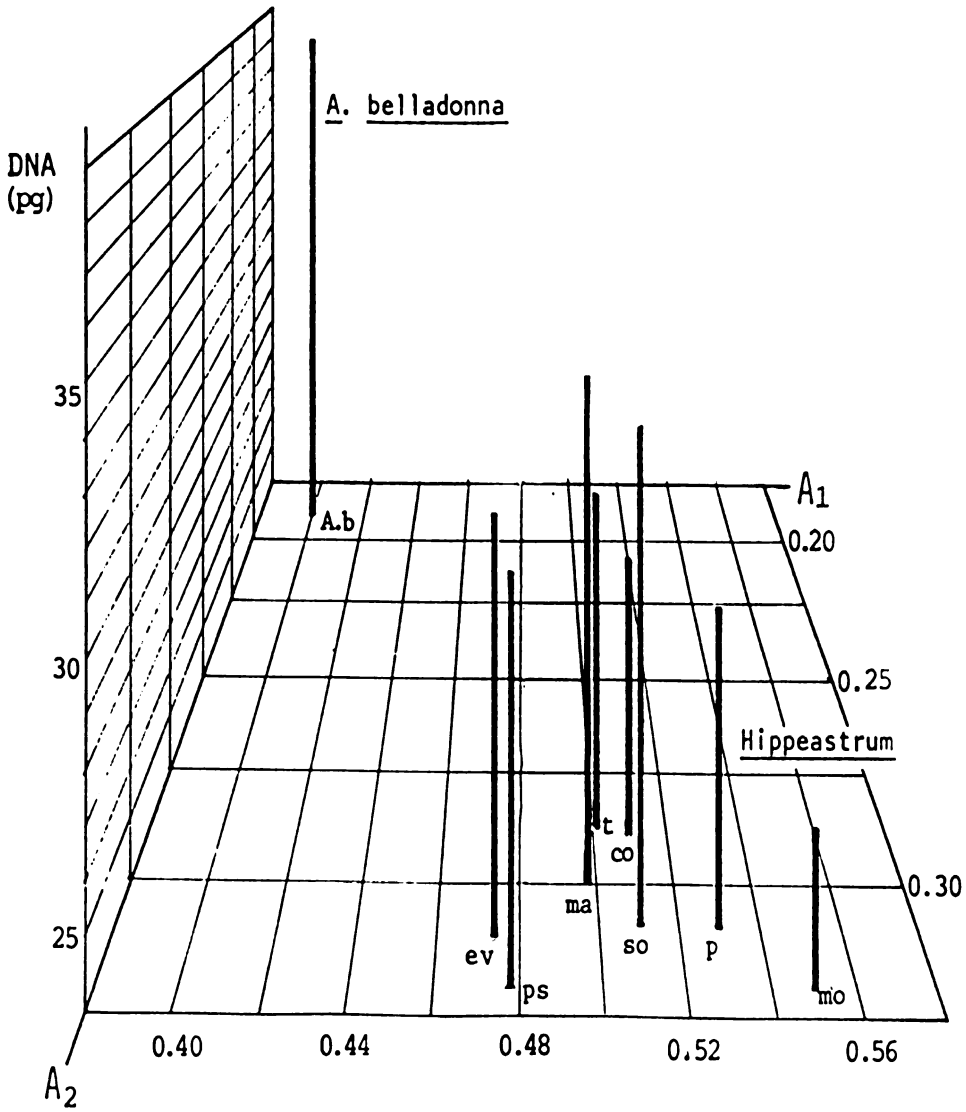



**Figure 6.** Scatter diagram showing asymmetry indices against DNA amounts of *Amaryllis belladonna* and *Hippeastrum* species. A.b, *A. belladonna*; co, *H. correiense*; ev, *H. evansiae*; ma, *H. machupijchense*; mo, *H. morelianum*; p, *H. parodii*; ps, *H. psittacinum*; so, *H. solandriflorum*; t, *H. tucumanum*.

## REFERENCES

**Albach DC, Greilhuber J. 2004.** Genome size variation and evolution in *Veronica*. *Annals of Botany* **94:** 897–911.

**Arroyo SC. 1982.** The chromosomes of *Hippeastrum*, *Amaryllis* and *Phycella* (Amaryllidaceae). *Kew Bulletin* **37:** 211–216.

**Arroyo SC, Cutler DF. 1984.** Evolutionary and taxonomic aspects of the internal morphology in Amaryllidaceae from South America and South Africa. *Kew Bulletin* **39:** 467–498.

**Bennett MD, Leitch IJ. 2005.** Nuclear DNA amounts in angiosperms: progress, problems and prospects. *Annals of Botany* **95:** 45–90.

**Bennett MD, Smith JB. 1976.** Nuclear DNA amounts in angiosperms. *Proceedings of the Royal Society of London, Series B* **274:** 227–274.

**Brandham PE. 1983.** Evolution in a stable chromosome system. In: Brandham PE, Bennett MD, eds. *Kew chromosome conference II*. London: George Allen & Unwin, 251–260.

**Brandham PE, Bhandol PS. 1997.** Chromosomal relationships between the genera *Amaryllis* and *Hippeastrum* (Amaryllidaceae). *Kew Bulletin* **52:** 973–980.

**Cerbah M, Montreau E, Brown S, Siljak-Yakovlev S, Bertrand H, Lambert C. 2001.** Genome size variation and species relationships in the genus *Hydrangea*. *Theoretical and Applied Genetics* **103:** 45–51.

**Garnatje T, Vallès J, Garcia S, Hidalgo O, Sanz M, Canela MA, Siljak-Yakovlev S. 2004.** Genome size in *Echinops* L. and related genera (Asteraceae, Cardueae): karyological, ecological and phylogenetic implications. *Biology of the Cell* **96:** 117–124.

**Kenton A, Dickie JB, Langton DV, Bennett MD. 1990.** Nuclear DNA amount and karyotype symmetry in *Cypella* and *Hesperoxiphion* (Tigridiae; Iridaceae). *Evolutionary Trends in Plants* **4:** 59–69.

**Klamt A, Schifino-Wittmann MT. 2000.** Karyotype morphology and evolution in some *Lathyrus* (Fabaceae) species of southern Brazil. *Genetics and Molecular Biology* **23:** 463–467.

**Levan A, Fredga K, Sandberg AA. 1964.** Nomenclature for centromeric position on chromosomes. *Hereditas* **52:** 201–220.

**Naranjo CA. 1969.** Cariotipo de nueve especies argentinas de *Rhodophiala*, *Hippeastrum*, *Zephyrantes*, *Habranthus* (Amaryllidaceae). *Kurtziana* **5:** 67–87.

**Naranjo CA, Andrada AB. 1975.** El cariotipo fundamental del género *Hippeastrum* Herb. (Amaryllidaceae). *Darwiniana* **19:** 556–582.

**Naranjo CA, Ferrari MR, Palermo AM, Poggio L. 1998.** Karyotype, DNA content, and meiotic behaviour in five South American species of *Vicia* (Fabaceae). *Annals of Botany* **82:** 757–764.

**Naranjo CA, Poggio L. 1988.** A comparison of karyotype, Ag-NOR bands and DNA contents in *Amaryllis* and *Hippeastrum* (Amaryllidaceae). *Kew Bulletin* **42:** 317–325.

**Raina SN, Rees H. 1983.** DNA variation between and within chromosome complements of *Vicia* species. *Heredity* **51:** 335–346.

**Rees H. 1984.** Nuclear DNA variation and the homology of chromosomes. In: Grant WF, ed. *Plant biosystematics*. Toronto: Academic Press, 87–96.

**Romero Zarco C. 1986.** A new method for estimating karyotype asymmetry. *Taxon* **35:** 526–530.

**Scheffe H. 1953.** A new method for judging all contrasts in the analysis of variance. *Journal of the Royal Statistical Society* **15:** 125–139.

**Seijo JG, Fernández A. 2003.** Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). *American Journal of Botany* **90:** 980–987.

**Siljak-Yakovlev S, Peccenini S, Muratovic E, Zoldos V, Robin O, Vallès J. 2003.** Chromosomal differentiation and genome size in three European mountain *Lilium* species. *Plant Systematics and Evolution* **236:** 165–173.

**Tito CM, Poggio L, Naranjo CA. 1991.** Cytogenetic studies in genus *Zea* 3. DNA content and heterochromatin in species and hybrids. *Theoretical and Applied Genetics* **83:** 58–64.

**White MJD. 1973.** *Animal cytology and evolution*. Cambridge: Cambridge University Press.