

# Ploidy dimorphism and reproductive biology in *Stenodrepanum bergii* (Leguminosae), a rare South American endemism

Irene Caponio, Ana M. Anton, Renée H. Fortunato, and G.A. Norrmann

**Abstract:** This is the first report on chromosome numbers and the reproductive behaviour in *Stenodrepanum* Harms, a rare endemic and monotypic legume genus from the arid and salty areas of central–western Argentina. Sixty individuals belonging to two populations from two salty areas (“salinas”) were surveyed and included mostly triploid ( $2n = 3x = 36$ ) and only two diploid ( $2n = 2x = 24$ ) plants. Meiosis in diploids is regular, with bivalent pairing and uniform and viable pollen. In contrast, meiosis in triploids is characterized by high trivalent pairing, with irregularly shaped pollen and variation in cytoplasm content and stainability, which is in agreement with an unbalanced segregation occurring in anaphases I and II. However, different triploid plants/individuals showed various degrees of pollen fertility, which may be attributed to particular genotypes. Research on reproductive biology events indicates sexual cross-pollinated reproduction enhanced by protogyny in both cytotypes. All plants produced seeds, but seedlings were only recovered from diploid plants pollinated with triploids, and even those eventually perished. Chromosome counts in these seedlings revealed aneuploid chromosome numbers owing to the combination of unbalanced gametes.

**Key words:** *Stenodrepanum*, triploids, diploids, endemic, Fabaceae, Argentina.

**Résumé :** Les auteurs présentent ici la première caractérisation du nombre chromosomique et du comportement reproductif au sein du *Stenodrepanum* Harms, un genre rare et monotypique au sein des légumineuses qui est originaire dans les régions arides et salées du Centre-Ouest de l’Argentine. Soixante individus appartenant à deux populations provenant de deux régions salines (« salinas ») ont été étudiés et comprenaient une majorité d’individus triploïdes ( $2n = 3x = 36$ ) et seulement deux diploïdes ( $2n = 2x = 24$ ). La méiose chez les diploïdes est normale, avec la formation de bivalents et de pollen uniforme et viable. Par contre, la méiose chez les triploïdes se caractérise par une grande fréquence de trivalents et des grains de pollen de forme irrégulière qui présentent de la variation pour le contenu cytoplasmique et la coloration, des caractéristiques qui concordent avec une ségrégation irrégulière au cours des anaphases I et II. Cependant, différents plants/individus triploïdes présentaient différents degrés de fécondité du pollen, ce qui pourrait être la conséquence de différents génotypes particuliers. Une investigation de la biologie reproductive indique une allofécondation sexuelle favorisée par la protogynie chez les deux cytotypes. Toutes les plantes ont produit des graines, mais des plantules n’ont été obtenues que chez les diploïdes fécondés par des triploïdes, mais même ces individus sont éventuellement morts. Des décomptes chromosomiques chez ces plantules ont indiqué une aneuploïdie due à la combinaison de gamètes déséquilibrés.

**Mots-clés :** *Stenodrepanum*, triploïdes, diploïdes, endémique, fabacées, Argentine.

[Traduit par la Rédaction]

## Introduction

Endemic species with restricted geographic distributions have become a central concern of biologists faced with the problem of preserving rare species endangered by habitat destruction and fragmentation (Ge et al. 2003).

*Stenodrepanum* Harms is an endemic and monotypic genus (Fig. 1A), with *Stenodrepanum bergii* Harms being a geophyte restricted to salt depressions in central–western Argentina in the provinces of Catamarca, Santiago del Estero,

Córdoba, La Rioja, and San Juan (Fig. 1C). These depressions are the bottoms of closed watersheds, known locally as “bolsones”, and are filled with evaporitic sediments. The climate is dry all year round, with a large thermal range, intense solar radiation, and scarce sporadic spring/summer rains. The soils are immature, very poor in organic matter, and salty (Fig. 1B). The prevailing vegetation is a halophytic shrubland, dominated by shrubs (Chenopodiaceae) and C<sup>4</sup> grasses, with scattered xerophytic trees.

Received 15 March 2011. Accepted 14 September 2011. Published at [www.nrcresearchpress.com/gen](http://www.nrcresearchpress.com/gen) on XX November 2011.

**I. Caponio.** Instituto de Botánica del Nordeste, CONICET-Universidad Nacional del Nordeste, Av. Sargento Cabral 2131, Casilla de Correo 209, 3400 Corrientes, Argentina.

**A.M. Anton.** Instituto Multidisciplinario de Biología Vegetal, CONICET-Universidad Nacional de Córdoba, Casilla de Correo 495, 5000 Córdoba, Argentina.

**R.H. Fortunato.** Instituto de Recursos Biológicos, CIRN-INTA, 1712 Castelar, Buenos Aires, Argentina.

**G.A. Norrmann.** Instituto de Botánica del Nordeste & College of Agriculture (UNNE), Corrientes, 3400 Corrientes, Argentina.

**Corresponding author:** Guillermo Norrmann (e-mail: [criadero@agr.unne.edu.ar](mailto:criadero@agr.unne.edu.ar)).

Recent collections of *Stenodrepanum* are not abundant in herbaria. After several years of systematic exploration over and at different times of the year in the distribution area of the species, in particular the boundaries of the type locality, we found in 2006 a well established population at the Salinas de Mascasín (San Juan province), and later on, another one was discovered in Salina La Antigua (La Rioja province).

The aim of this paper is to report our results concerning the chromosomes and reproductive behaviour in two well-established populations of *S. bergii* at the Salinas de Mascasín and Salina La Antigua (Fig. 1C).

### Materials and methods

Fresh material from sixty individuals belonging to two populations was collected directly in situ, through several field trips performed during the years 2006–2008. However, attempts to grow plants and make them flower outside their natural habitat during the first year were not successful either from transplanted individuals or from tubers. Therefore, we focused our attention on natural populations at both sites, labelling the plants to be able to perform checks at later dates.

Identification of populations and plants was simplified to just two populations, one for each salina, bearing the numbers of the collector and also letters to identify each plant within the population. Vouchers are as follows: **Argentina: Prov. La Rioja:** Dpto. Chamental, Salina La Antigua, Ruta Nacional 79, Km 282, Bertone & Bringas s.n. (CORD 1218), 25–04–2006; **Prov. San Juan:** Dpto. Caucete, Ruta Nac. 141, Salinas de Mascasín, aprox. a 500 m del cruce interprovincial con La Rioja, Fortunato et al. 9144, 24–I–2008 (fl) (BAB, CORD). The populations are kept at the following herbaria: Herbarium Universidad Nacional de Córdoba (CORD) and Herbarium Instituto Nacional de Tecnología Agropecuaria, Castelar, Argentina (BAB). Each marked individual was analyzed for meiosis, reproductive events, and seed set over different periods. Inflorescences were fixed in an absolute ethanol – glacial acetic acid solution of 3:1. Pollen mother cells (PMC) for meiotic analysis were stained with 1% acetocarmine. Pollen stainability and cytoplasm detection were achieved through a 50% solution of glycerol–acetocarmine.

To analyse the megasporogenesis and megagametogenesis, pistils were removed at different stages of development. The pistils were dehydrated in butyl alcohol series, embedded in paraffin, sectioned at 12  $\mu$ m, and then stained in the safranin-0-fast green series.

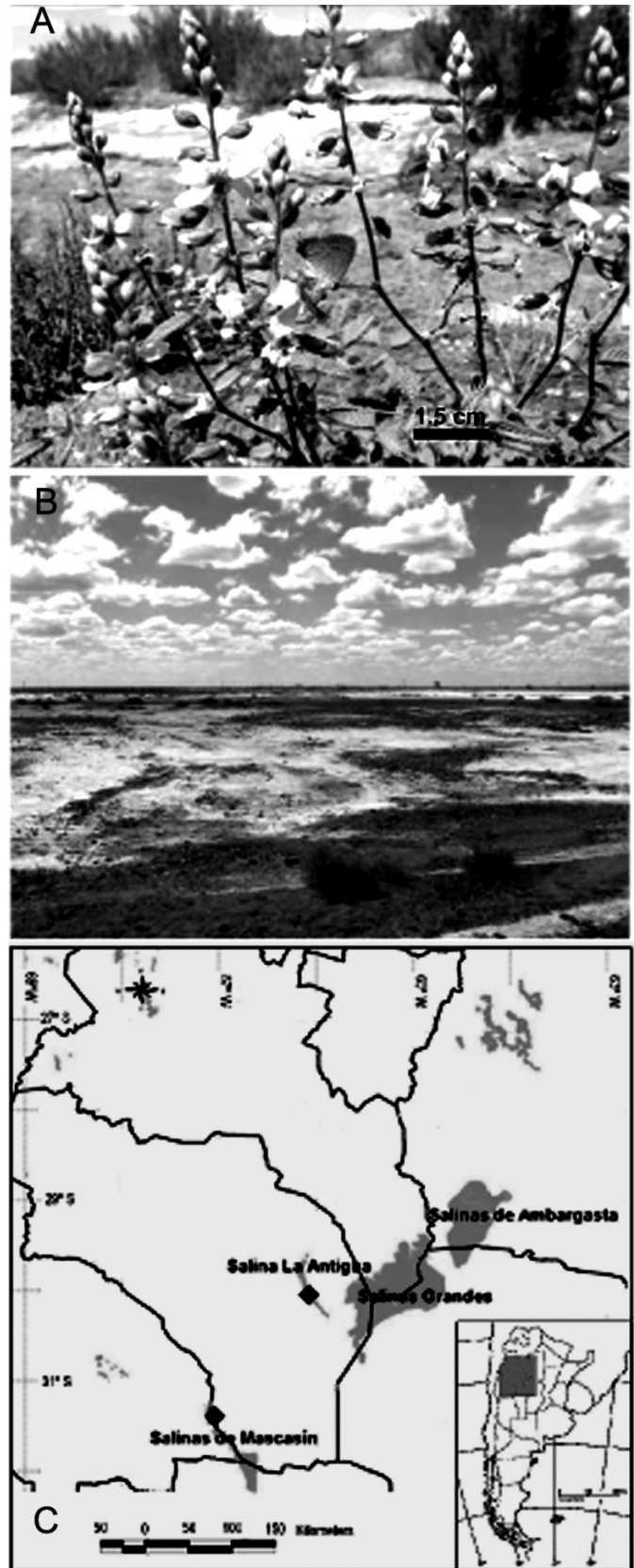
### Results

#### Chromosomes

From the 60 individuals analyzed, we were able to determine the chromosome numbers of 15 individuals from the Mascasín population and 22 individuals from the La Antigua population. Two plants from La Antigua were diploids with  $2n = 2x = 24$  chromosomes, whereas the other 20 individuals were triploid ( $2n = 3x = 36$ ). Plants from Mascasín were uniformly triploid, with no other ploidy level detected (Table 1).

Meiotic chromosome behaviour was studied in all 37 individuals, with a mean of 20 PMC per plant. The meiosis in

**Fig. 1.** (A) Inflorescence and (B) habitat of *Stenodrepanum bergii*. (C) Location of populations analyzed in this study are indicated by  $\blacklozenge$ . Dark grey areas refer to salt depressions (salinas) in central–western Argentina.

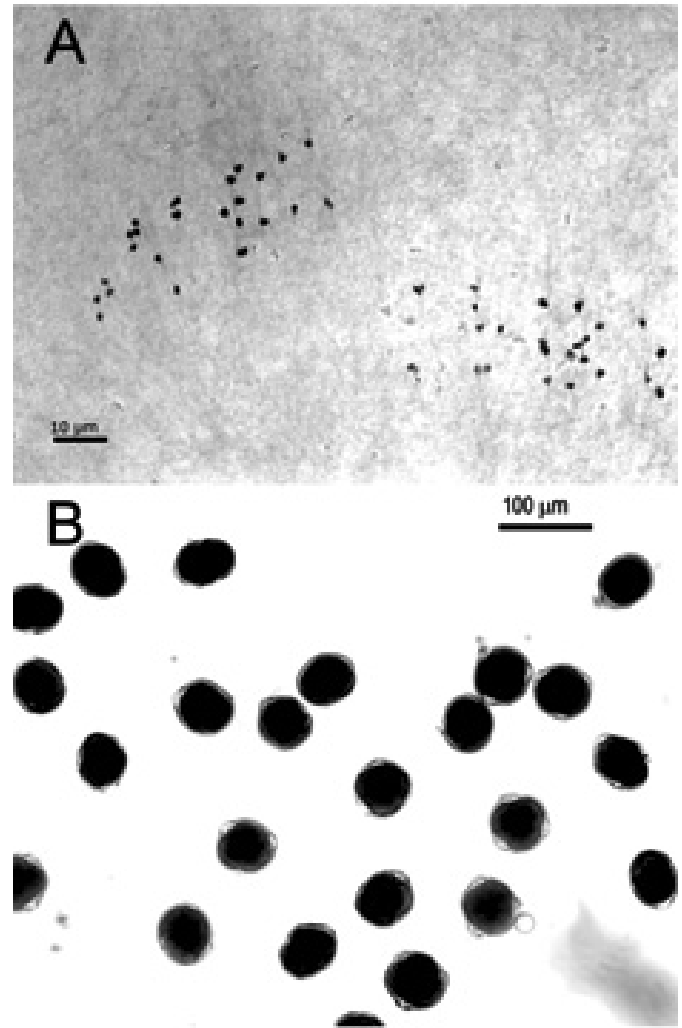




**Table 1.** Mean frequencies and ranges of univalents, bivalents, and trivalents at metaphase I; segregation ratios at anaphase I; and pollen viability in *Stenodrepanum bergii*.

Population location	No. of studied plants (scored PMC)	Univalents	Bivalents	Trivalents	Segregation ratios at anaphase I	Pollen viability (600 scored grains per plant)	
						Mean stainable pollen %	Variation among plants
Mascasín (San Juan)	15 (298)	3.6±3.0 (0-12)	2.45±2.56 (0-13)	8.93*±1.91 (4-12)	17:19 (13:23)	59.36	38.2-68.7
La Antigua (La Rioja)	20 (320)	3.17±2.74 (0-12)	2.47±2.1 (0-9)	9.31*±1.85 (3-12)	17:19 (14:22)	—	—
La Antigua (La Rioja)	2 (50)	—	12	—	12:12	98	—

Note: PMC, pollen mother cells.



**Fig. 2.** (A) Anaphase II showing standard bivalent pairing and

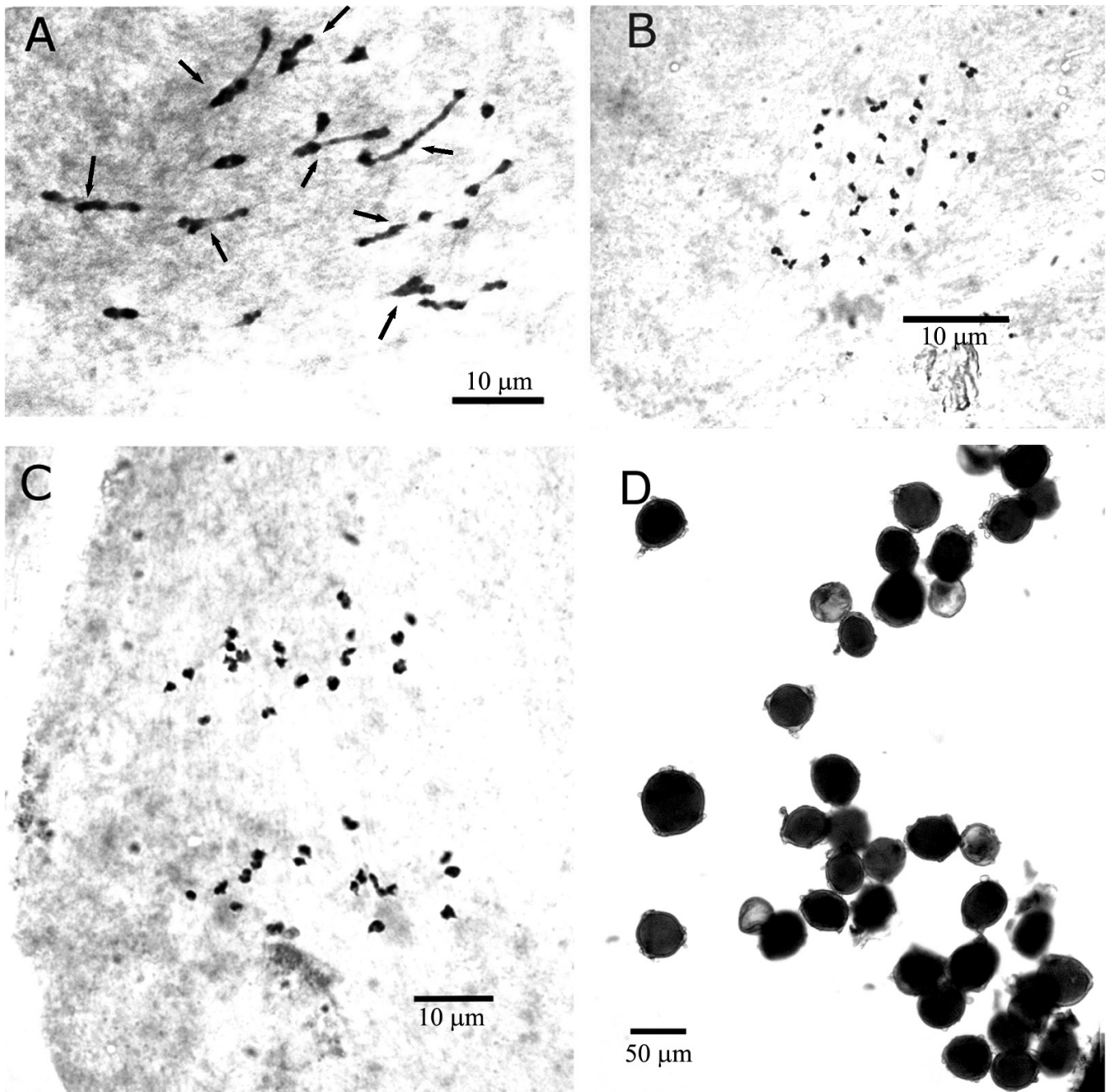
diploids was regular, with standard bivalent pairing and normal segregation (Table 1; Fig. 2A). As a consequence, the pollen was uniform and fully stained (Table 1; Fig. 2B).

On the other hand, the meiosis in triploids presented a high frequency of trivalent chromosome associations (Table 1; Fig. 3A). We frequently observed up to 12 trivalents, with a mean frequency of 8.9 (Mascasín) and 9.3 (La Antigua) in the two populations analyzed (data from individual plants not shown).

The segregation in anaphase I from triploids did not produce many gametes with equal chromosome numbers: less than 26.6% of cells analyzed showed an 18:18 ratio. Other segregation numbers were 13:23 and 14:22, with the most frequent being 17:19 (Table 1, Fig. 3C). Even though we could not find a 12:24 segregation, the eventual production of euploid gametes is possible. These irregularities affected pollen grains that were irregular in shape and showed low tincture (Fig. 3E). However, small differences were found in pollen size and stainability between triploid plants, which are in agreement with the distortions observed in the segregation process. Despite these abnormalities, unbalanced male gametes were able to fertilize, as shown below.



**Fig. 3.** (A) Metaphase I showing univalent, bivalent, and trivalent chromosome associations in triploid *Stenodrepanum bergii*. Arrows indicate trivalents. (B) Anaphase I showing 36 chromosomes in the equatorial plate. (C) Anaphase I with 17:19 segregation. (D) Pollen grains showing low staining and different sizes.



**Reproductive behavior**

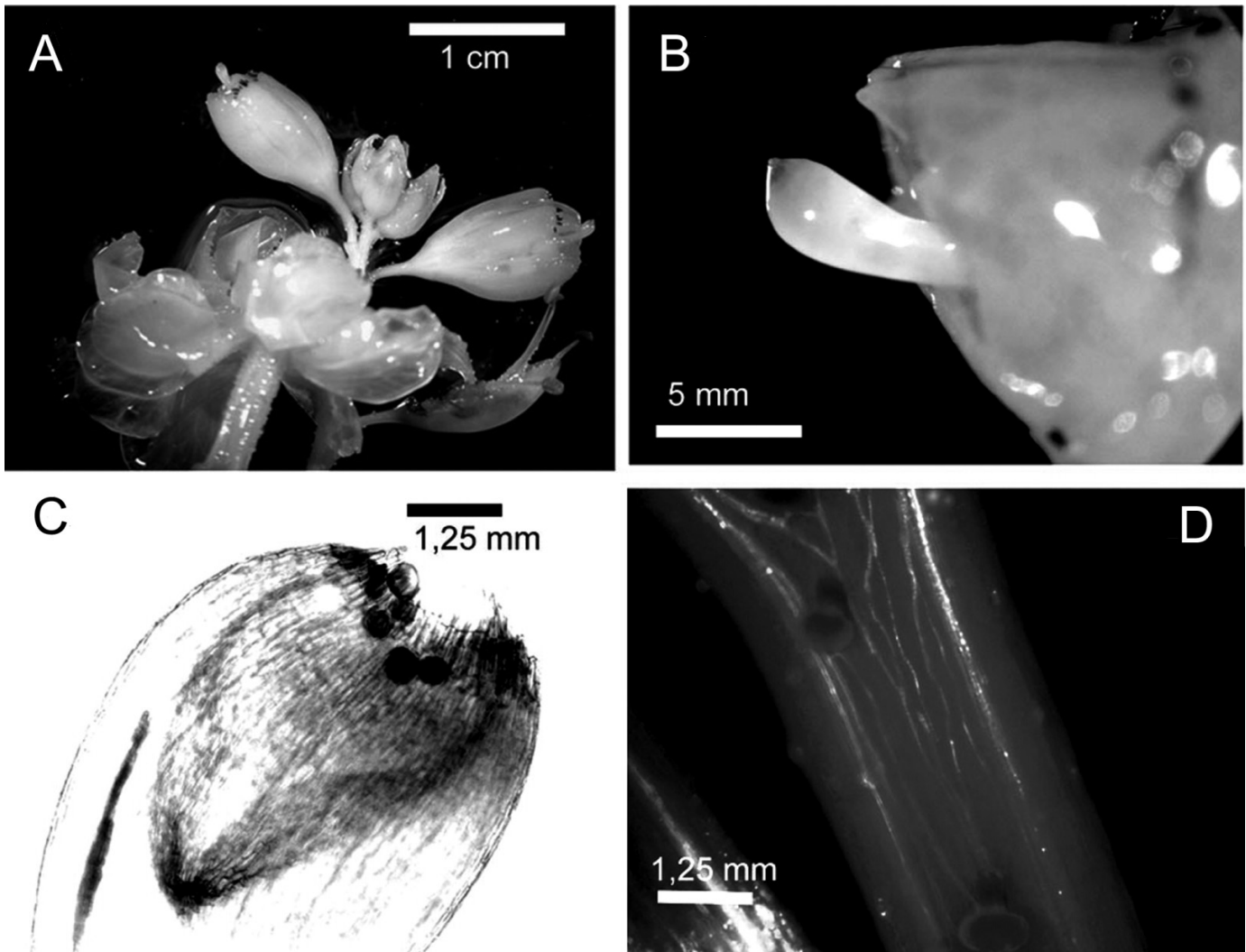
**Diploids and triploids**

Direct observations of flowering events and fixed material revealed that the stigmata protruded from the flower at least 1 day before the stamen filaments elongated, thus configuring a protogynous scenario (Figs. 4A, 4B). Cross-pollination was therefore enhanced by this feature, as shown by the lack of diploid progenies from diploid individuals (see below). However, occasional self-pollination through geitonogamy cannot be discounted. We could not detect any incompatibility pol-

len–stigma system when pollen from triploid plants fertilized either the diploid or triploid stigmata (Figs. 4C, 4D).

Sequences of megasporogenesis and embryosac development were scored in diploids as well as in triploid plants. In both, meiosis occurred in the archesporial cell (Fig. 5A) with a linear tetrad of megaspores being formed. Three of these later degenerated leaving the functional one. In due course, this developed into a one-nucleate embryo sac, followed by a two-, four- and, finally eight-nucleate embryo sac of the Polygonum type (Fig. 5B–5G).

**Fig. 4.** (A–B) Triploid *Stenodrepanum bergii* with stigmata protruding from the flowers. (C) Pollen into stigmata. (D) Growth of pollen tube in style, by fluorescence.



A difference between diploid and triploid plants was that in the latter deterioration of young embryo sacs could be immediately observed (Fig. 5H), thus leading to a high frequency of sterile ovules (data not shown).

### Progenies

Seeds were collected from plants of both Mascasín and La Antigua populations. The Mascasín plants, all triploids, produced different amounts of seed that would either not germinate or start to do so and then collapse. From a total of 118 seeds, 26 germinated and 5 produced seedlings, but none continued its development

In parallel, at La Antigua, triploid and diploid plants coexist; the triploids behave as those in Mascasín (from 98 seeds, only 15 germinated and 2 produced seedlings), i.e., most seeds do not succeed, and diploids (pollinated by triploids) produced seeds that grew into seedlings, but they did not last more than a week. However, from 54 seeds collected, 17 germinated and we were able to count chromosomes in root tips from 2 of these germinating seeds. One seedling had  $2n = 26$  chromosomes, a number that can be explained through

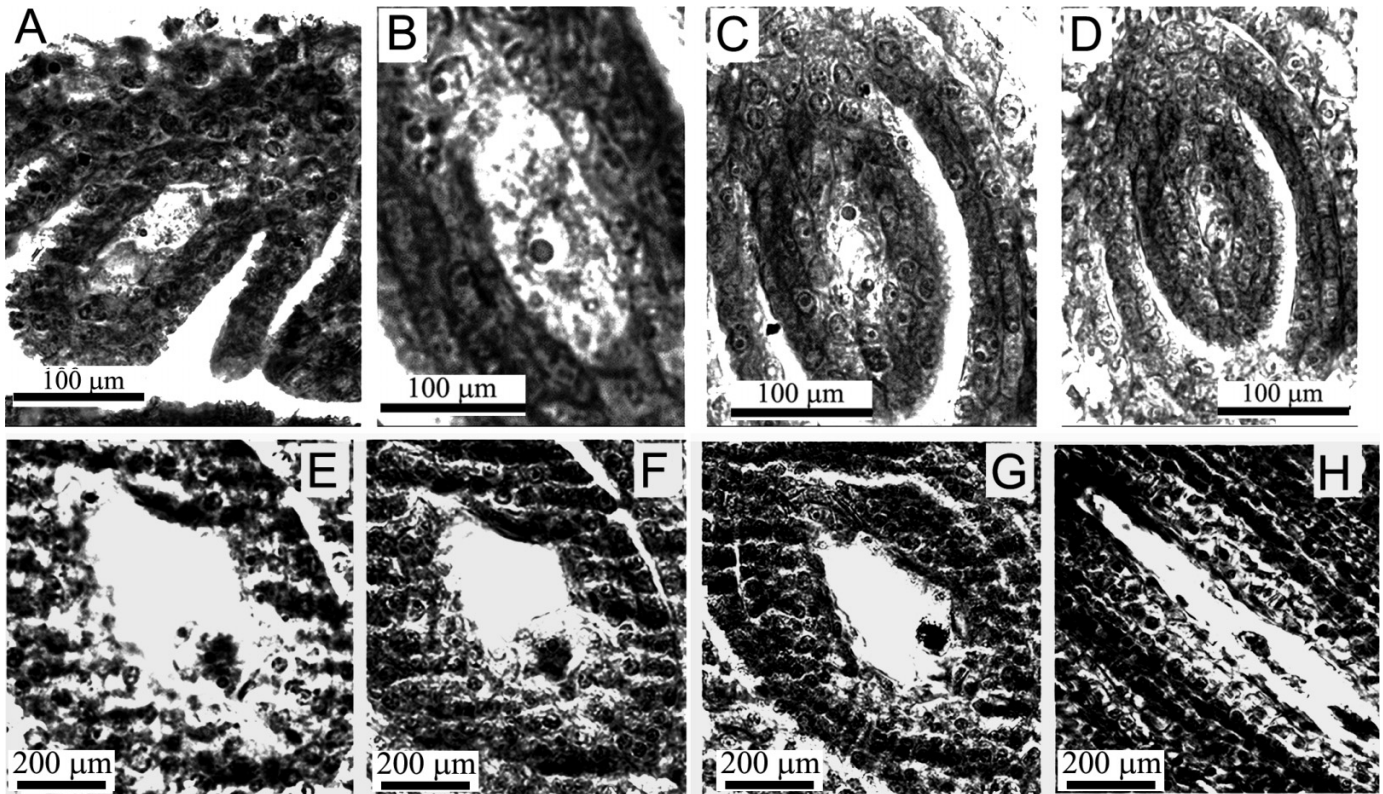
the combination of a reduced gamete from the mother plant ( $n = 12$ ) and an unbalanced gamete ( $n = 14$ ) from a triploid plant. The other seedling had  $2n = 27$  chromosomes, based on the same explanation. Finally, we could not find diploid progenies from diploid plants, likely owing to the shortage of diploid pollinators in an otherwise triploid neighbourhood.

### Discussion

Although triploids often occur in natural populations, only a few are able to become established by their own means, usually by being vegetatively spread, and even fewer can breed true by sexual means, i.e., permanent odd polyploidy—sensu Grant (1981) and John (1990). Nevertheless, triploids that acquire the capability to survive and spread often become a large portion of the available cytotypes and sometimes the main ploidy level of the species. Most triploid karyotypes are composed of three sets of the same basic chromosomes and are generally originated by the combination of nonreduced gametes, i.e., autopolyploids (Grant 1981; Ramsey and Schemske 1998). Few, if any (e.g., *Andropogon*



**Fig. 5.** Megasporogenesis and embryo sacs in diploid and triploid *Stenodrepanum bergii*: (A) Archesprial cell. (B) One-nucleate embryo sac. (C) Two-nucleated embryo sac. (D) Four-nucleated embryo sac. (E–G) Eight-nucleated embryo sac in three sections. (H) Triploid-collapsed mature embryo sac.



*ternatus* see Norrmann and Quarin 1987), possess two sets of homologous chromosomes plus another set of different origin and can still breed true.

Autotriploidy is often considered to be a maladaptive behaviour in plants because of its association with high levels of sterility. However, sterility is variable (see Comai 2005 and references therein), and in addition, autotriploids are considered important in tetraploid formation (Harlan and deWet 1975; deWet 1980; Ramsey and Schemske 1998). Triploids also are involved in the incorporation of genetic diversity in polyploid complexes (Lord and Richards 1977; Lumaret and Barrientos 1990), and recently, new features related to the advantages of triploidy itself have been uncovered (see Comai 2005).

According to the present study, *S. bergii* constitutes a new case of spontaneous triploidy in a legume, which is expressed as the most frequent chromosome number of the species. The meiotic chromosome behaviour studies and morphological resemblance among cytotypes suggest the triploid cytotype is an autopoloid.

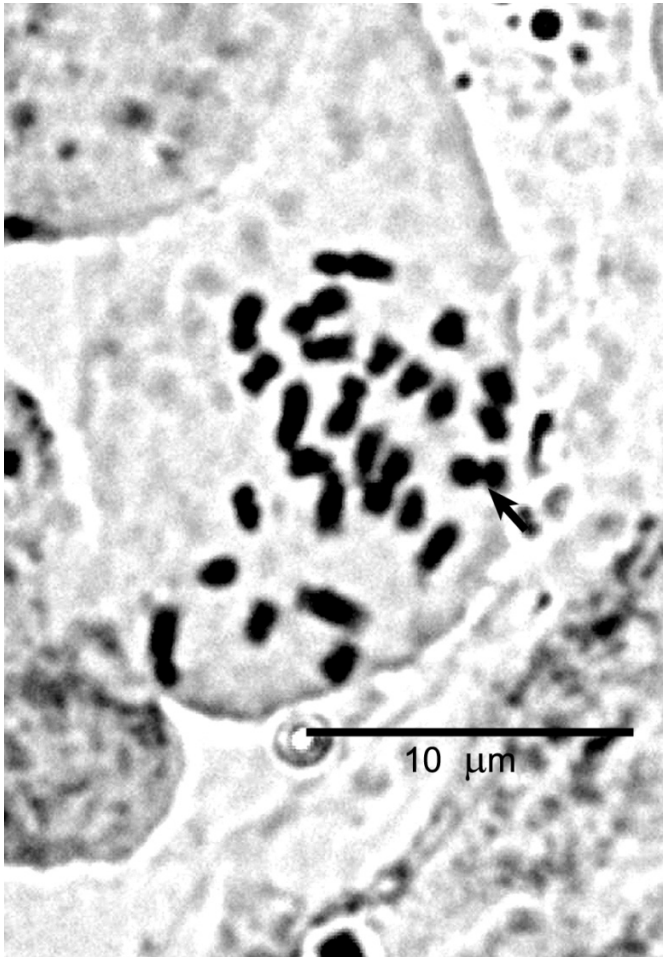
Upon comparing our findings with other known autotriploids and odd polyploids, the similarities with two American species are worth investigating. One of these is groundnut (*Apios americana* Medik.), a perennial twining legume (Papilionoideae: Phaseoleae) that grows along riverbanks in eastern North America (Joly and Bruneau 2004). This species has both triploid ( $2n = 3x = 33$ ) and diploid ( $2n = 2x = 22$ ) individuals distributed in the north and the south of its range, respectively (Seabrook and Dionne 1976; Bruneau and Anderson 1988). Triploids are thought to be au-

topolyploids because of their morphological similarity with diploids (Seabrook and Dionne 1976). Crossing experiments have also shown that triploid individuals are completely sterile, whereas diploids are allogamous, although fruit production in these individuals is scarce (Bruneau and Anderson 1988). Groundnut takes advantage of an efficient asexual means of reproduction via tubers, which seem to be carried and distributed by water currents. These tubers were extensively used as food by Native American people and early American settlers (Duke 1983).

In general, *S. bergii* has similar chromosomal and reproductive behaviour as groundnut, but there are apparent differences in the way both legumes explore spontaneous triploidy to become successful, or at least survive. Extreme ecological conditions coupled with cross-pollination have been considered to be fertile soil for the establishment of newly produced polyploids (see Stebbins 1950; Grant 1981). In this sense, the edaphic, climatic, and ecological conditions of the sites in which *Stenodrepanum* grows have probably influenced and determined its life form and its ploidy level. Once triploidy occurs, a source of propagation and perpetuation is required. In *S. bergii*, vegetative reproduction by means of tuberous roots is usual. Then, being a geophyte, the plant survives during the winter as dormant root tubers that sprout after late spring rains. Therefore, with such a successful multiplication scheme, seeds are apparently no longer needed.

North American bluestem (*Andropogon gerardii* Vitman) is another species worth comparing with *S. bergii*. *Andropogon gerardii* is a cross-pollinated hexaploid grass that often produces enneaploid progenies (Keeler 1990, 1992; Norr-

**Fig. 6.** Progeny from a diploid *Stenodrepanum bergii*. Mitotic chromosomes from seedling:  $2n = 27$ . Arrows indicate two different chromosomes. Bar = 10  $\mu\text{m}$ .



mann et al. 1997). For this comparison, *A. gerardii* cytotypes can be treated as diploid-like allohexaploids and triploids like enneaploids. On the edge of *A. gerardii* distribution, enneaploids behave better than hexaploids and therefore survive and spread thanks to a rhizomatous system (Keeler 1990, 1992). Once hexaploids and enneaploids of *A. gerardii* are in contact, all kinds of aneuploids are produced but none become established (Norrman and Keeler 2003). Similarly, the  $3x$  level in *Stenodrepanum* seems to be the main chromosome number for the species, in which aneuploid progenies are produced but not established. This suggests that triploid lineages may exhibit an evolutionary potential of their own, which does not serve solely as a first step toward tetraploid formation.

Having sexual reproduction restrained because of low fertility or poor adaptation implies that multiple origins could constitute an important contribution to genetic diversity. Although recurrent polyploidization has certainly not been proved yet for *S. bergii*, diploids do exist and therefore this process is more likely to occur.

For the moment, the particular ploidy level of *S. bergii* may explain the paucity and rareness of the species or vice versa, i.e., given that diploid populations are living under ex-

treme ecological conditions, autotriploidy is one of the evolutionary processes best suited for creating more diversity and (or) better adapted individuals.

### Acknowledgements

We thank G. Bertone, A. Bringas, and A. Romanutti for technical assistance; L. Sánchez and G. Cruzate helped with the illustrations; and N. Nagahama with statistical advice. This work was supported by grants as follows: Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET, PIP 6190 and 5560), SECyT-Universidad Nacional de Córdoba, Argentina, and SGCyT-Universidad Nacional del Nordeste, Argentina.

### References

- Bruneau, A., and Anderson, G.J. 1988. Reproductive biology of diploid and triploid *Apios americana* (Leguminosae). *Am. J. Bot.* **75**(12): 1876–1883. doi:10.2307/2444742.
- Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* **6**(11): 836–846. doi:10.1038/nrg1711. PMID:16304599.
- de Wet, J.M.J. 1980. Origins of polyploids. In *Polyploidy: biological relevance*. Edited by W.H. Lewis. Plenum Press, New York. pp. 3–15.
- Duke, J.A. 1983. Handbook of energy crops. Available from <http://www.hort.purdue.edu/newcrop/dukeenergy/dukeindex.html> [accessed 14 December 2010].
- Ge, X.-J., Yu, Y., Zhao, N.-X., Chen, H.-S., and Qi, W.-Q. 2003. Genetic variation in the endangered Inner Mongolia endemic shrub *Tetraena mongolica* Maxim. (Zygophyllaceae). *Biol. Conserv.* **111**(3): 427–434. doi:10.1016/S0006-3207(02)00312-9.
- Grant, V. 1981. *Plant speciation*. 2nd ed. Columbia University Press, New York.
- Harlan, J.R., and de Wet, J.M.J. 1975. On Ö. Winge and a prayer: the origins of polyploidy. *Bot. Rev.* **41**(4): 361–390. doi:10.1007/BF02860830.
- John, B. 1990. Meiosis. Edited by P. Barlow, D. Bray, P.B. Green, and J.W. Slack. Cambridge University Press.
- Joly, S., and Bruneau, A. 2004. Evolution of triploidy in *Apios americana* (Leguminosae) revealed by genealogical analysis of the histone H3-D gene. *Evolution*, **58**(2): 284–295. PMID:15068346.
- Keeler, K.H. 1990. Distribution of polyploid variation in big bluestem (*Andropogon gerardii*, Poaceae) across the tallgrass prairie region. *Genome*, **33**(1): 95–100. doi:10.1139/g90-015.
- Keeler, K.H. 1992. Local polyploid variation in the native prairie grass *Andropogon gerardii*. *Am. J. Bot.* **79**(11): 1229–1232. doi:10.2307/2445049.
- Lord, R.M., and Richards, A.J. 1977. A hybrid swarm between the diploid *Dactylorhiza fuchsii* (Druce) Soó and the tetraploid *D. purpurella* (T. and T.A. Steph.) Soó in Durham. *Watsonia*, **11**(3): 205–210.
- Lumaret, R., and Barrientos, E. 1990. Phylogenetic relationships and gene flow between sympatric diploid and tetraploid plants of *Dactylis glomerata* (Gramineae). *Plant Syst. Evol.* **169**(1–2): 81–96. doi:10.1007/BF00935987.
- Norrman, G.A., and Quarín, C.L. 1987. Permanent odd polyploidy in a grass (*Andropogon ternatus*). *Genome*, **29**(2): 340–344. doi:10.1139/g87-056.
- Norrman, G.A., Quarín, C.L., and Keeler, K.H. 1997. Evolutionary implications of meiotic chromosome behavior, reproductive biology, and hybridization in  $6x$  and  $9x$  cytotypes of *Andropogon gerardii* (Poaceae). *Am. J. Bot.* **84**(2): 201–207. doi:10.2307/2446081. PMID:21712199.

Ramsey, J., and Schemske, D.W. 1998. Pathways, mechanisms, and rates of polyploidy formation in flowering plants. *Annu. Rev. Ecol. Syst.* **29**(1): 467–501. doi:10.1146/annurev.ecolsys.29.1.467.

Seabrook, J., and Dionne, A. 1976. Studies on the genus *Apios*. I.

Chromosome number and distribution of *Apios americana* and *A. priceana*. *Can. J. Bot.* **54**(22): 2567–2572. doi:10.1139/b76-276.

Stebbins, G.L. 1950. Variation and evolution in plants. Columbia University Press, New York.