Meiotic irregularities in *Alstroemeria andina* var. *venustula* (Alstroemeriaceae)

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ABSTRACT. *Alstroemeria andina* Phil. var. *venustula* (Phil.) M. Muñoz *(sub nom. A. andina* Phil. subsp. *venustula* (Phil.) Ehr. Bayer) is a perennial, small herb, 5-16 cm tall, that occurs mainly at 2,800-3,700 meters above sea level, in populations of limited distribution from Argentina and Chile. The course of the meiosis was analyzed in a population of this taxon *(2n* = 2x = 16), and it proved to be highly irregular. It was characterized by presenting bridge and fragment configurations both at anaphases I and II. The highest number of bridges at anaphase I found in one cell was two, suggesting heterozygosity for as many as two paracentric inversions. Typical chiasmata were almost not detectable, even though they actually existed. The chiasma-like structures observed may be regarded as concealed chiasmata as it has been described in cryptochiasmate meiosis. A high frequency of tetrads with micronuclei was observed, implying significant levels of unbalanced gametes. Pollen stainability ranged between 28 and 30%. In *Alstroemeria* species the meiotic behaviour is highly regular, and the presence of rearrangements is very uncommon. The whole situation led us to suggest that some environmental factors have drastically affected the chromosome structure and the control of the meiotic process. The present study constitutes the first report of remarkable meiotic irregularities found in a wild population of this genus.

Keywords: Alstroemeriaceae; Chromosomes; Cryptochiasmate meiosis; Meiotic behaviour; Pollen staining; Structural heterozygosity.

INTRODUCTION

Alstroemeria andina Phil. var. *venustula* (Phil.) M. Muñoz (sub nom. *A. andina* Phil. subsp. *venustula* (Phil.) Ehr. Bayer) is a perennial, small herb, 5-16 cm tall, that occurs at 2,800-3,700 meters above sea level, exceptionally at 2,300-2,400 meters above sea level, in populations of limited distribution from Argentina and Chile (Sanso, 1996). This taxon inhabits the IV Region of Coquimbo in Chile and the departments of Iglesias and Calingasta, San Juan province, in Argentina (Bayer, 1987; Sanso, 1996). Its habitat comprises stony or sandy slopes and screes of the Anden mountains (Sanso, 1996).

The basic karyotype structure in the entire genus *Alstroemeria* is apparently uniform, and most species of this one have normal male meiotic behaviour with eight bivalents and the larger pair showing up to three visible chiasmata (Sanso, 2002). The karyotype formula of *A. andina* var. *venustula* was: 3 m pairs, 1 sm pair, 3 t and 1 t (st) pair, with microsatellites observed on pairs n° 3 and n°

6. Chromosome lengths ranged from 5.55 μm to 22.78 μm (Sanso, 2002).

This paper reports the first meiotic characterization of a population of *A. andina* var. *venustula,* with results that are totally different from all previous karyological studies of the genus.

MATERIALS AND METHODS

Plant materials for this study were collected from San Juan province, Calingasta department, Puesto de Gendarmería, Las Juntas, at 31°41'49" S-70' 14'13" O, Argentina. Voucher specimens, Fortunato & Kiesling 5631, were deposited at Herbarium of Instituto Darwinion (SI).

Flower buds of individuals collected from the wild population were fixed in ethanol-chloroform-glacial acetic acid (6:3:1) and later transferred into 70% ethanol and stored at 4-5°C. The cytogenetic analysis of this reduced population of *A. andina* var. *venustula* revealed that only five buds presented appropriate cells to analyze the course of meiosis, even when flower and anther sizes were suitable for it. Immature anthers were dissected out and

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slides were performed by the squash method in propionic acid haematoxylin (2%) using ferric citrate as a mordant (Nuñez, 1968).

For estimation of pollen viability, pollen grains of flowers at anthesis were stained with Alexander's differential stain (Alexander, 1969).

RESULTS

A strong disturbance of synchronization was detected in the development of pollen mother cells leading to a broad range of variation in meiotic stages. Cells from the diplotene stage (Figure 1A), microspores, and even pollen grains (Figures 3E and 3F) were present within the same anther, which is uncommon in *Alstroemeria* species.

The individuals showed eight bivalents, two of them were easily identifiable because of their notably larger size (pairs n° 1 and 2). Striking differences in the morphology of the bivalents compared with the previously studied *Alstroemeria* species were observed. Chromosome pairing seemed to be normal since univalents were seldom observed at diakinesis or metaphase I. From diplotene until diakinesis, or even metaphase I, the eight bivalents could be observed with the homologue chromosomes so intimately associated, that they gave the appearance of being composed of a single element (Figures 1A-D). The opening-out of the bivalents in the reductional plate, which usually occurs at the very beginning of diplotene, was postponed until first metaphase and in some cells it even did not occur at all until the homologues were pulled apart at anaphase. Separation of non-sister chromatids was suppressed, and there was thus no typical diplotene-diakinesis, and synapsis of homologue chromosomes was prolonged up to metaphase I (Figures 1A-D). Chiasmata were almost undetectable at these stages, even though they actually existed. The reductional split opened up at metaphase (exceptionally late diakinesis), revealing some chiasmata (Figures 1E and 1F). The centromeres co-orientated, and gradually became separated, and the paired segments were consequently pulled apart towards the distal ends by the centromere movement (Figures 1G, 2A and 2B).

Different abnormalities were observed at anapahase I (Table 1). In summary, only 46 out of 213 cells (21.60%) at first anaphase showed neither bridges nor fragments. The highest number of bridges at anaphase I found in one cell was two (34 cells = 20.36%), suggesting heterozygosity for as many as two paracentric inversions. Most of these cells with two bridges (Figures 2C, D) presented also two fragments (28 out of 34). The fragments usually lagged at the equator and in some cells, non congressed, lagged univalents or bivalents were found at anaphases I near them (Table 1, Figure 2D). Bivalents forming double bridges and fragments were also observed. (They could be explained as a four-strand double crossover within the inversion.) (Figure 2A). In that same cell (Figure 2A), a chromatid loop (arrowhead) indicated the presence of another inversion in heterozygosis in a different chromosome pair. A possible explanation for this meiotic figure is if, in addition to a chiasma in a paracentric inversion loop, one occurs in the interstitial segment between the centromere and the inversion. In this case, the anaphase I bridge is converted into a loop plus a fragment in one of the chromosomes of the original bivalent (Sybenga, 1992, page 115). At anaphase II the loop becomes a bridge that is not formed when a bridge is present at first anaphase. The remaining chromosome of the pair is present in the other pole of this cell, but it was difficult to individualize it.

At anaphases II one (Figure 2E) or two bridges (Figure 2F), bivalents, chromosome fragments, and/or chromatid fragments (Figure 2E) could also be observed. With regard to the relationship between the bridges and the fragments, it was noted that in most cases, every bridge was accompanied by one fragment (two dicentric bridges, two fragments). However, some cells were found to have only bridges (with no fragments) and other ones, with more bridges than fragments (Table 1).

In the tetrad analysis, we found that only about 18% of the tetrads lacked micronuclei, and most of them presented from 1 to 4 micronuclei (Table 2): 1 (56%), 2 (27%, Figure 3A), 3 (19%, Figures 3B and 3C), 4 (3%, Figure 3D). A very low pollen grain number was present within the anthers, and in some cases they were almost

Table 1. Number and percentage of cells presenting or not presenting bridges, fragments and/or univalents/bivalents at anaphase I.

Normal cells	Cells presenting dicentric bridges								Total number of cells
	One bridge[a]				Two bridges[a]				
	Only	+1f	+1f+2I or 1II	+1f+1I	Only	+2f	+2f+1-2I or 1II	+1f	
	39	79	12	3	2	22	6	4	
46		133				34			213
		79.64%				20.36%			
21.60%			78.40%						100%

[a]f = acentric fragment; I = univalent; II = bivalent.

completely absent. Although pollen stainability ranged between 28 and 30%, the pollen fertility value is suspected to be significantly lower, bearing in mind the percentage of observed tetrads presenting micronuclei. Furthermore, two kinds of pollen grains were found: small sized with supposedly normal chromosome complements (Figure 3E) and very large ones with abnormal chromosome numbers and fragments (Figure 3F).

DISCUSSION

In *Alstroemeria* species meiotic behaviour is highly regular and the presence of rearrangements very

Table 2. Number and percentage of tetrads with or without micronuclei.

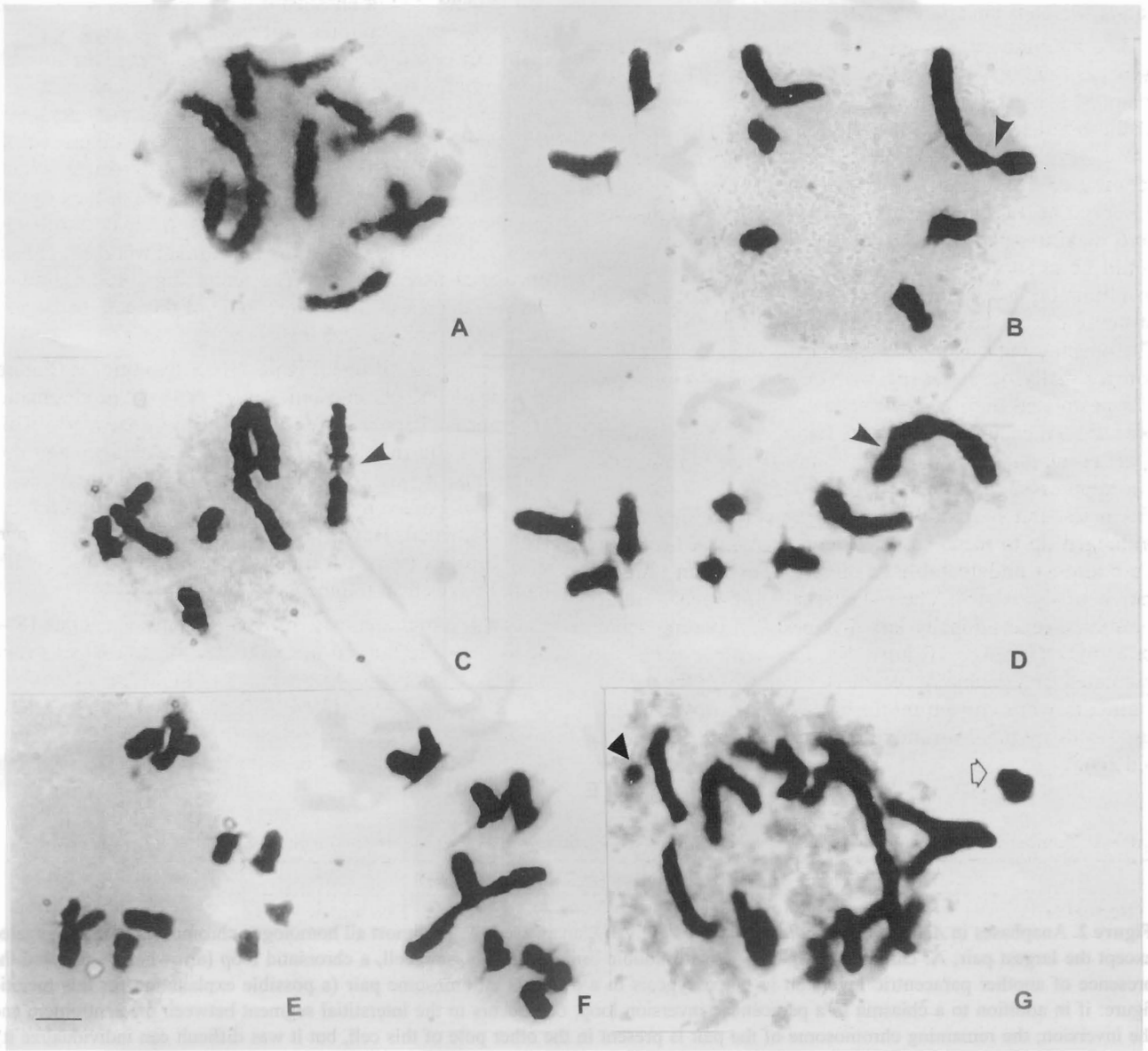| Micronuclei number	0	1	2	3	4	Total
Tetrads number	24	62	30	16	3	135
Tetrads percentage		56%	27%	19%	3%	
	17.78%		82.22%			100%

Figure 1. Meiosis in *Alstroemeria andina* var. *venustula* ($n = 8$). A: diplotene; B, C, and E: diakinesis; D and F: metaphase I; E and F: bivalents showing clearly some of the chiasmata; G: early anaphase I: seven opening-out bivalents, plus one bivalent (empty arrow) and one fragment out of equatorial plane (full triangle). In B and D, arrowheads point out the positions of secondary constrictions of chromosome pair n° 1, in C, the region between two homologous chromosomes of a bivalent which had been almost pulled apart. All photomicrographs are with the same enlargement. Bar = 10 μm.

Figure 2. Anaphases in *Alstroemeria andina* var. *venustula*. A-D: anaphase I; A, B: almost all homologue chromosomes are segregated except the largest pair; A: chromosome pair n° 1 with double bridges. In the same cell, a chromatid loop (arrowhead) indicated the presence of another paracentric inversion in heterozygosis in a different chromosome pair (a possible explanation for this meiotic figure: if in addition to a chiasma in a paracentric inversion loop, one occurs in the interstitial segment between the centromere and the inversion; the remaining chromosome of the pair is present in the other pole of this cell, but it was difficult can individualize it); B: chromosome pair n° 1 with a dicentric bridge; C: cell with two dicentric bridges, one acentric fragment (arrowhead) possibly accompaning a bridge and at least three chromosome fragments, probably derived from breakages (empty triangles); D: cell with two dicentric bridges, one acentric fragment (arrowhead), one possible univalent (empty triangle) and one small chromatid fragment (full triangle) (incomplete complement); E-F: anaphase II, E: cell with a dicentric bridge, an acentric fragment (arrowhead), a small chromatid fragment (full triangle), and two bivalents (stars), F: cell with two dicentric bridges (incomplete chromosome complement). Figures A, B, D and figures E, F, with the same enlargement. Bar = 10 μm.

uncommon (Sanso and Hunziker, 1998; Sanso, 2002). The present study constitutes the first report of meiotic irregularities from a wild population of this genus. Although a few meiotic irregularities had been observed in *A. hookeri* Lodd. subsp. *cummingiana* (Herb.) Ehr. Bayer (Sanso, 2002), the studied material of this taxon endemic to Chile was obtained from plants cultivated in a botanical garden.

One of the striking observed features in this population of *A. andina* var. *venustula* was the particular morphology of bivalents during meiotic prophase. Although almost no typical chiasmata were detected, the chiasma-like structures observed may be regarded as concealed chiasmata as it has been described in cryptochiasmate meiosis. In this kind of atypical chiasmate meiosis, numerous chiasmata are concealed between closely
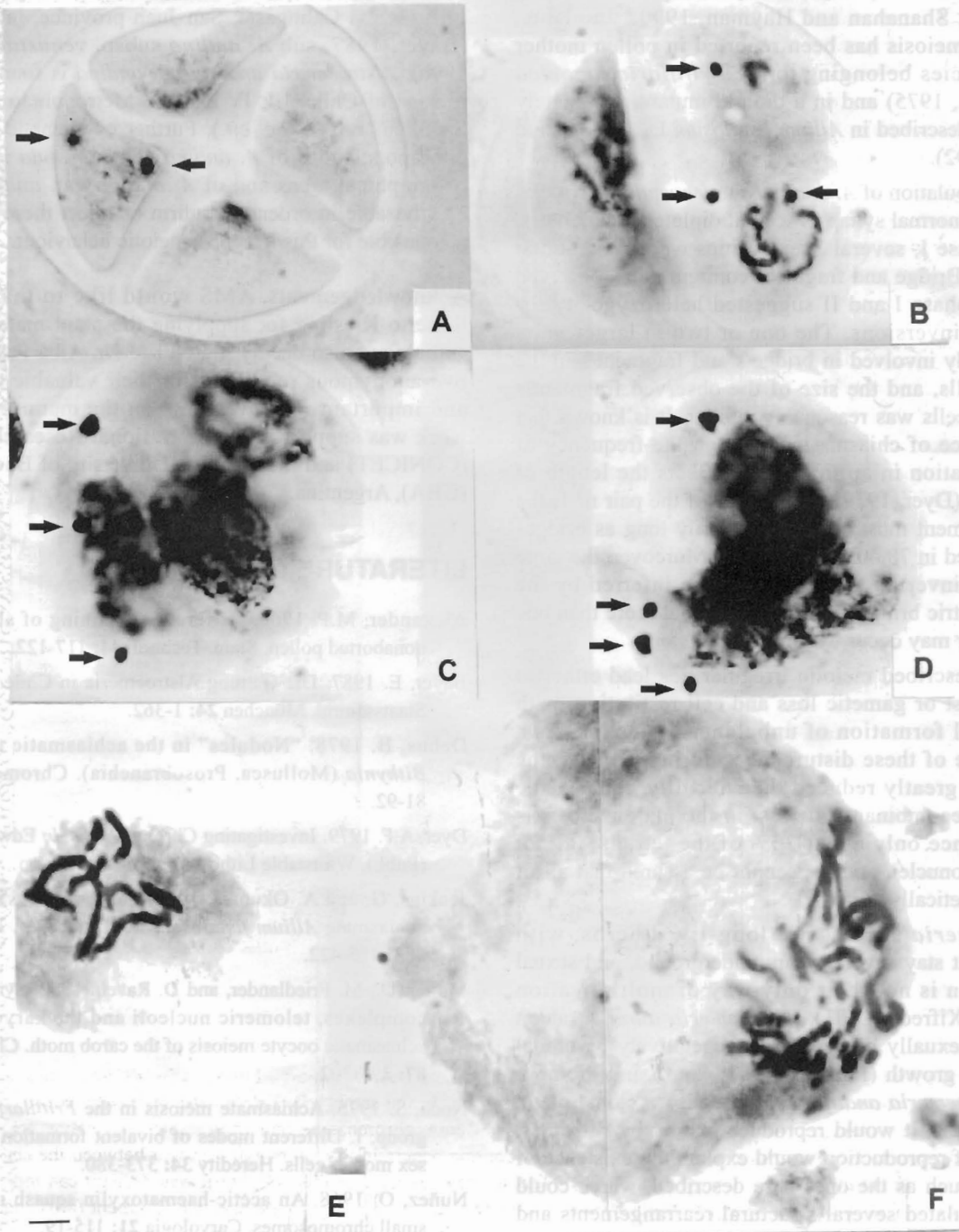

Figure 3. Tetrads and pollen grains in *Alstroemeria andina* var. *venustula*. A-D: tetrads with two (A), three (B, C), or four micronuclei (D); E, F: pollen grains, E: normal, n = 8; F: abnormal, with larger size, anomalous number of chromosomes, and chromosomal fragments. Micronuclei are indicated by arrows. All photomicrographs are with the same enlargement. Bar = 10 μm.

synapsed homologues, and they are apparently not responsible for, or play a subsidiary role in, maintaining the bivalent up until the later meiotic stages (White, 1965). Cryptochiasmate meiosis has been described in *Thericles* males (*loc. cit.*) and has been seen previously only in animals. This kind of meiosis is assumed to be a transitional step from chiasmate to achiasmate meiosis (Noda, 1975). The achiasmate condition, in which the recombination may be virtually abolished, is usually confined to one sex (Debus, 1978; Oakley, 1982; Morag et al., 1982; Shanahan and Hayman, 1990). In plants, achiasmate meiosis has been reported in pollen mother cells of species belonging to the *Fritillaria japonica* group (Noda, 1975) and in a diploid mutant, completely male steril, described in *Allium fistulosum* L. (Jenkins and Okumus, 1992).

In this population of *A. andina* var. *venustula*, in spite of the apparent normal synapsis seen at diplotene, diakinesis, and metaphase I, several irregularities were observed at later stages. Bridge and fragment configurations observed both at anaphase I and II suggested heterozygosity for paracentric inversions. The one or two m larger pairs were probably involved in bridge/s and fragment/s in the anaphase cells, and the size of the observed fragment/s in different cells was reasonably similar. It is known that in the absence of chiasma localization the frequency of bridge formation in an inversion reflects the length of the segment (Dyer, 1979). In the case of the pair n° 1, the inverted segment must be extraordinarily long as bridges were observed in 78.40% of the cells. Moreover, the large size of this inverted segment could be inferred by the double-dicentric bridges, which evince that more than one crossing over may occur within the inversion.

All the described meiotic irregularities lead either to meiotic arrest or gametic loss and cell restitution, with the eventual formation of unbalanced gametes. As a consequence of these disturbances during meiosis, the plants have greatly reduced their fertility. Indeed, the number of recombinants at least in the male meiosis is very low since only about 20% of the tetrads did not present micronuclei, and we cannot be assured that all of them are genetically normal.

Alstroemeria species are long-lived herbs, with rhizomes that stay several years underground, and sexual reproduction is not their only way of multiplication (Sanso and Xifreda, 2001). *Alstroemeria aurea* Graham reproduces sexually by seeds and vegetatively by clonal rhizomatous growth (Puntieri, 1991; Souto and Premoli, 2003). *Alstroemeria andina* would be also a clonal plant, but in this case, it would reproduce mainly by rhizomes. This mode of reproduction would explain the existence of individuals such as the ones here described, which could have accumulated several structural rearrangements and mutations.

The possibility that these plants may be hybrids combining genomes from different populations or species must be put aside since the population is geographically isolated from other *Alstroemeria* populations. The whole situation led us to suggest that some environmental factors have drastically affected the chromosome structure and the control of the meiotic process. A plausible explanation is that the cell division is being disturbed by natural soil pollution, probably the proximity of a mineral bed. The studied population was collected from a mountain region, where mining exploration projects are being evaluated. *Alstroemeria andina* var. *venustula* inhabits the IV Region of Coquimbo in Chile and the departments of Iglesias and Calingasta, San Juan province, in Argentina (Bayer, 1987, sub *A. andina* subsp. *venustula*; Sanso, 1996). *Alstroemeria andina* var. *andina* is found in three regions of Chile, III, IV and the Metropolitan Region of Santiago (Bayer, *loc. cit.*). Further cytogenetic studies of other populations of *A. andina* var. *venustula* in different geographical areas and of *A. andina* var. *andina* would be advisable in order to confirm or reject these factors as responsible for this irregular meiotic behaviour.

LITERATURE CITED

Alexander, M.P. 1969. Differential staining of aborted and nonaborted pollen. Stain. Technol. **44:** 117-122.

Bayer, E. 1987. Die Gattung Alstroemeria in Chile. Mitt. Bot. Staatssamml. München **24:** 1-362.

Debus, B. 1978. "Nodules" in the achiasmatic meiosis of *Bithynia* (Mollusca, Prosobranchia). Chromosoma **69:** 81-92.

Dyer, A.F. 1979. Investigating Chromosomes. *In* Edward Arnold (Publ.), Whitstable Litho Ltd., London. 138 pp.

Jenkins, G. and A. Okumus. 1992. Indiscriminate synapsis in achiasmate *Allium fistulosum* L. (Liliaceae). J. Cell Sci. **103:** 415-422.

Morag, D., M. Friedlander, and D. Raveh. 1982. Synaptonemal complexes, telomeric nucleoli and the karyosphere in achiasmatic oocyte meiosis of the carob moth. Chromosoma **87:** 293-302.

Noda, S. 1975. Achiasmate meiosis in the *Fritillaria japonica* group. I. Different modes of bivalent formation in the two sex mother cells. Heredity **34:** 373-380.

Nuñez, O. 1968. An acetic-haematoxylin squash method for small chromosomes. Caryologia **21:** 115-19.

Oakley, H.A. 1982. Meiosis in *Mesostoma ehrenbergii ehrenbergii* (Turbellaria, Rhabdocoela) II. Synaptonemal complexes chromosome pairing and disjunction in achiasmatic oogenesis. Chromosoma **87:** 133-147.

Puntieri, J.G. 1991. Vegetation response on a forest slope cleared for a ski-run with special referente to the herbs *Alstroemeria aurea* Graham (Alstroemeriaceae). Argentina Biol. Conserv. **56**: 207-221.

Sanso, A.M. 1996. El género *Alstroemeria* (Alstroemeriaceae) en Argentina. Darwiniana **34**: 349-382.

Sanso, A.M. 2002. Chromosome studies in Andean taxa of *Alstroemeria* (Alstroemeriaceae). Bot. J. Linn. Soc. **138**: 451-459.

Sanso, A.M. and J.H. Hunziker. 1998. Karyological studies in *Alstroemeria* and *Bomarea* (Alstroemeriaceae). Hereditas **129**: 67-74.

Sanso, A.M. and C.C. Xifreda. 2001. Generic delimitation between *Alstroemeria* and *Bomarea* (Alstroemeriaceae).

Ann. Bot. **88**: 1057-1069.

Shanahan, C.M. and D.L. Hayman. 1990. Synaptonemal complex formation in male scorpions exhibiting achiasmatie meiosis and structural heterozygosity. Genome **33**: 914-926.

Souto, C.P. and A.C. Premoli. 2003. Genetic divergence among natural populations of *Alstroemeria aurea* D. Don: a dominant clonal herb of the understory in subalpine *Nothofagus* forests. Bot. Bull. Acad. Sin. **44**: 329-336.

Sybenga, J. 1992. Cytogenetics in Plant Breeding. Springer-Verlag, Berlin Heidelberg, 469 pp.

White, M.J.D. 1965. Chiasmatic and achiasmatic meiosis in African Eumastacid grass-hoppers. Chromosoma **16**: 271-307.

Alstroemeria andina var. *venustula* (Alstroemeriaceae) 的減數分裂不規則性

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Alstroemeria andina 是一種多年生，小型草本 (5-16 公分高)，主要分佈在海拔 2,800~3,700 公尺阿根廷及智利的有限山區。我們分析此族群之個體的減數分裂過程 ($2n=2x=16$)，發現其過程極不規則。其特徵為末期 (anaphase) I 及 II 均有染色質之接橋及斷裂組態。在某一特定細胞於末期 I 接橋組態之最高數目為 2 個 paracentric inversions （發生在可能之異質結合染色質上）雖然異質嵌合體的確存在，但典型的嵌合體幾乎無法偵測到。我們觀察到的類似異質嵌合體可視同為穩藏的異質嵌合體（因為文獻上曾在隱型異質嵌合體減數分裂過程描述過。）我們觀察到高頻率之具小核之四倍體，意謂不平衡孢子之產生頻率相當高。花粉可染色度介於 28 及 30% 之間。*Alstroemeria* 屬植物，減數分裂之行為極為規則而且染色質之重組是很罕見的。綜上所述，我們懷疑某些環境因子劇烈地影響到染色體之構造以及減數分裂之調控。本研究乃第一個在野生 *Alstroemeria* 屬下族群具明顯減數分裂不規則性之報告。

關鍵詞：Alstroemeriaceae；染色體；隱藏的異質嵌合體減數分裂；減數分裂行為；花粉染色；構造異質結合。