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LARREA INTERSPECIFIC HYBRIDS REVISITED (ZYGOPHYLLACEAE)

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ABSTRACT: Hunziker, J. H. & Comas, C. 2002. *Larrea* interspecific hybrids revisited (Zygophyllaceae). *Darwiniana* 40(1-4): 33-38.

Evidence from recent molecular studies suggested a reappraisal of genome relationships between *Larrea ameghinoi* and the other South American species of the genus. As a consequence, new attemps to study the meiotic pairing of the *L. ameghinoi* x *L. cuneifolia* hybrids were undertaken (2n = 3x = 39). Mean number of bivalents and univalents were 9.8 and 19.4, respectively. Since the basic number *x* is 13, the interpretation that is favored is that *L. ameghinoi* (2n = 2x = 26) (AA) and *L. cuneifolia* (2n = 4x = 52) (DDCC) share partially homeologous genomes (A and C) that are involved in most bivalent pairing and this would explain the partial seed fertility of these triploid hybrids (15-19.4 %). *L. ameghinoi* genome (A) could have retained several parts of an ancestral genome which makes possible at least some pairing with those of the other species and enable it to function as a pivotal genome producing some fertility (from partial to complete) in all the possible hybrid combinations with its three South American congeners. An appendix is included with the captions of the figures of chapter 2 from the book edited by Mabry et al. (1977) which were erroneously omitted in the editorial and printing process.

Key words: Zygophyllaceae, *Larrea* hybrids, Cytogenetics.

RESUMEN: Hunziker, J. H. & Comas, C. 2002. Revisión de los híbridos interespecíficos de *Larrea* (Zygophyllaceae). *Darwiniana* 40(1-4): 33-38.

Estudios moleculares recientes nos han inducido a realizar una reinterpretación de las relaciones genómicas entre *Larrea ameghinoi* y las otras especies sudamericanas del género. Se hicieron nuevos intentos para estudiar el apareamiento de los cromosomas del híbrido *L. ameghinoi* x *L. cuneifolia* (2n = 3x = 39). El promedio de bivalentes y univalentes fue de 9.8 y 19.4, respectivamente. Dado que el número básico x es 13 la interpretación más razonable es que *L. ameghinoi* (2n = 2x = 26) (AA) y *L. cuneifolia* (2n = 4x = 52) (DDCC) comparten genomas parcialmente homeólogos (A y C) que forman la mayoría de los bivalentes y eso explicaría la producción de semillas (15-19.4 %) de estos híbridos triploides. El genoma de *L. ameghinoi* (A) podría haber retenido varias partes de un genoma ancestral que hace posible algún apareamiento con aquellos de las otras especies y que lo capacitaría para funcionar como un genoma pivote que permite alguna fertilidad (desde parcial a completa) en todas las posibles combinaciones híbridas con sus tres congéneres sudamericanos. Se incluye un apéndice con las leyendas completas del capítulo 2 del libro de Mabry et al. (1977) que fueran omitidas por error en el proceso editorial y de impresión.

Palabras clave: Zygophyllaceae, Híbridos de *Larrea*, Citogenética.

INTRODUCTION

The genus *Larrea* (Zygophyllaceae) is composed of evergreen shrubs of wide geographical distribution in the major deserts of the New World, covering large or isolated arid regions of Argentina, Chile, Bolivia, Perú, México and the Southwestern United States. It comprises five species: four in South America: *L. ameghinoi* Speg., *L. nitida* Cav., *L. divaricata* Cav. and *L. cuneifolia* Cav. ("jarillas"), and one in North America, *L. tridentata* (DC.) Coville ("gobernadora", "creosote bush") (Hunziker et al., 1977, 1978).

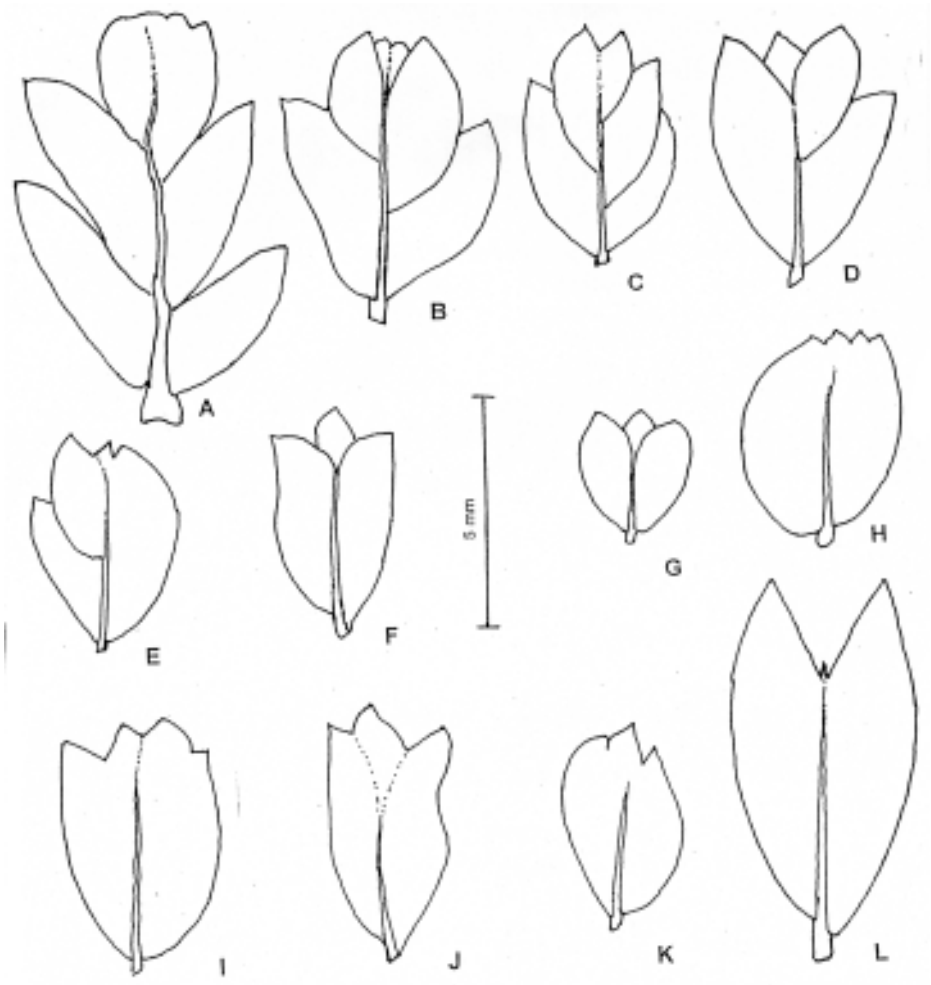

Fig. 1.- Leaf forms of the *Larrea ameghinoi* x *L. cuneifolia* hybrid and its parental species. A: *L. ameghinoi* (J. H. Hunziker 8508, BAFC). B-K: *L. ameghinoi* x *L. cuneifolia* (J. H. Hunziker 13275, SI). B-D: multifoliolate leaves with 6, 5 and 4 leaflets. E: 3- foliolate leaf with 1 tooth. F-G: 3- foliolate leaves. H-K: cuneiform leaves with 4 and 3 teeth. L: *L. cuneifolia* (*J. H. Hunziker 8504, BAFC*).

Two sections are recognized within the genus (Palacios and Hunziker, 1972). Section Larrea includes diploid species *L. ameghinoi* and *L. nitida* (2n=26) with multifoliolate leaves and rather small flowers. The remaining three species constitute section Bifolium and are characterized by the presence of bifoliolate leaves and larger flowers, exhibiting differnt levels of polyploidy: *L. divaricata* (2n=26), *L. cuneifolia* (2n=52), and *L. tridentata* (2n=26,52,78) (Hunziker et al. 1977; Yang, 1970).

In recent years through the study of DNA sequences of the Rubisco large subunit (rbc*L*) and the internal transcribed spacers (ITS) of nr DNA the probable time of creosote bush (*Larrea tridentata* (DC.) Coville) arrival to the North America was postulated at 4,2 to 8,4 mybp (Lía et al., 2001). As a consequence of these paper cladograms, where tetraploid *Larrea cuneifolia* Cav. stands close to diploids *L. ameghinoi* Speg. and *L. nitida* Cav. (Lía et al. 2001, Fig. 2, 3), a renewal of interest regarding *Larrea* genome relationships occurred.

In October of 2001 the senior author returned to the original site where the 5 interspecific natural hybrids between the 4 South American species were detected and individualized some presumed *L. ameghinoi* x *L. cuneifolia* F_1 hybrids.

In this contribution we present some partial results of the cytological analysis of one of these hybrids. Moreover, as the illustrations of chapter 2 by Hunziker et al. (1977) in the *Larrea* book, edited by Mabry, Hunziker and Di Feo (1977) were unfor-

Table 1.- Chromosome behavior at metaphase I in *Larrea ameghinoi* x *L. cuneifolia* putative hybrid and its parental species. Data for *L. ameghinoi, L. cuneifolia, L. divaricata and the L. cuneifolia* x *L. divaricata* hybrid from Hunziker et al., 1978.

Species or hybrid	Herbarium number	Chromosome number	Chromosome associations. Mean and range per cell				
			Prometaphase and Metaphase I			Metaphase I	
			II	I	Nº of cells	Ring II	Nº of cells
L. ameghinoi	9001 (BAFC)	26	13	0	70	12.6 ± 0.12 (11-13)	26
L. divaricata	8587 (BAFC)	26	12.83 (11-13)	0.34 (0-4)	47	10.36 ± 0.33 (6-13)	30
L. cuneifolia	8955 (BAFC)	52	25,99 (25-26)	0,02 (0-2)	100	23.07 ± 0.16 (19-26)	100
L. ameghinoi x L. cuneifolia	13275 (SI)	39	9.8 (7-13)	19.4 (13-25)	20	6.75 ± 0.53 (3-11)	20
L. cuneifolia x L. divaricata	8619 (BAFC)	39	12.35 (10-14)	14.03 (11-19)	66	11.79 ± 0.14 (9-13)	63

tunately published without the complete captions, we add now an appendix with the complete text that was omitted. The senior author of the chapter never received the proofs of the book and one of the editors of the book, in charge of the final printing, chose a simple list of the illustrations and omitted inadvertently the entire text of the detailed captions. Research workers and students of *Larrea* will be able now to fully understand the illustrations and the text of chapter 2.

MATERIAL AND METHODS

Materials were fixed in 6:3:1 ethanol, chlorophorm and acetic acid mixture and kept in the fixative for several months at –18°C. Before squashing they were treated with ethanol 70° and stained with acetic haematoxylin (Sáez, 1960; Núñez, 1968).

For examination of pollen fertility a 1:1 mixture of aceto carmine and glycerine was used and at least 500 pollen grains were counted (Hunziker et al., 1978).

Herbarium specimens were deposited at the Instituto Darwinion (SI) or at the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFC). All materials were collected at a depression near a Km sign (formerly 1193, at present 1198), Route N° 3, 69 Km South West of San Antonio Oeste, Rio Negro, Argentina. The depression is easily found because there is small culvert under the road.

RESULTS

The putative *L. ameghinoi* x *L. cuneifolia* hybrids were intermediate in growth habit to both parents reaching about 20 cm high. The leaves formed a great array of different forms, from cuneiform with 3-4 apical teeth to those with 3-6 leaflets (Fig. 1). Several plants were fixed but only *J.H. Hunziker n° 13275* yielded metaphase I plates that could be analyzed and was, as expected, a triploid with 2n=3x=39.

The cytological analysis is based on a limited number of cells since many of them were not clear enough for an interpretation (Table 1). Mean number of bivalents and univalents were 9.8 and 19.4, respectively. Figure 2 shows one of the configurations that were found.

Pollen and seed fertility could not be studied in these plants but there are data from morphologically similar plants studied earlier (Hunziker et al., 1978, p. 295) and are dealt with in the following section.

DISCUSSION

It is unfortunate that the numerous putative natural triploid hybrids between *Larrea ameghinoi* and *L. cuneifolia* studied earlier presented evidence of cytomixis and that the fixations were not favorable for meiotic studies (Hunziker et al., 1978). However, in several presumed F_1 hybrids 4-19 univalents were easily detected out of the metaphase plate in 230 cells. Regarding pollen fertility one of the hybrids showed none, two, 51-59% and

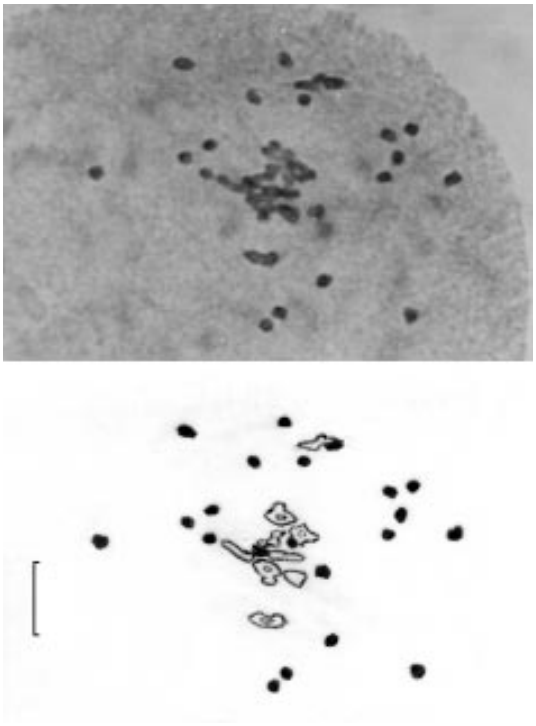

Fig. 2.- Meiotic behavior in putative *L. ameghinoi* x *L. cuneifolia* hybrid (13275). MI= 9 II + 21 I. The bar represents 10 µm.

five, 71-86 % stainable pollen. These hybrids reached 15, 17.7 and 19.4% of seed bearing mericarps. From 111 seeds collected after open pollination on one hybrid 38 plants were recovered. This showed an astonishing segregation for vigour, growth habit and leaf characteristics. Of 23 plants that remained alive 1 year after germination, 10 showed cuneiform leaves (Hunziker et al. 1978, fig. 2 A, B, C), 4 had multifoliolate leaves with acute apex (Fig. 2 E-G) and 9 both types of leaves within the same individual (Fig. 2 H-J). Some of the progeny plants showed transgressive segregation with leaflets more fused than in *L. cuneifolia* and having a 3-dimensional navicular conformation (Hunziker et al. 1978, fig. 2 D).

The results of the presently studied triploid intersectional *L. ameghinoi* x *L. cuneifolia* hybrid, with averages of 9.8 bivalents and 19.4 univalents (Table1), are quite different than those of the intrasectional triploid hybrids *L. cuneifolia* x *L. divaricata*, in which 13 bivalents and 13 univalents were found in nearly 50% of the cells, and with means of 12.35 bivalents and 14.03 univalents (Hunziker et al., 1978). The number of ring bivalents in both triploid hybrids (Table 1) is quite different: 11.79 ± 0.14 in *L. cuneifolia* x *L. divaricata* (higher than its parental *L. divaricata*, which has 10.36 ± 0.33) and 6.75 ± 0.53 in *L. ameghinoi* x *L. cuneifolia* (Table 1). In the case of the hybrid *L. cuneifolia* x *L. divaricata* it was concluded that *L. cuneifolia* was a allopolyploid and that it had one genome homologous to that of *L. divaricata* (Hunziker et al., 1978). In the present study we propose genome formulae for all species and hybrids of *Larrea* (Fig. 3). We have named A the genomes of *L. ameghinoi* and *L. nitida*, D the genomes of *L. divaricata* and *L. tridentata* (Chihuahuan diploid) and DC the genomes of allopolyploid *L. cuneifolia*. The *L. ameghinoi* x *L. cuneifolia* hybrid would have a genome formula ACD. In this intersectional hybrid allosyndesis would be responsible for most of the pairing (A with C, these genomes being partially homeologous). However, it is possible that some occasional pairing between A and D may occur since the AD diploid hybrid *L. ameghinoi* x *L. divaricata* shows 4% seed fertility (Fig. 3).

Figure 3 shows the crossing relationships based on the present available information on *Larrea* interspecific hybrids (mean chromosome association at metaphase I and seed fertility). *Larrea ameghinoi* shows seed fertility (s. f.= 4-71%) in the hybrids with all other South American species. In that sense its A genome perhaps shares some homeology with all other genomes and therefore has a unique pivotal ability to produce partially fertile hybrids with *L. nitida*, *L. divaricata* and *L. cuneifolia* (Fig. 3).

Lía et al. (2001) have proposed that one of the parental species of the tetraploid *L. cuneifolia* was an ancestor of section *Larrea* and not any of its extant members. As a matter of fact both the phylogeny obtained from ITS sequence data and the tree derived from the combined analysis support a sister group relationship for *L. nitida* and *L. ameghinoi*, with *L. cuneifolia* as sister to this clade (Lía et al. 2001, figs. 2 and 3).

Larrea ameghinoi genome could have retained several parts of an ancestral genome (A) that makes possible at least some pairing and seed fertility in the hybrids with those of the three other South American species.

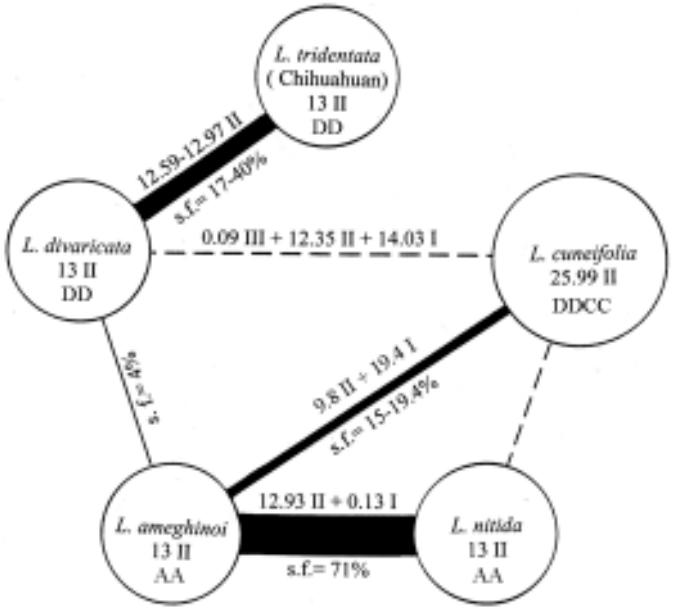

Fig. 3.- Crossing relationships of the species of *Larrea*. Modified from Hunziker et al., 1977 and based on data from Hunziker et al., 1978, from Yang et al., 1977, and from results of the present work. Mean number of chromosomal associations is indicated as well as seed fertility (s. f.). The width of the black bars connecting species are proportional to the seed fertility of the hybrids. Broken lines represent complete sterility.

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Appendix 1.- Captions of the figures of chapter 2 from the book edited by Mabry et al. (1977), which were erroneously omitted.

Fig. 2.4- Geographic distribution and chromosome numbers of creosote bush populations in North America (*L. tridentata*). Diploid populations 1-17 occur in the Chihuahuan Desert and adjacent desert grassland. Tetraploid populations 19-33 are situated in the Sonoran Desert. Hexaploid populations 35-41 are located in the Mojave Desert. Shaded area shows the contemporary distribution of *L. tridentata* in North America. Reproduced from Yang (1970).

Fig. 2.6- Leaves of species of *Larrea* and their natural hybrids. All leaves of a single collection number belong to a single individual and show phenotypic plasticity. A= *L. nitida* (8503), B-F= *L. ameghinoi*, B, E, F (8508); C, D (LP 11958). G-H= *L. divaricata*, G (Kurtz 9495), H (8506). I= *L. tridentata* (diploid, Pringle 10223). J= *L. cuneifolia* (8504). K= *L. cuneifolia* x *L. divaricata* (8683). L-P= *L. ameghinoi* x *L. nitida* (8513). Q-R= *L. ameghinoi* x *L. cuneifolia* (8532), transgressive segregant showing extreme fusion of leaflets. S-Y= *L. ameghinoi* x *L. cuneifolia*, putative F_1 (8531). Z-C'= segregant of *L. ameghinoi* x *L. cuneifolia*, with a predominance of leaves of types intermediate between B' and C' (8519). D'-H': *L. ameghinoi* x *L. divaricata* (8524). I'-L': *L. cuneifolia* x *L. nitida* (8957) (From Hunziker et al., 1978).

Fig. 2.7- Geographic distribution of some interspecific *Larrea* hybrids in northern Patagonia (From Hunziker et al., 1978).

Fig. 2.8- *Larrea* vegetation at the depression of Km 1193, Ruta 3, 59 Km South of San Antonio Oeste, Río Negro Prov., Argentina. A= *L. ameghinoi* at the bottom of the depression; B and C= *L. ameghinoi* x *L. cuneifolia* hybrids in the intermediate zone of the depression. In the background in the higher parts of the depression, the shrubby "jarillas" (*L. divaricata*, *L. cuneifolia*, *L. nitida*). Photograph B in South direction. Photo C in N-NW direction. In C in front *L. ameghinoi*. D= *L. cuneifolia*. E= *L. nitida.* In A, D and E the prominent part of the knife is 22 cm long, approximately. (From Hunziker et al., 1969).

Fig. 2.9- Distribution of species of *Larrea* and hybrid plants or swarms in the depression of Fig. 2.8 (Modified from Hunziker et al., 1969).

Fig. 2.10- Vegetation profile of the depression represented in Fig. 2.8 S-SE to N-NW direction, in the same scale as Fig. 2.9. The slopes appear exagerated 5 times. In the case of the 3 hybrids the leaves drawn belong to the same individual (Modified from Hunziker et al., 1969).

Fig. 2.11- Leaves, growth habit and phenolic compounds of North Patagonia species and hybrids of *Larrea*. (From Hunziker et al., 1972, modified).

Fig. 2.12- Polyacrilamide gel electrophoresis of seed albumins of *L. ameghinoi*, *L. nitida* and their hybrid derivatives. Anode to the right. a= *L. ameghinoi* (8522). 8678, 8702, 8873 and 8818 are hybrid derivatives. n= *L. nitida* (8852). Explanation in the text. (From Hunziker et al., 1978).

Fig. 2.13- First meiotic metaphase of *L. divaricata*, *L. tridentata* and their hybrid. A and B= *L. divaricata* (A-T 219). C, D and E= *L. divaricata* x *L. tridentata* (A-T 219-110). F and G= *L. tridentata* (T-Z 151). A= 13 bivalents (9 closed), 22 chiasmata; B= 13 bivalents (8 closed), 22 chiasmata. C= 13 bivalents (12 closed) 26 chiasmata; D= 13 bivalents (9 closed); E= 13 bivalents (11 closed), 26 chiasmata; F= 13 closed bivalents, 26 chiasmata and G= 13 closed bivalents, 26 chiasmata (Modified from Yang et al., 1977).

Fig. 2.14- Putative crossing relationships of the species of *Larrea* and their hybrids. Mean chromosome associations are indicated for species and hybrids. The connection between circles give an idea of fertility as measured by the percent of seed-bearing mericarps: black, >20%; hatched, partially fertile (15-19%); solid line, flowering sporadical, almost sterile; broken line, sterile. The width of the connections is proportional to the value of fertility.

Fig. 2.15- Leaves fron segregants from progeny trials originating from seed collected on natural *L. ameghinoi* x *L. cuneifolia* hybrids. Numbers given are culture numbers. A-B= plant 822-12. C= Plant 822-5. D= 822-1. The 822 plants shown here are part of the progeny of 8517 (Presumably an F_1). E-F= plant 824-1. G= 824-2. H-J= 824-14. The 824 plants are part of the progeny of 8527 (Presumably an F_1 hybrid). Explanation in the text. (From Hunziker et al., 1978).

Fig. 2.16- Seed protein electrophoresis of *L. divaricata* and *L. tridentata*. A= Polyacrylamide gel electrophoresis of seed albumins of *L. tridentata* (New Mexico) and *L. divaricata* (Peru, Salta, Rio Negro). Anode to right. B= schematic interpretation of the previous photographs, with the numbers of the protein fraction according to presumed homologies (From Yang et al., 1977).