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Cytogenetic and Electrophoretic Evidence for a Polyploid Origin of the Basic Chromosome Number x = 14 in the Genus *Asphodelus (Liliaceae)*

By

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Abstract: Cytogenetic and electrophoretic analyses on 2n = 28 strains of *Asphodelus cerasiferus* strongly suggest that the basic number x = 14 of the genus *Asphodelus* is of secondary polyploid origin from x = 7.

There are two fundamental mechanisms by which living beings have increased their chromosomal numbers during the evolutionary process. The first one is polyploidy, the multiplication of entire chromosomal complements, that has played *a* preponderant role in the evolution of higher plants (STEBBINS 1950,1971,1974, GOTTLIEB 1976, DOBZHANSKY & al. 1977). They have pointed out that "there is good evidence to suggest that all genera or families having basic numbers of x = 12 or higher have been derived originally by polyploidy from groups having lower number, and that even the numbers x = 10 and x = 11 may often be of polyploid derivation". The second mechanism is aneuploidy and dysploidy, variation in number affecting individual chromosomes, a phenomenon that has occurred in different families of plants (GRANT 1971).

On the basis of previous data from *Asphodelus microcarpus* and *A. tenuifolius* (RUIZ REJÓN 1978) we are trying to establish the nature of the basic chromosome number x = 14 of the genus. In the present study, we have applied different cytogenetic and electrophoretic techniques to 2 n = 28 strains of *A. cerasiferus* GAY. There are two different chromosome numbers (2n = 28 and 2n = 56) in Spanish natural populations of this species (SAÑUDO & RUIZ REJON 1975, RUIZ REJON 1976, 1978, RUIZ REJON & al. 1978).

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Material and Techniques

This study has been performed on a total of 199 individuals from two natural populations :

CZ: Cabra Montés, Sierra de Cázulas, Granada, Spain (86 individuals).

PA: Padul, Granada, Spain (113 individuals). Voucher specimens are preserved in the herbarium GDA.

Roots of seedlings were used for karyological analysis. The root tips were pretreated in 8-hydroxyquinoline 2 mM for 2-4 hours, and then fixed in Carnoy (3 : 1) for 2 hours. After 5 minutes hydrolysis in 1 N HCl at 60 °C they were stained in acetic orcein (1 per cent) one hour. Root tips were then squashed in fresh acetic orcein. Meiosis was studied in squash preparation of young anthers. Young buds were fixed in Carnoy (3: 1) for 24 days at 4 °C and stained in acetic carmine.

The esterase isozymes were studied on crude extracts obtained after crushing roots and leaves from two week old seedlings. Horizontal starch gel electrophoresis was carried out using a modified POULIK's (1957) system of buffers. Modification consists in using Tris-citrate buffer pH 8.65 in the electrode trays as well as in the gel. Electrophoresis was performed under an applied voltage of 350 V and a current of 25 mA until the Bromophenol blue migrated a fixed distance of 4cm. A refrigerant maintained at 4°C was circulated through the apparatus during electrophoresis. Visualization of esterase activity after electrophoresis was performed according to the method of JOHNSON & al. (1966) with phosphate buffer pH modified to 5.60.

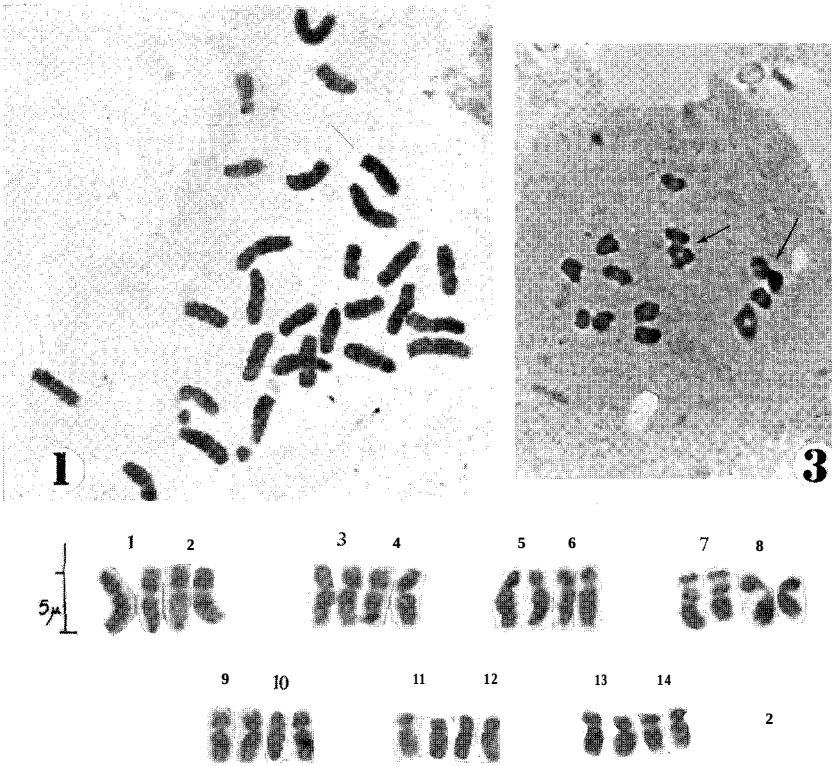
Results

Mitosis. The two populations investigated have the same chromosomal number 2n = 28 (Fig. 1). Karyotype analyses (Fig. 2) have revealed the presence of 12 pairs of heterobrachial chromosomes (arm ratio between 1: 1 and 1:3, according to BATTAGLIA 1955) and two hyperheterobrachial pairs (the fifth and sixth ones, with arm ratio of 1:3 or more).-Only one satellited pair can usually be seen (Fig. 1). However, some cells presented two satellited pairs (Fig. 2).

Meiosis reveals fourteen bivalents in all individuals analyzed. The nuclei of pollen mother cells contain only one nucleolus associated with 2-3 bivalents which present 2-3 chiasmata.-No significant anomalies of the meiotic process were observed, except the existence of secondary associations between some bivalent pairs (Fig. 3).

Electrophoretic analysis of esterase isozymes in the sampled individuals has uncovered five bands which, accompanied by their respectives mobilities, are shown in the Fig. 4. The pattern is the same in roots and leaves of two week old seedlings.

Sixteen distinct esterase zymogram phenotypes, differing with respect to the presence or absence and the relative staining intensities of their bands, were observed among the 199 individuals sampled (Fig. 5). Individual plants show at least one band and a maximum of



Figs. 1-3. Chromosomes of *Asphodelus cerasiferus*. — Fig. 1. Metaphase chromosomes of root mitosis with one satellited pair (2 n = 28). — Fig. 2. Karyotype from metaphase chromosomes with two satellited pairs (2 n = 28). — Fig. 3. Diakinesis : 14 bivalents. At arrows : secondary associations between two pairs of bivalents

three bands. Differences exist in the relative staining intensities of the distinct bands which can be ascribed to gene dosage effects.

The inheritance of each of the electrophoretic variants was determined by examining a progeny of seedlings grown from seed collected from a single outcrossed plant in nature. The results of these analysis, including only those cases where a common band exists at least in all daughter seedlings, are shown in Table 1. Since each progeny had one common parent, the presence of a common band in the progeny suggests that the parent also possessed this band. The remaining bands may be inherited from pollen grains from other plants with different genotypes.

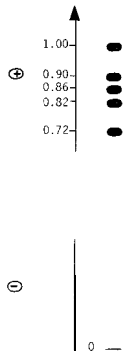


Fig. 4. Composite schematic representation of the electrophoretic variants of esterase in *Asphodelus cerasiferus*. The variant bands are named according to their relative migration. The origin is indicated by 0

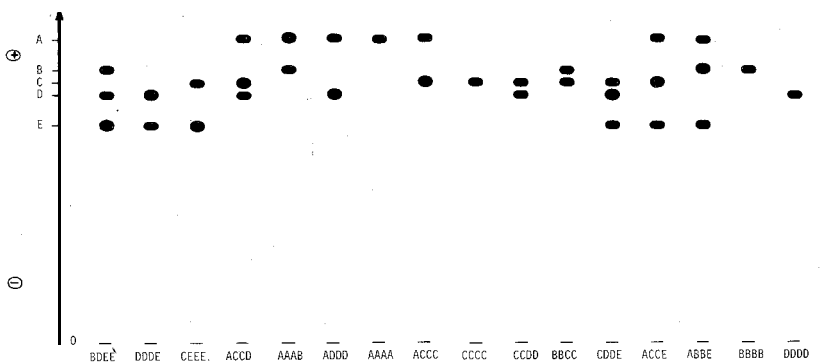


Fig. 5. The sixteen different esterase zymogram phenotypes observed among the 199 sampled individuals. The respectives possible genotypes are indicated also. A = Est-$1^{1.00}$, B = Est-$1^{0.90}$, C = Est-$1^{0.86}$, D = Est-$1^{0.82}$, and E = Est-$1^{0.72}$. The origin is indicated by 0

Discussion

The simplest hypothesis which is in full agreement with the different esterase zymogram phenotypes and with the results of progeny analysis is one which assumes: a) that the genetic control of this enzyme corresponds to a one-locus model, with each esterase band controlled by a different codominant allele : Est-**100**, Est-$1^{0.90}$, Est-$1^{0.86}$, Est-$1^{0.82}$, and Est-$1^{0.72}$; b) that this locus in duplicated, so that each individual has four, instead of two genic sites; in this case polysomic inheritance and gene dosage effect can be expected; and c) that the active esterase enzymes are monomers.

Table 1. Results of progeny analyses of single outcrossed plants for the duplicated locus Est-1, including only those cases where a common band exist in all daughter seedlings. A = $Est\text{-}1^{1.00}$, B = $Est\text{-}1^{0.90}$, C = $Est\text{-}1^{0.86}$, D = $Est\text{-}1^{0.82}$, and E = $Est\text{-}1^{0.72}$

Mother plant	Progeny					Number of seedlings	Common band
	A	B	C	D	E		
PA-1	—	*		*	*	9 BDEE	E
	—			*	*	7 DDDE	
	—	—	*		*	4 CEEE	
PA-2	*	—	*	*		10 ACCD	A
	*	*				7 AAAB	
	*	—				7 AAAA	
	*	—		*		8 ADDD	
PA-3	*	—	*			2 ACCC	c
	—	—	*			5 cccc	
	—	—	*	*	—	8 CCDD	
	—	*	*			1 BBCC	
PA-4	—	—	*		*	5 CEEE	C and E
	—	—	*	*	*	1 CDDE	
PA-5	—	*		*	*	1 BDEE	D
	—	—	*	*		7 CCDD	
	—	—		*	*	4 DDDE	
cz-1	—	—	*	*		7 CCDD	C
	—	—	*		*	6 CEEE	
	—	*	*		—	8 BBCC	
cz-2	*	—	*	*	—	2 ACCD	A
	*	*			—	8 AAAB	
	*	—				5 AAAA	

* = Present — = Not present.

The observed dosage effect in individual esterase zymogram phenotypes and the results of the progeny analyses support these assumptions. Moreover, our hypothesis also explains the appearance of the observed electrophoretic phenotypes, as shown in Fig. 5. Other phenotypes could have been expected ; their absence in our sample can be attributed to the fact that the number of individuals examined was not large enough in view of the high number of possible phenotypes.

The alternative hypothesis of an un-duplicated locus with dimeric enzymes does not explain the zymogram phenotypes observed. In particular, some heterozygous individuals have only two bands instead of three, as one would have to expect if the enzyme were dimeric

(Fig. 5). Furthermore, the progeny tests do not support this alternative hypothesis.

The cytogenetic research accomplished until now in different species of the genus *Asphodelus (Liliaceae)* shows the existence of a polyploid series of chromosomal numbers ranging from 2 n = 28 (x = 14) to 2 n = 84, with some intermediate numbers (DARLINGTON & WYLIE 1955, LOVE & LOVE 1961, and other indices of plant chromosome numbers). The results of our cytogenetic and electrophoretic study suggest that this high basic number (x = 14) has a remote polyploid origin. We believe that the following points support this hypothesis: 1) The karyotype analysis of the 2 n = 28 strain of *A. cerasiferus* shows that the chromosomes of this species can be grouped four by four, as it is shown in the Fig.2, instead of two by two. There are two satellited pairs, and at least two bivalents are associated with the nucleolus. Some secondary associations between bivalent pairs appear during meiosis (Fig. 3). RUIZ REJÓN (1978) has observed a similar meiotic behavior at the same chromosomal level (2 n = 28) of two other species of this genus *(A. microcarpus* and *A. tenuifolius*). All these facts indicate partial structural homology between different chromosome pairs and the existence of relictual polyploidy.

2) The existence of polysomic inheritance in the locus Est-1 of 2 n = 28 chromosomal level suggests the presence of duplicated genetic material. This duplication might have originated in three ways: a) a restricted duplication at this locus; b) a duplication affecting individual chromosomes (aneuploidy-dysploidy) ; and c) a duplication of entire chromosomal complements (polyploidy). This last alternative is the only one that is consistent with the above mentioned cytogenetic data. The duplicated esterase gene therefore may be considered as a relic of a remote polyploid origin which has not yet been reduced to a diploid-like state.

In conclusion, all of these cytogenetic and electrophoretic data support the hypothesis that the basic number x = 14 of the genus *Asphodelus* is secondary and derived through polyploidy from x = 7, the ancestral basic number of the genus, with all diploids now being extinct. The secondary basic number x = 14 would therefore correspond to a tetraploid level more or less transformed to a diploid-like state.

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