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THE RELATION BETWEEN ISOZYMES AND PLOIDY LEVEL. ITS APPLICATION TO BIOGEOGRAPHICAL STUDIES OF *MUSCARI ATLANTICUM* (LILIACEAE)

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Summary

In this paper a correlation between polyploid level and esterase isozyme pattern in individuals from different Spanish populations of ***Muscari atlanticum*** has been established. We have taken advantage of this correlation to make a study of the distribution of the reported polyploid levels in 1,017 individuals from thirteen natural populations. The interest in and application of this method for biogeographical analysis are discussed.

Introduction

It is fairly frequent to find in plants different ploidy levels of the same species occupying the same or different geographic areas. The study of the geographic distribution of the different polyploid levels within a species is of great interest in clarifying the evolutionary changes in the species and its relation to neighbouring taxa. Likewise, the biogeographical study of the different polyploid levels in any taxon can be very useful for determining its adaptation to different environments.

Up to now attempts have been made to solve this problem through cytogenetic analysis. However, if a representative sample of a species throughout its distribution area is analyzed, it is necessary to study a large number of individuals, and the cytogenetic method is extraordinarily tedious and requires very specific materials. In the following study, performed in different natural populations ***of Muscari atlanticum*** Boiss. et Reuter ***(M. racemosum*** (L.) Miller), a method of attack has been developed through isozyme analysis which we believe avoids the difficulties of the cytogenetic approach.

Materials and Methods

This study has involved a total of 1,017 individuals in thirteen natural populations which are indicated with their respective codes in Table 1. For the cytogenetic study, roots were used, their formation initiated by placing the bulbs in glass test tubes with tap water. However, for the electrophoretic analysis, roots, leaves, flowers and unripe seeds were used. Somatic mitosis was observed in root meristems by the squash method using acetic-orcein stain (Tjio and Levan, 1950).

Isozyme analysis of the esterases was accomplished by means of horizontal starch gel electrophoresis, using the Poulik technique (1957) with the modification that in the gel as well as in the electrodes, Triscitrate buffer pH 8.65 has been used. Development was made at 350 V and 25 mA through the required time so that the Bromophenol Blue indicator could reach a fixed distance of 4 cm. The gel incubation

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was carried out according to the Johnson et al. (1966) method with the difference that the buffer used was phosphate pH 5.60.

To estimate the proportions of each polyploid level in populations where various ploidy levels coexist, the Maxlik program, written in Fortran IV for a general estimation by maximum likelihood was used.

Table 1. Populations sampled, size of samples and proportions of the different polyploid levels of Muscari *atlanticum*. Note that 5n and 6n levels are shown grouped in one class. The keys of populations are: HV=Huetor-Vega (Granada); GD=Guadahortuna (Granada); IH=La Iruela. Cazorla (Jaen); NV=Nava de San Pedro. Cazorla (Jaen); PC=Parador. Cazorla (Jaen); PA=Padul (Granada); HU =Huelma (Jaen); SE=Seseña (Toledo); PL=Peal de Becerro (Jaen); CN=Púlpito de Canales. Sierra Nevada (Granada); GB=Gabia Grande (Granada); VI=Villafranqueza (Alicante); TV=Torre del Vinagre. Cazorla. (Jaen).

Population	*Sample*	4n	*5n*	*5n+6n*
Hv	284	1.00	**0.00**	
GD	97	0.00	1.00	
IH	83	0.00	1.00	
NV	76	0.449	———	0.551
PC	59	0.291		0.709
PA	19	1.00	0.00	
HU	54	1.00	0.00	
SE	104	1.00	0.00	
PL	76	0.910	———	0.090
CN	71	1.00	0.00	
GB	38	0.00	1.00	
VI	10	1.00	0.00	
TV	46	0.095	———	0.905

Total = 1,017

Results

During a first phase we simultaneously studied cytogenetically and electrophoretically a total of 75 individuals in 13 natural populations of ***Muscari atlanticum***.

Of these individuals, 32 were tetraploid (4n=36), 22 were pentaploid (5n=45), and 21 were hexaploid (6n=54). Of the 13 populations in which these individuals occur, six are only tetraploid, three populations are pentaploid, and in the remaining four populations there is a mixture of the three ploidy levels.

Electrophoretic analysis of the esterase isozymes in these individuals revealed the bands which, grouped by zones and accompanied by their respective mobilities, are shown in Fig. 1. In Fig. 2, these zones of esterase activity in various organs (root, leaf, flower, and unripe seed) of plants at the different polyploid levels are represented.

Having proven the existence of correspondence between the ploidy level and the electrophoretic pattern, in a secondphase we broadened our electrophoretic study to include a larger number of individuals (1,017) of ***Muscari atlanticum***. In Table 1 the proportions of individuals at each polyploid level in the 13 populations studied, together with the sample size in each case, are presented.

Discussion

The results obtained permit us to establish a correspondence between the chromo-

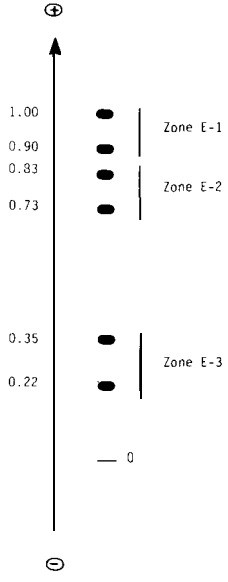

Fig. 1. Composite schematic representation of the electrophoretic variants of esterase in *Muscari atlanticum*. The zones of activity are designated E-l, E-2, and E-3, and the variant bands are named according to their relative migration. The origin is indicated by 0.

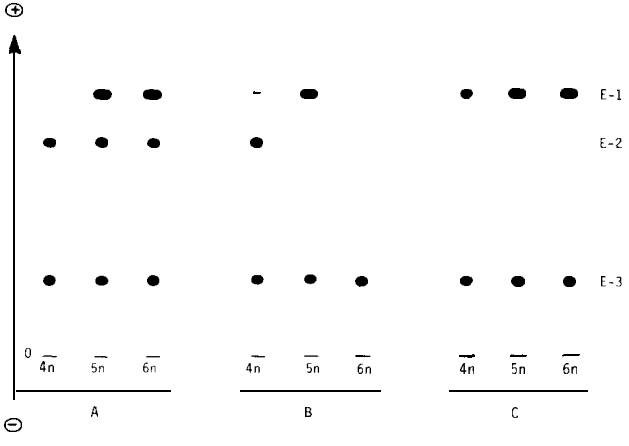

Fig. 2. Differential expression pattern of the three esterase isozymes, E-l, E-2, and E-3, in root (A), leaf(B), flower and unripe seed (C) of the different polyploid levels (4n, 5n, and 6n) of *Muscari atlanticum*. Note that the flowers and unripe seeds present the same pattern.

some level and the electrophoretic pattern of the esterase isozymes.

There are two criteria by which the tetraploid individuals can be distinguished electrophoretically from the penta- and hexaploid individuals: 1. The intensity of the coloring of the esterase activity bands in the penta- and hexaploids is visibly greater than in the tetraploids, especially in the zone E-l. 2. The pattern of the different esterase activity zones varies according to the ploidy level. For example, the E-l

zone is not present in the roots nor in the leaves of the tetraploid individuals, but it is shown in the penta- and hexaploids. On the other hand, the zone E-2 is only shown in the leaves of tetraploids but not in the leaves of the penta- and hexaploids (Fig. 2).

In this way, using both criteria it is possible to determine by electrophoretic methods whether an individual is a tetraploid or one of the other two ploidy levels (penta- or hexaploid). Making a distinction between penta- and hexaploids is not feasible since they are not differentiated by the intensity of coloring of their bands (at least not visually) nor by the pattern of expression in the different zones. For this reason, as can be seen in Table 1, in the populations of mixed ploidy levels, the 5n and 6n individuals are grouped into one class.

However, it is possible to calculate quite accurately the proportions of each level, 4n, 5n and 6n, in the four mixed-level populations. It is necessary to assume that plants of the three levels hybridize among themselves when found in the same population. We believe that this assumption is justified first by the observation that in all populations in which levels 4n and 6n are present, 5n individuals always appear. On the other hand, the presence in all the three ploidy levels of identical zones of esterase activity, even identical electromorphs, independently of above-mentioned changes in the genic expression, indicates the existence of the same structural alleles in all ploidy levels, implying the occurrence of gene flow between them and therefore the existence of hybridization. Using this assumption, if we call the proportion of 2n gametes x and the proportion of 3n gametes y, the expected proportions of the 4n, 5n and 6n individuals in the next generation would be represented by x^2, $2xy$ and y^2 respectively. To estimate the proportions in those populations where the three ploidy levels coexist, we used the Maxlik program of estimation by maximum likelihood. The results are expressed in Table 2.

Table 2. The four mixed level populations and the proportions of the different polyploid levels. Note that now the proportions of 5n and 6n are shown separately.

Population	*4n*	*5n*	*6n*
NV	0.449	0.442	0.109
PC	0.291	0.497	0.212
PL	0.910	0.088	0.002
TV	0.095	0.426	0.479

Using these data, we have determined the geographic distribution of the different polyploid levels of this species in the populations (Fig. 3). The following points we consider are worth pointing out: a) There are some populations with individuals at only one ploidy level, but there are also others in which the three levels coexist in the same populations, in particular, those located in the Cazorla Mountains and its surroundings (PC, NV, TV and PL). b) Although there are homogeneous populations of tetra- and pentaploid individuals, there are no populations of only hexaploid. When this level exists, it is always accompanied by tetra- and pentaploids. c) Concluding, we must point out that the tetraploid level is the most extensive geographically in the populations analyzed, while the hexaploid level is limited to the Cazorla Mountains and its surroundings (populations PC, NV, TV and PL). Since the latter populations occur at greater altitudes, we believe that this fact supports the observations of Love and Love (1969), Favarger (1957) and Küpfer (1974).

We conclude that once the electrophoretic pattern of esterase isozymes is determined, the ploidy level of each individual can be deduced. Because electrophoresis techniques permit a relatively rapid study of large samples, we believe that the method constitutes a means of solving this type of problem in many other species, and that studies that have as their main objective the analysis of polyploidy in the

evolutionary process should be encouraged. Polyploidy is a phenomenon frequently observed in plants (Gottlieb, 1976) and acquiring each day more importance in understanding the evolution of the animal kingdom (Ohno, 1970).

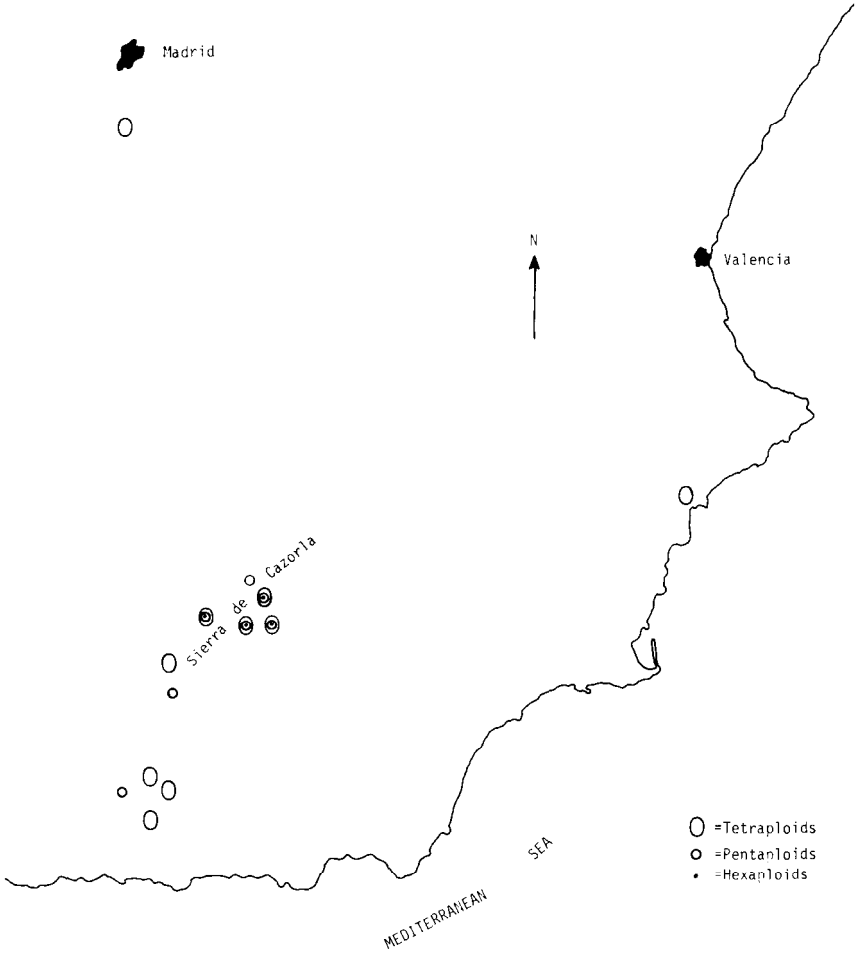

Fig. 3. Map showing the distribution of the different polyploid levels of *Muscari atlanticum* in the Iberian Peninsula.

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