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Genetic variability in *Muscari comosum (Liliaceae)*. III. Enzyme polymorphism in European and Canarian populations

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ABSTRACT

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Electrophoretic variation at six loci from 33 European and Canarian populations of *Muscari comosum* (Liliaceae) were studied. Only GDH was monomorphic, being polymorphic the remaining loci (GOT-1, GOT-2, GOT-3, IDH and ADH). Although apparently the populations are differentiated, such as showed the values of chi-square of heterogeneity, the values of genetic identity and the Fst indicate that this heterogeneity is only moderated. The most likely explanations of this result could be related to the biological characteristics (sexual reproduction, predominantly outcrossing with mechanism of pollination by insects, widespread geographic range) joint with other factors additionally related to life history of the species, such as the plastic response to the environment, and the high longevity. Furthermore the analysis with F-statistics shows that there is a deficit of heterozygotes for four loci (GOT-1, GOT-2 GOT-3 and IDH) and this is explained by partial self-pollination, Wahlund effect or consanguineous mating, among other factors. However this analysis shows a clear excess of heterozygotes at the ADH locus. Although many reasons could cause an excess heterozygotes (negative assortive mating, differences in allele frequencies between male and female gamete pools) we think that this observation can be explained by selection.

INTRODUCTION

Plants are unique when compared with animals. They are sessile and have limited mobility via pollen and seed dispersal. Isozyme studies suggest that generally plants have somewhat more variation than invertebrate animals and considerable more variation than most vertebrates (Gottlieb 1977; Brown 1979; Hamrick et al. 1979). Furthermore, different plant species contain varying amounts of genetic variation. Trees, for example, contain significantly more variation than

herbaceous plants (Hamrich et al. 1979). Differences in isozyme variation among and within species is accounted for by variation in their life history and ecological characteristics. Species with large range, high fecundities, an outcrossing mode of reproduction, wind pollination, a long generation time, and from habitats representation later stages of succession tipically have higher amounts of isozyme variation. Selfing species, annuals, early succession species, and those with animal –or gravity– dispersed seed typically have high interpopulation variation. There are exceptions to the above observations and the literature is biased towards temperate species, usually annuals, short-lived perennials, or conifers. Long-lived herbaceous perennials, angiosperm trees, and monocots (except grasses) have not been studied extensively, and the population genetics of tropical or alpine species is virtually unknown.

In this sense we present here our results and conclusions on the protein polymorphism in a bulbiferous monocot species *Muscari comosum* (Liliaceae), a diploid perennial plant, (2n = 18), that comprises a number of continuous morphological types. *Muscari comosum* L. (*Leopoldia comosa* Parl.) is the only species of the subgenus *Leopoldia* with an extensive geographical and ecological distribution. In fact, it has a wider ecological tolerance than others *Leopoldia* and grows on dry grasslands as well as on cultivated ground of most of the Mediterranean countries, living also in North Africa and Canary Islands. It is mainly allogamous, with pollination by means of insects, high fecundities and seed dispersed by animals. Analyses of the chromosome variability showed that this species displays a striking widespread chromosomal polymorphism in the second pair of chromosomes (Garbari, 1969; Bentzer, 1972; Bentzer and Ellmer 1975; Ruiz Rejón and Oliver, 1981; Ruiz Rejón et al., 1987). A pericentric inversion and an unequal interchange or an insertional translocation, are the most likely origins of these polymorphism (Ruiz Rejón et al., 1987).

MATERIALS AND METHODS

A total of 1289 individuals were studied from 33 natural populations (with a mean of 40 individuals per population) from throughout the range of the species (Appendix 1). The populations were sampled by us collecting whole bulbs from the field. In addition, some bulbs were obtained from other researchers or scientific institutions.

The bulbs were grown in hydroponic culture. The meristems of the roots tips were cut and squashed directly on 9 x 3 mm. pieces of Whatman paper number 3 which were placed into slots on the gel. Flowers and unripe seeds were also employed in several localities.

Two buffer systems were used: LiOH pH 8.3 (Selander et al., 1971) and Histidine pH 8.0 (Brewer, 1970). The ionic strengh was adjusted one–half in the Histidine buffer. Electrophoresis was carried out for 6 and 5 hours, respectively. Enzymatic systems studied were: Alcohol dehydrogenase (ADH) E.C. (1.1.1.1.), Glutamate Oxaloacetate transaminase (GOT) E.C. (2.6.1.), Isocitrate dehydrogenase (IDH) E.C. (1.1.1.42) and Glutamate dehydrogenase (GDH) E.C. (1.1.4.3.). The electrophoresis was carried out indifferently in LiOH and Histidine buffers except for GOT, which was carried out in LiOH, and IDH, which was run in Histidine. The staining solutions were those of Shaw and Prasad (1969), Selander et al., (1971) and Brewer (1970).

The isozyme loci were numbered according to their mobilities: the gene coding the fastest migrating isozyme designate 1. The alleles at a locus were designated according to their mobilities: *a* for the fastest, *b* for the next, etc.

The fixation index (F_I) was estimate using the original parameter suggested by Wright (1951). The values of F_{st} which are given in this paper have been corrected for bias using the formula of Eanes and Koehn (1978). Two estimates of F_{IT} were calculated. One was calculated according to Wright's formula starting from the observed data (F_{IT}), and the other one starting from the values of F_{st} and F_{IS} (F_{IT}) in the usual form under the assumption that there is an alea-

tory differentiation among populations (Workman and Niswander, 1970). The chi-square test for heterogeneity in allelic frequencies was computed according to the method of Snedecor and Irwin (1933) cited in Wokman and Niswander, (1970). Finally, Nei's indices of genetic identity and genetic distance (Nei, 1972) were estimated.

RESULTS

The different systems studied are controlled by a total of six loci, three of which correspond to enzymes of GOT activity. Only the GDH locus is monomorphic in all populations.

All the GOT loci which we call GOT-1, GOT-2 and GOT-3 are diallelic. At every one of the three loci is the same allele in very high frequency throughout; and in fact this allele is actually fixed in many samples (Table 1).

The IDH locus is also diallelic, with the *a* allele in high frequency (mean of 0.86) in all populations, but only occasionally fixed (Table 1).

The ADH locus is tetrallelic with mean frequencies for each of the alleles of 0.06, 0.18, 0.46 and 0.30 for *a, b c* and *d* alleles, respectively. The *c* allele is the most common in a majority of samples (Table 1).

Respect to genetic variation among populations the fixation indices (f_i) (Table 2) are very heterogenous among loci. Mean values of F_{IS} (Table 3) vary from 0.218 for ADH to 0.530 for GOT-2. The F_{IS} of ADH reflects the evident negativity of fixation indices for this locus in large majority of samples, indicating an excess of heterozygotes in the populations for this locus. Furthermore is important emphasize that four of the five surveyed loci showed significant excess of homozygotes and the mean over all loci was 0.215, which is appreciably positive. This indicated that exist a 22% deficiency of heterozygotes relative to Hardy-Weinberg expectations.

The values of chi-square test for homogenity in allelic frequencies are high in all the loci: gene frequency heterogenity is highy statistically significant in all loci ($p < 0.001$, 32 d.f.; Table 1).

Having in mind the heterogenity in gene frequencies among populations it was of interest to determine whether gene frequency clines ocurred within populations. Gene frequencies of populations were regressed aginst latitude in order to ascertain the presence of clines which might be related with temperature differential or an east-west moisture differential. No significant relations were detected. Moreover, there was no correlations between the absolute distance between populations an their gene frequencies. The mean genetic identity of *M. comosum* populations is 0.98 with pairwise values ranging from 0.94 for samples AL and POD to 1.00 for a great number of comparisons (the data corresponding to these three last results are not showed).

DISCUSSION

Althought apparently the analysed populations of *M. comosum* are differentiated from the genetic point of view, such as showed the values of chi-square test of homogenity, the values of genetic identity and the F_{ST} indicate that this heterogenity is only moderated. These data indicate that althought there is heterogeneity at single locus level genome as a whole are only moderately differentiated between populations. The F_{ST} values are within the general range of plants wich presents biological and ecological characteristics such as life history similar to *M. comosum* (Loveless and Hamrich, 1984).

M. comosum is a species which present biological characteristics such as predominatly outcrossing, dioecious, sexual reproduction, mechanism of pollination by general entomophily, life cycle long-lived and widespread geographic range, which may explain the pattern of isozyme variation in this plant. Furthermore, some characterictics related to the life history of the species also could be invoked to explain the genetic similarity among populations of *M. comosum*. An

important life history characteristics of *M. comosum* is that this species is transported as a "camp-follower" weed with agricultural crops. However, although *M. comosum* can be viewed as a "sinantropic" species in some countries (for example it is cultivated in some regions of Italy), it is very difficult to think that all the Mediterranean populations of *M. comosum* are stablished from some clones scaped from the cultivations. Other hypotheses related to the life history characteristics of *M. comosum*. that could explain the lack of genetic differentiation among populations, are its plastic response to the environment (it is a very polymorphic species from the morphological point of view, Tutin et al., 1980), joined to the wide distribution and the high longetivity and fertility of the species. These characteristics will increase the effective population size and thus slow down the rate of selection (Levin, 1978). Phenotypic plasticity, for example, will allow to individuals to respond to different enviroments without changes in the genotype (Bradshaw, 1965).

The analysis with F statistics shows that four of the five loci surveyed presents significant excess of homozygotes; the mean for the four loci was 0.215 indicating deficiency of heterozygotes in relation to that expected by panmixia. This is a generalized fact in allogamous plants and the opposite is true in autogamous species; this have been called "heterozygosity paradox" (Brown, 1979). Whalund effect, consanguineous mating, partial self-pollination and another factors could contribute to explain this observation.

However, the estimations with the F statistics for the ADH locus are appreciably negative, indicating an excess of heterozygotes in the populations for this locus. The f_i statics show negative values in the majority of populations (Table 2) and the F_{IS} also presents a value negative in the pool of populations. Although many reasons could be the origin of a heterozygotes excess such as negative assortive mating, differences in allele frequencies between male and female gamete pools, among others, we think that our observation can be explained by selection (heterosis). The significance of F indices is tested by means of the X^2 respect to the frequencies of Hardy-Weinberg equilibrium. These tests are significatives in very few cases. However, this results is not scarce for several reasons. Firstly the region of highest samplig variance for F is $0 < F < 0.35$ when the gene frequencies are in the most experimentally useful range ($0.2 < p < 0.5$) (Brown, 1979). Generally this is the case of all the loci of *M. comosum*. Second, it is necessary a very high sample size, for detect significatives deviations for values of F importants (Cavalli-Sforza and Bodmer, 1971). For example for values of F of 0.10 is necessary to analize a minimal of 384 individuals for detect significatives deviations by means of X^2 test, to level of 5%. For these reasons is possible to think that the values found in the populations of *M. comosum* point to an excess of heterozygotes individuals.

In relation with this observation is important to mentioned that natural selection has been also invoked in the maintenance of chromosome variability of *M. comosum* (Ruiz Rejón and Oliver, 1891). As far as this is concerned it is important to mentioned that ADH locus shows significant associations of the same kind with second chomosome arrangements in all the populations (Ruiz Rejón et al., submitted). This may also be taken as proof of selection for allozymes of ADH.

In conclusión, we think that interaction between biological and life history characterictics of *M. comosum*, such as sexual reproduction, predominantly outcrossing with pollination by general entomophily and widespread geographic range, joined to Whalund effect and adaptative factor as selection have played a major role in determing the geographical distribution and the amount of isozyme variability present in this species.

RESUMEN

Se analiza la variabilidad electroforética en seis loci enzimáticos de 33 poblaciones naturales de *M. comosum*. Excepto el sistema GDH todos los demas loci (GOT-1, GOT-2, GOT-3, IDH y ADH) eran polimórficos. Aunque aparentemente las poblaciones están diferenciadas tal como

muestran los valores de X^2 de heterogeneidad esta diferenciación es solamente moderada de acuerdo con los valores de identidad genética y el estadístico F_{ST} calculados. Las explicaciones más pausibles de este resultado pueden estar relacionadas con características biológicas (reproducción sexual, alogamia predominante, polinización por insectos, amplio rango de distribución geográfica) junto con otros factores adicionales relacionados con el pasado histórico de la especie, tales como su plasticidad fenotípica y su alta longevidad. Además el análisis realizado con estadísticos F muestra que en cuatro de los loci analizados existe un deficit de individuos heterocigotos, que se puede explicar por autopolinización parcial, efecto Whalund o apareamientos consanguíneos. También este análisis muestra que en el locus ADH existe un claro exceso de individuos heterocigotos. Varias causas pueden producir exceso de heterocigotos (apareamientos negativos, diferencias en las frecuencias alélicas entre los acervos gaméticos de machos y de hembras). Pero nosotros pensamos que esta observación podría explicarse por acción de la selección.

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APPENDIX 1

List of local populations sampled and their locations

PAD = Padul (Granada, Spain)
AL = Almaizar (Granada, Spain)
COZ = Cozvijar (Granada, Spain)
CAN = Balcón de Canales, Sierra Nevada (Granada, Spain)
HIG = Higuera, Sierra Nevada (Granada, Spain)
SIL = Silleta, Sierra Nevada (Granada, Spain)
SAB = Sabinas, Sierra Nevada (Granada, Spain)
PE = Peña de los Enamorados, Archidona (Málaga, Spain)
COL = Colmenar (Málaga, Spain)
BNS = Baños de Salcedo (Jaén, Spain)
MZ = Manzanares el Real (Madrid, Spain)
GU = Guadalix de la Sierra (Madrid, Spain)
GAR = Gargantilla (Madrid, Spain)
LAC = La Cabrera (Madrid, Spain)
ZA = Zafra (Badajoz, Spain)
PB = Puerto de Bejar (Salamanca, Spain)
BM = Baños de Montemayor (Salamanca, Spain)
CP = Cruce de Piedrahita (Salamanca, Spain)
SA = Salamanca (Spain)
POA, POB, POC, POD = Portugal populations without specific location
MOY = Moya (Las Palmas de Gran Canaria, Spain)
SUR = Santa Ursula (Tenerife, Spain)
MEN-1, MEN-2 = Menorca, Spain
LLUV = Lluvi (Mallorca, Spain)
DUR = Durance (France)
AVI = Avignon (France)
ROM = Roma (Italy)
GRE = Greece

Table 1.- Allelic frequencies in 33 natural populations of *Muscari comosum*

Gene	IDH		ADH			GOT-1	GOT-2	GOT-3
Allele	a	a	b	c	d	b	a	a
PAD	.88	.05	.15	.54	.26	.83	1.00	.96
AL	.79	.03	.30	.56	.11	.94	.98	.75
COZ	.68	.04	.25	.40	.31	.93	1.00	.99
CAN	.83	.09	.15	.55	.21	.95	.97	.94
HIG	.88	.10	.14	.52	.24	.94	1.00	.98
SIL	.82	.11	.17	.42	.30	.90	.99	.98
SAB	.90	.09	.22	.49	.20	.92	.97	1.00
PE	.79	.02	.31	.55	.12	.88	.97	.72
COL	.89	.02	.26	.48	.29	.76	.9	.98
BNS	.95	.10	.10	.44	.36	.92	.92	.97
MZ	.86	.06	.26	.35	.33	.92	.92	.91
GU	.82	.02	.19	.61	.19	.86	.97	.93
GAR	.84	.15	.21	.47	.17	.93	.98	.92
LAC	1.00	.05	.35	.40	.20	.95	.96	1.00
ZA	.90	.05	–	.48	.47	1.00	1.00	1.00
PB	.79	.04	.08	.55	.33	1.00	1.00	.97
BM	.77	.07	.04	.62	.27	.90	1.00	.84
CP	.83	.08	.14	.51	.27	1.00	1.00	.95
SA	.73	–	.17	.46	.37	1.00	.99	.91
PDA	.92	.08	.04	.60	.28	1.00	.97	
POB	.76	.01	.09	.50	.40	.92	.92	1.00
POC	.94	.06	.17	.58	.19	.96	1.00	1.00
POD	.70	–	–	.38	.62	1.00	1.00	.97
MOY	.72	.05	.10	.35	.50	1.00	1.00	1.00
SUR	.95	.11	.07	.56	.36	1.00	1.00	.98
ME-1	.89	.03	.20	.22	.55	.97	1.00	.92
ME-2	.92	.12	.27	.46	.15	.96	1.00	1.00
LLUV	.98	.12	.27	.22	.39	.82	.99	.97
VAL	1.00	.08	.39	.25	.27	.92	.97	1.00
AVI	.90	.13	.25	.43	.19	.95	.98	.95
DUR	.83	.09	.15	.46	.30	.90	.98	.94
ROM	.85	.09	.18	.40	.33	.94	1.00	.94
GRE	.97	–	.18	.52	.30	.97	1.00	1.00
Average	.86	.06	.18	.46	.30	.93	.98	.95
Standard errors	.015	.017	.019	.021	.01	.001	.012	
X^2Het	112.54		63.30	133.49	54.87	176.48		

Table 2.- Estimates of fixation indexes (f_i) in the 33 samples of *Muscari comosum*.

Locus	IDH	ADH	GOT-1	GOT-2	GOT-3
PAD	-.13	-.15	+.35	+1.00	-.03
AL	+.24	-.27	+.64	-.01	+.56
COZ	+.33	-.47	+.10	+1.00	-.49
CAN	+.08	-0.1	+.03	+.05	+18
HIG	+.25	-.19	+37	+1.00	+.07
SIL	+.86	-.21	+.35	+.21	-.20
SAB	-.16	.-31	+.77	-.15	+1.00
PE	+.37	-.06	.-13	-.04	+.85
COL	+.43	-.11	+.33	-.03	.03
BNS	+.04	-.18	-.13	-.13	+.05
MZ	-.16	-.06	+.64	-.09	+.29
GU	-.07	-.23	+.42	-.04	+.64
GAR	+.83	-.37	+.18	+.19	+.06
LAC	+1.00	-.41	-.08	0.00	+1.00
ZA	+.26	-.13	+1.00	+1.00	+1.00
PB	+.19	+.62	+1.00	+1.00	+1.00
BM	+.07	-.36	+.38	+1.00	+1.00
CP	-.11	+.24	+1.00	+1.00	+.79
SA	-.05	-.04	+1.00	-.03	+1.00
PDA	-.02	-.22	+1.00	+1.00	+.18
POB	-.34	-.12	-.21	+1.00	+1.00
POC	+.02	-.11	0.00	+1.00	+1.00
POD	-.24	-.59	+1.00	+1.00	+.46
MOY	+.33	-.16	+1.00	+1.00	+1.00
SUR	+1.00	-.15	+1.00	+1.00	-.16
MEN-1	-.13	-.12	+.05	+1.00	-.13
MEN-2	-.05	-.08	0.00	+1.00	+1.00
LLUV	+.12	-.49	+.01	+.13	-.18
VAL	+1.00	-.52	-.13	+.-05	+1.00
AVI	-.22	-.68	-.25	-.24	-.03
DUR	+.29	-.53	-.16	+1.00	-.06
ROM	+.17	-.46	-.13	+1.00	-.13
GRE	+.38	+.08	+.12	+1.00	+1.00

Table 3.- Values of F-statistics

	F_{IS}	F_{ST}	F_{IT}	F_{IT}
IDH	0.144	0.043	0.151	0.180
ADH	-0.218	0.021	-0.190	-0.192
GOT-1	0.303	0.048	0.234	0.336
GOT-2	0.530	0.048	0.174	0.539
GOT-3	0.316	0.062	0.375	0.359
Average	0.215	0.039	0.149	0.244
Standard errors	0.1244	0.0081	0.0933	0.1231