GENETIC STRUCTURE AND SPATIAL DISTRIBUTION OF A NARROW ENDEMIC PLANT: *ANDROCYMBIUM EUROPAEUM* (LANGE) K. RICHTER (COLCHICACEAE)

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SUMMARY

Allozyme differentiation at 10 loci was used to ascertain, by means of the application of the conventional mathematical methods to assess gene frequency data, the manner in which the genetic variability of *A. europaeum* is structured in all its known populations. In spite of its conditions of narrow, threatened and endangered endemic plant, it shows no sign of genetic depauperation, having been clearly able to maintain very high levels of variation. A conception to determine allelic rarity was used in an attempt to establish relations of ancestrality between the populations. These relations were further substantiated by the data concerning the paleontological history of the distribution area.

RESUMEN

La diferenciación en 10 loci aloenzimáticos entre la poblaciones conocidas de *A. europaeum*, evaluada mediante los métodos convencionales para procesar datos de frecuencias alélicas, se ha utilizado para averiguar la distribución de la variabilidad genética en esta especie. A pesar de su condición de endemismo con distribución restringida y en peligro de extinción, *A. europaeum* no muestra signos de depauperación genética, siendo capaz de conservar niveles muy altos de variación. Un concepto de rareza alélica fué utilizado para intentar el establecimiento de relaciones de ancestralidad entre la poblaciones. Estas relaciones fueron adicionalmente cotejadas con datos sobre la historia paleontológica del área de distribución.

INTRODUCTION

Androcymbium europaeum (Lange) K. Richter (Colchicaceae) is a narrow threatened and endangered endemic plant species (GOMEZ CAMPO, 1987) that

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lives in the temporal pastures of the calcareous and desertic steppes of Almería (Spain), where annual rainfall is 100-300 mm. *A. europaeum* has a strikingly short period of seasonal growth; it surfaces, blooms and fruits between November and February (when the rain periods occur). During the unfavourable dry season, it remains with its cormus buried, experiencing a long period of dormancy. It forms clumps which are clearly defined at the exhausted populations but quite intertwined at the less spoiled ones. Since no documentation is known on the reproductive biology of the genus *Androcymbium*, we advance some data (PEDROLA & CAUJAPE, in prep.) to support the interpretation of the results. Generation length is very high (more than 20 years). Its reproduction is predominantly sexual, though occasionally there is asexual reproduction by means of the emission of droppers which can form new corms. The only observed visitor insects during the day were small flies (family Milichiidae) which, because of their minute size and other considerations about flower morphology, are unlikely to be effective pollinators. In the course of recent field observations during dusk, a great number of bugs and beetles carrying considerable amounts of pollen of *Androcymbium* on their abdomens were observed. They are, most probably, their effective pollinators. Wind pollination and agamospermy are discarded. Self-compatibility was obtained artificially, but the plants are predominantly outcrossing. Fecundity is high, but the experimental germination rate is very low (4%). Seeds are not endowed with special adaptations for long distance dispersal and they are toxic for some animals. Moreover, the valvae of the capsules don't split open to ease scattering, a trend that differentiates *A. europaeum* from other related species (GREUTER, 1967). Regarding these data and the disposition in clumps, assortative mating is expected. Therefore, gene flow and genetic variability are likely to be limited in *A. europaeum*. Apart from the current values of genetic parameters, an important component of the genetic structure of any species arises as a consequence of the interaction between historic events and genetic substrate. It is possible that, considering the probably recent speciation of this group, some evidence of its pristine spatial distribution could still remain, reflecting the oscillations in size which its occupation area underwent through the Quaternary. For this reason, we selected rare alleles as genetic markers to trace the possible relationships of ancestrality among the studied populations. The purpose of this paper is, then, to study the possible influence both of the limited gene dispersal and of the proposed paleontological history of the distribution area of this species upon its genetic structure.

MATERIALS AND METHODS

Ten populations of this species are known, whose sizes are of some hundreds of individuals, although some of them (populations 5, 6 and 8) are greatly exhausted (owing to an intense anthropic pressure). These populations were sampled in December 1989 (Fig. 1). Individuals are arrayed in a row of a width

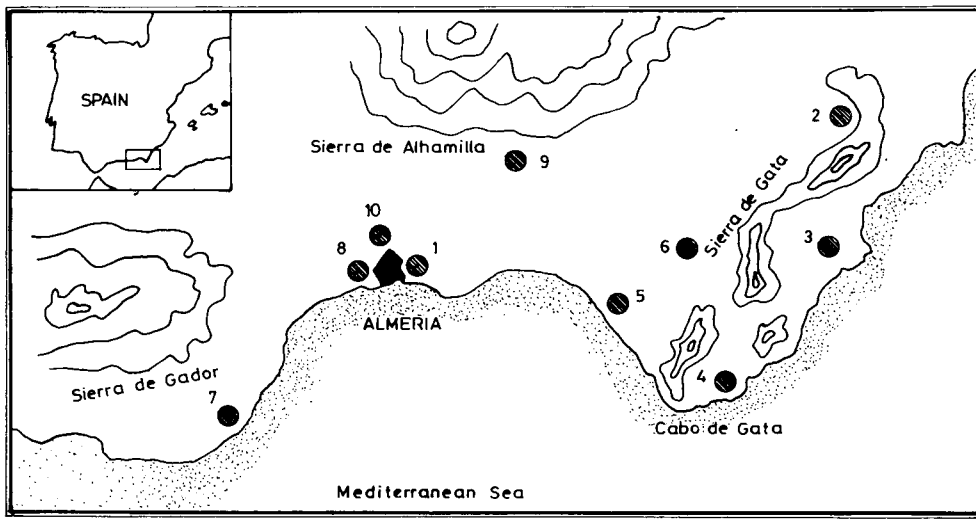

Figure 1.- Map of Almeria with the location of populations (1: Los Molinos, 2: Charco del Lobo, 3: Cerro de Los Lobos, 4: Playa de Monsul, 5 :Barranco La Curria, 6: El Barranquete, 7: El Solanillo, 8: Centro Zonas Aridas, 9: Cerro de Los Peligros, 10: Cerro de San Cristobal).

ranging from one to 10 metres which is always found along a north-south axis, this being the direction of sampling. Clump areas are narrow (6 to 10 plants) with a distance of 40 to 100 cm between two consecutive nuclei, showing an overlapping external region. We sampled ten clumps per population, taking 2-3 plants from their cores. A total of 300 individuals were transported to the green-houses of the Botanical Garden Marimurtra (Blanes).

Fragments of leaves were directly taken to the laboratory, where we proceeded as described in VALLEJOS (1983) and SHIELDS *et al.* (1983). Of all the enzyme systems assayed, only ten (Table 1) were included in the calculations. Owing to the fact that these plants deteriorate very quickly and that 20 of them were not considered for possessing ambiguous bandingpatterns, the effective sampling size was of 186 individuals (Table 2). The analyses carried out in different developmental stages didn't reveal detectable expression differences at the level of electrophoretic bands, whose intensities for polymorphic loci were consistent with the mendelian hypothesis of codominant inheritance. Genetic experiments in order to check the mendelian inheritance of banding patterns were not done, but the results of the electrophoreses of the offspring and of the only resolved system for the seeds (alcohol dehydrogenase-1, ADH1) were in accordance with those obtained with the parental generation.

RESULTS

As a first introduction to the genetic variation of the samples, the proportion

Table 1.- Optimum gel buffers and electrode buffers for the enzymes analysed in *A. europaeum*. (ADH = alcohol dehydrogenase; MDH = malate dehydrogenase; PGI = phosphoglucose isomerase; PGM = phospho-glucomutase; GOT = glutamate-oxaloacetate transaminase; ACO = aconitase; ME = malic enzyme).

Enzyme	Gel buffer		Electrode buffer
PGI	Histidine-citrate	pH 5.7	Tris-citrate 0.1M.
	Histidine	pH 7.0	
MDH	Histidine-citrate	pH 5.7	Tris-citrate 0.1M.
	Histidina	pH 7.0	
6PGD	Histidine-citrate	pH 5.7	Tris-citrate 0.1M
	Histidine	pH 7.0	
ADH	Histidine	pH 7.0	Tris-citrate 0.1M
PGM	Histidine-citrate	pH 5.7	Tris-citrate 0.1M
	Histidine	pH 7.0	
ACO	Histidine-citrate	pH 5.7	Tris-citrate 0.1M
	Histidine	pH 7.0	
GOT	Tris-citrate	pH 8.2	Borate pH 8.2
	Histidine	pH 7.0	Tris-citrate 0.1M
ME	Tris-citrate	pH 8.2	Borate pH 8.2

of polymorphic loci (P)(assuming that a locus was polymorphic when the frequency of its most frequent allele was ≤ 0.95) and the mean number of alleles per locus (A), were worked out for each population (Table 2). Both the values of P (0.52) and of A (1.86) averaged over all populations are high for a species holding the traits of *A. europaeum*, though they conform to the values that endemic species are expected to span (HAMRICK *et al.*, 1979). These values are also high when compared with the available data on bulbous plants (i.e. genus *Allium*) (VASSEUR *et al.*, 1990).

Table 2.-Genetic summary statistics for the ten populations of *A. europaeum*. Mean number of alleles per locus (A), proportion of polymorphic loci (P) and expected and observed heterozygosities (H_e and H_o) for each studied population. The number of individuals used in the analyses (N) is also shown.

Populations	N	A	P	He	Ho
1. Los Molinos	23	1.8	.60	.176	.220
2. Charco de Los Lobos	23	1.9	.50	.199	.185
3. Cerro de Los Lobos	25	1.5	.30	.101	.116
4. Playa de Monsul	15	1.8	.50	.185	.164
5. Barranco La Curria	15	1.8	.40	.230	.324
6. El Barranquete	11	1.9	.50	.251	.288
7. El Solanillo	16	2.2	.70	.275	.164
8. Centro Zonas Aridas	14	2.0	.50	.237	.307
9. Cerro de Los Peligros	21	2.2	.60	.306	.226
10.Cerro de San Cristobal	23	1.9	.60	.205	.270

The most of the overall genetic variability in *A.europaeum* is attributable to gene diversity within populations ($H_s = 0.217$), whereas gene diversity among populations is quite lower ($D_{st} = 0.051$). We note that the proportion of genetic variation explained by the among populations component (NEI, 1973) is quite high ($G_{st} = 0.191$) in comparison with data for endemic plants (PRENTICE, 1984; LOVELESS & HAMRICK, 1984). This enables us to adopt the view that the populations of this species act as variability pools where the most frequent alleles are always shared by the totality of populations, as GOTTLIEB (1975, 1977) reports for the alleles whose frequency is above 0.20, being the observed differences among them due both to divergences among the frequencies of the non-predominant alleles and to phenomena of presence-absence of rare alleles. Genetic variability is scarce within each clump, but noticeably larger when considering the whole population, because clumps present low but qualitatively different levels of variability. The observed variation of banding patterns among clumps through the ten populations and a few sampled groups of seedlings at the more variable loci supports this view. We calculated F_{is} (WRIGHT, 1951, 1969) in order to check if these clumps could be assumed to be restrictive reproduction areas. Though the values of H_o and H_e shown in Table 2 could suggest an erratical behaviour of frequencies respect to Hardy-Weinberg expected proportions, none of the *chi*-square heterogeneity tests that we performed for each locus was significant, so deviations from a value of $F_{is} = 0$ are assumed to occur by chance.

The values of Nei's distance (NEI, 1972) for every pairwise combination of populations (Table 3) are within the range that conspecific plant populations are expected to show (CRAWFORD, 1983) and evidence that some populations are more related than what could be thought from their geographical separation (Fig.1) and the low levels of gene flow among them. This suggests that some of these populations could have been part of a more widspread populations a short period of time ago. The divergence times obtained from $t = D/2\alpha$ (NEI, 1975) allow, taking $\alpha = 10^{-7}$ and with the caution that must be derived from the assumptions entailed by the application of Nei's formula, to relate the current separation of these populations and the paleoclimatic events of the Quaternary (the lesser of the obtained values is 140,000 years between populations 2 and 5 and the higher 1,610,000 years between populations 9 and 10). We thought that rare alleles would perform well as genetic markers of ancestrality, for the probability that two or more populations that share at least one effectively rare allele be of common origin must be higher than the probability that the shared rare alleles have arisen by chance at the populations in which they are detected, particularly when considering that, for short term evolution, mutation can be neglected and divergence between populations assumed to have arisen only by drift (REYNOLDS *et al.*, 1983). We find it feasible to suppose that the difference between these probabilities increases as the number of populations which share one or more rare alleles grows. Applying the method that is put forward in the model of allelic ubiquity (CAUJAPE & PEDROLA in prep.) we ascertained which of the alleles belonging to loci in which we detected three or more alleles were rare, and used them as genetic markers of communality. This was possible because the allele frequency data were processed to determine which was the

Table 3.-Genetic distances. Number of loci = 10; Average distance = .075; Average SD = .040; Variance of the distances = .005 (se: standard error)

Populations	1	2	3	4	5	6	7	8	9	10
1 Los Molinos	-	.091	.080	.034	.091	.141	.066	.068	.158	.051
se=	-	.063	.074	.015	.053	.073	.044	.041	.065	.044
2 Charco del Lobo		-	.033	.046	.026	.056	.066	.090	.128	.041
se=		-	.021	.031	.018	.037	.024	.049	.072	.022
3 Cerro de Los Lobos			-	.042	.058	.065	.035	.058	.161	.014
se=			-	.031	.032	.034	.011	.040	.073	.006
4 Playa de M"nsul				-	.067	.087	.067	.073	.160	.035
se=				-	.033	.046	.023	.045	.082	.020
5 Barranco La Curria					-	.030	.077	.093	.116	.050
se=					-	.019	.030	.049	.060	.028
6 El Barranquete						-	.089	.113	.127	.054
se=						-	.030	.059	.071	.033
7 El Solanillo							-	.047	.101	.034
se=							-	.019	.034	.013
8 Centro Zonas Aridas								-	.092	.044
se=								-	.046	.033
9 Cerro de los Peligros									-	.129
se=									-	.060
10 Cerro de San Cristobal										-
se=										-

value of ubiquity of each allele, defined as the property of being found with a high frequency in a high number of populations. Altogether, the threshold value of ubiquity for each different locus allowed us to determine which of those alleles was rare (Table 4), this rare alleles being used for the graphic representation (Fig. 2).

DISCUSSION

The species with the narrowest ecological amplitudes are generally expected to be the least variable. However, the proportion of polymorphic loci and the mean number of alleles per locus found in the *A. europaeum* populations show no sign of genetic depauperation, with a level of variability much higher than expected. Two explanations can account for this fact. First, the subjection of this species to an environment that has been heterogeneous in space and time may have promoted genetic polymorphism. Survival in such adverse conditions might have been greatly aided by a more predominant role of asexual reproduction which, allowing the extension of generation length, would have sustained the reached levels of variability. Second, the strong recent antropic pressure which some of the populations have suffered could have acted as a random selective force, diminishing these populations in bulk but keeping a high variability, which is quite difficult to explain if assuming that they hold a regular number of individuals since their stablishment. Whatever the reason, *A. europaeum* has been clearly able to maintain considerable levels of variation, which

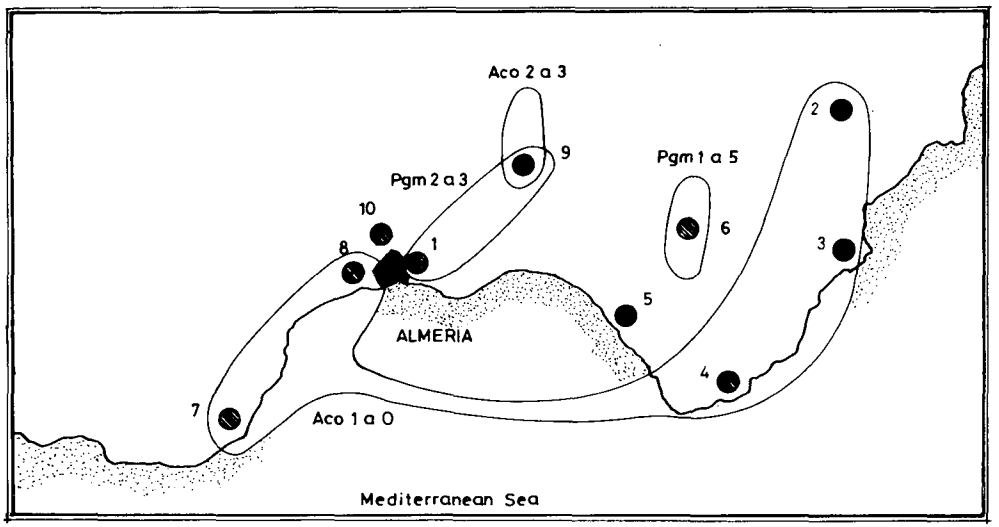

Figure 2.- Relationships between the populations based upon the sharing of rare alleles. Populations which possess a private rare allele are also considered.

seem to be quite independent among populations, as the value of G_{st} show. This must be so because gene flow among populations is very limited and moreover handicapped by the fact that seeds are not dispersed easily. However, it seems from the distribution of rare alleles that some of the presently separated, small populations could descend from a more widespread one. The outcome of the analysis of ubiquity (Fig. 2) enabled us to establish two groups. The first one comprised the peripheral populations (2, 3, 4, 7 and 8). The rare allele which allows to build this group is a null allele, belonging to the Aco1 system (Aco10), which does not make it possible to establish reliable hypothesis on the common origin of these populations. The second group clusters the populations 1 and 9. This cluster can be interpreted as an evidence of the existence of an ancestral and more widely extended population, from which these two would have derived.

Noticeably, this cluster is discordant with the obtained value of Nei's genetic distance. It must be stressed that the shared rare alleles are more likely to reflect proximity among populations in terms of coancestry if both sample sizes and the number of analysed loci is high, precisely as a consequence of their rarity. We used them on the basis that the different populations of an area can descend from an ancestral deme and not be able to interchange migrants. Rare allele frequencies in these demes would be determined by the time since the radiation event took place instead of by the existence of gene flow (SLATKIN, 1985). Then, an allele can be found in low frequencies in all the demes because its frequency was also low in the ancestral deme. Consistent with this, it is feasible to hypothesize a pristine distribution of our populations in a common area which spanned the southern Iberian Peninsula and the north of Africa. This circumstance could only have ocurred during the Messinian Salinity Crisis (HSÜ, 1972; HSÜ *et al.*, 1973; HSÜ *et al.*, 1977; AZZAROLI & GUAZZONE, 1978,

Table 4.-Matrix of allele frequencies (the order of populations is the same as that showm in the other tables). Rare alleles are underlined.

	1	2	3	4	5	6	7	8	9	10
mdh11	1.000	0.610	1.000	0.870	0.570	0.560	1.000	1.000	0.710	1.000
mdh12	0.000	0.390	0.000	0.130	0.430	0.440	0.000	0.000	0.290	0.000
mdh31	0.880	1.000	1.000	1.000	1.000	1.000	0.770	1.000	1.000	0.930
mdh32	0.120	0.000	0.000	0.000	0.000	0.000	0.230	0.000	0.000	0.070
got11	0.830	1.000	1.000	1.000	0.960	1.000	0.690	0.430	0.360	0.920
got12	0.170	0.000	0.000	0.000	0.040	0.000	0.310	0.570	0.640	0.080
me11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
me12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
pgi11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
pgi12	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
adh11	0.090	0.000	0.000	0.000	0.000	0.000	0.270	0.000	0.000	0.000
adh12	0.910	1.000	1.000	1.000	1.000	1.000	0.730	1.000	1.000	1.000
pgm11	0.820	0.020	0.000	0.540	0.140	0.064	0.190	0.250	0.210	0.170
pgm12	0.180	0.520	0.880	0.420	0.390	0.690	0.650	0.620	0.210	0.650
pgm13	0.000	0.350	0.040	0.000	0.390	0.063	0.080	0.065	0.210	0.040
pgm14	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.160	0.000
pgm15	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000
pgm16	0.000	0.110	0.080	0.040	0.080	0.120	0.040	0.065	0.210	0.140
pgm21	0.000	0.090	0.000	0.170	0.000	0.250	0.080	0.060	0.280	0.170
pgm22	0.960	0.910	1.000	0.830	0.960	0.620	0.880	0.880	0.640	0.830
pgm23	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000
pgm20	0.000	0.000	0.000	0.000	0.040	0.130	0.040	0.060	0.000	0.000
aco11	0.500	0.260	0.330	0.270	0.500	0.500	0.230	0.440	0.080	0.500
aco12	0.500	0.645	0.560	0.365	0.500	0.500	0.730	0.440	0.920	0.500
aco10	0.000	0.095	0.110	0.365	0.000	0.000	0.040	0.120	0.000	0.000
aco21	0.000	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
aco22	0.870	0.860	0.870	0.920	0.570	0.420	0.710	0.620	0.250	0.700
aco23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000
aco24	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.170	0.000
aco25	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.310	0.000	0.000
aco26	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.180
aco27	0.000	0.020	0.130	0.040	0.430	0.580	0.290	0.070	0.330	0.120

1979-1980; BOCQUET & WIDLER, 1978; BOCQUET *et al.*, 1978; CARDONA & CONTANDRIOPOULUS, 1979) between 7.5 and 5.2 million years BP. The physiographic characteristics of the zone of Cabo de Gata were most probably developed during the Pliocene. The "Pliocenian Flood" of the Mediterranean Sea, after the Messinian event, caused the geographic isolation between Europe and Africa. We can then substantiate that the factors which, most probably, allowed the processes of speciation of these populations with regard to African related species could have appeared in this period. The subsequent oscillations in the temperature and degree of moisture that the climatic variations which occurred through the Quaternary provoked in this zone could have allowed the *A. europaeum* populations to expand during the auspicious periods, forcing them to concentrate in privileged reducts when general conditions were unfavourable.

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