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The pattern of esterase variation in the *Mimulus glabratus* complex (Scrophulariaceae)

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THE PATTERN OF ESTERASE VARIATION IN THE *MIMULUS GLABRATUS* COMPLEX (SCROPHULARIACEAE)

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Abstract.— Disc gel electrophoretic assays of *Mimulus* plants showed young stems to contain at least 15 esterase bands. The 6 bands in the center of the gels were highly reproducible. Eighty-one populations representative of all the cytotaxonomic groups and of the Western Hemisphere geographic range of the *Mimulus glabratus* complex were assayed for those 6 bands. There was no apparent correlation between the distribution of the esterase bands and important environmental parameters such as elevation and latitude. Hence, their distribution did not appear to reflect environmental adaptive values. Each population was monophonic for its particular combination and intensity of esterase bands which is consistent with our previous suggestion that genetic drift is a strong component of the evolutionary pattern of the complex.

The demonstration of widespread inter- and intra-population variation in the distribution of electrophoretically detectable enzymes (Hubby and Lewontin 1966; Selander 1970; Lewontin 1974) continues to raise interesting evolutionary questions. For example, Are these variations of adaptive advantage? Do they reveal evolutionary patterns, such as genetic drift? As to the first question, Koehn's finding (1969 and pers. comm.) of a close correspondence between habitat temperature, optimum temperature for enzyme activity, and the distribution of esterase alleles in *Catostomus clarkii* gave a clear yes answer for esterases of the Gila mountain sucker. Since our studies of the *Mimulus glabratus* complex (Alam and Vickery 1973) suggest the presence of genetic drift in the distribution of barriers to gene exchange, would an analysis of the distribution of the *Mimulus* esterases confirm the genetic drift pattern of evolution in the complex? Or would it reveal an adaptive basis as in *Catostomus*?

The putative origin of the *Mimulus glabratus* complex is in the Great Basin. It appears to be derived possibly via a genetic revolution (Mayr 1963) from an aneuploid peripheral population of the California-centered *M. guttatus* complex (Vickery et al., in press). Then, the successful aneuploid, n=15, radiated throughout the Great Basin. Successive radiations carried it into the Great Plains, Great Lakes region, and Rio Grande drainage (Fig. 1). At their southern limit the diploids apparently gave rise to a small radiation of tetraploids, n=30, and an extensive radiation of aneuploid tetraploids, n=31. The latter extends throughout the temperate regions of Mexico and Guatemala. Possibly hexaploids evolved in the same area as the tetraploids and aneuploid tetraploids, but were transported by migratory birds to South America (Vickery et al., in press). In fact, there is an extensive radiation of polymorphic hexaploid, n=46, forms throughout most of temperate South America. This unusual pattern of radiation from the Great Basin east to Quebec and south to Patagonia must have been facilitated by the colonizing nature of these plants. The members of the complex occur in a variety of moist habitats from sea level to altitudes of over 5,000 m. The populations of the complex form four genetically isolated groups with n=15, 30, 31, and 46 chromosomes, respectively, corresponding to the major radiations (Vickery 1969). Each group is divided by a variety of barriers to gene exchange into a diversity of subgroups (Vickery et al., in press). These consist of from few to many, small—often very small—geographically isolated populations. Due to the Founder effect and/or genetic drift coupled with vegetative reproduction, some of these must consist of only a few, possibly even a single, genotype.

Materials and Methods

Sample populations of 81 natural populations (see Hsu 1973 for details) that well represent the geographic range of the complex (Fig. 1), its morphologic and cytologic diversity (Alam and Vickery 1974), and its various taxa (Humboldt

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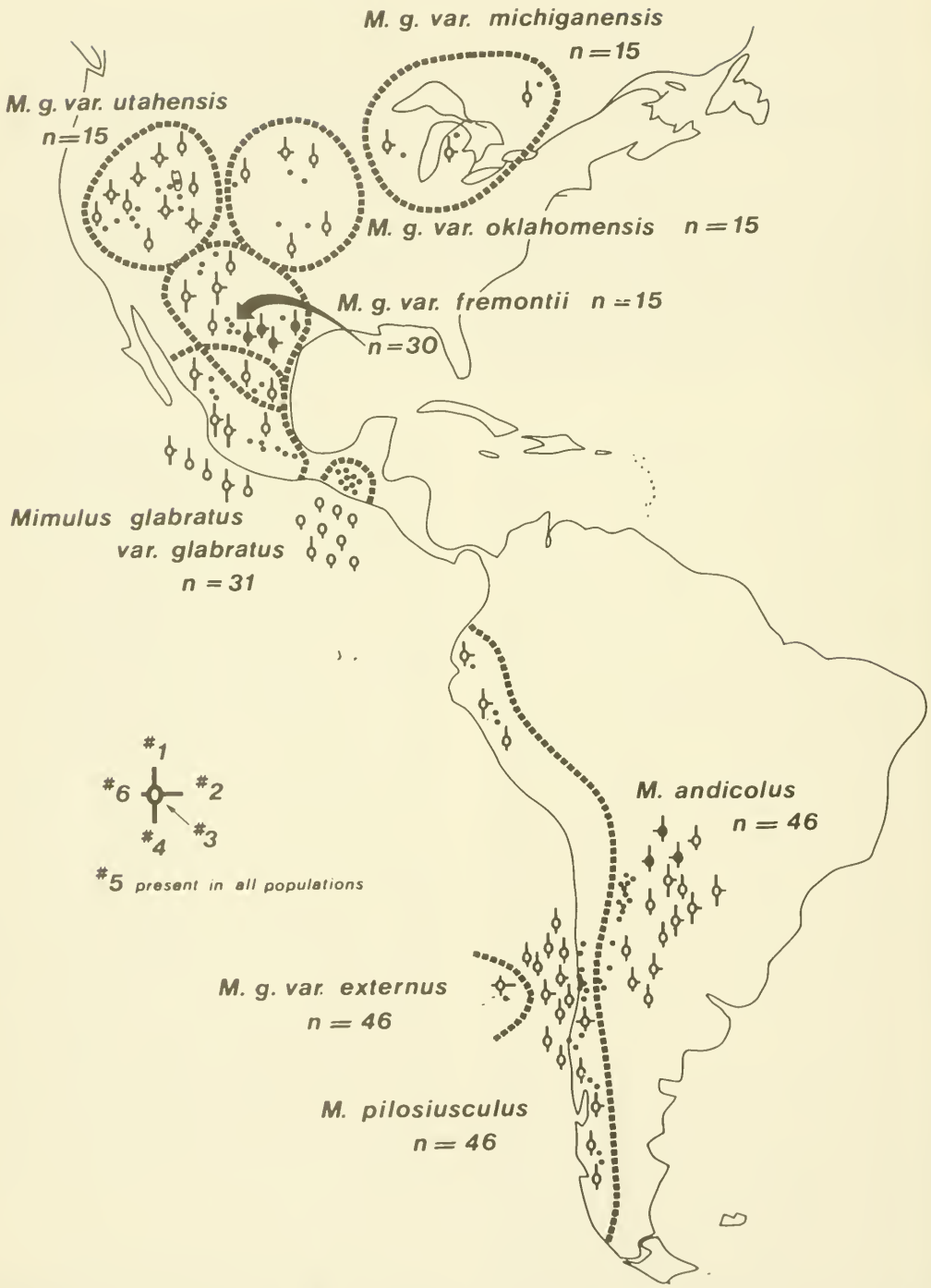

FIGURE 1. Distribution of the *Mimulus glabratus* complex and its related species showing the populations assayed for the esterases of their stems. The dots give the actual locations of each population studied and the corresponding symbols give the esterase composition. No arm indicates that that esterase does not occur or is not expressed in that population, a short arm indicates an esterase of moderate activity, and a long arm indicates a strongly active one based on the intensity of staining of the electrophoretic bands. The #5 esterase (not indicated) was always present at high intensity. The #3 was always present either at moderate intensity, (the hollow circle) or at high intensity (the solid circle). Note: In the area where the n = 15, n = 30, and n = 31 populations overlap, the two populations studied electrophoretically have n = 15 chromosomes.

et al. 1817; Reiche 1911; Grant 1924; Pennell 1935; Fassett 1939; Skottsberg 1953; Descole 1954) were assembled in the University of Utah greenhouse. The sample populations or cultures were grown from seeds collected in the wild or from plants grown from such seeds.

Of the various plant organs assayed electrophoretically for their esterases, young stems produced the clearest, most reproducible results when repeated assays of the same stem were run. The stems of five plants were tested for each of 77 of the populations. For the other four populations from Wendover, Utah (culture number 5852); Limpia Canyon, Texas (6617); Quezaltenango, Guatemala (7301); and Aisen, Chile (6328)—one from each heteroploid level—the stems of 24 plants each were checked in order to investigate intra-population variability.

The esterases were extracted from the stems according to standard methods (Brewer and Sing 1970). Their electrophoretic separation followed the procedures of Ornstein (1964) and Davis (1964) with minor modifications required by our material (Hsu 1973). We used disc eltecrophoresis employing a Canalco 12 apparatus with 6.5 cm x 5 mm glass tubes, 7.5 percent polyacrylamide gels, 2.5 ma/tube, and up to 300 v during each run of from 75 to 85 minutes. Alphanaphthylacetate was used as the substrate and Fast Blue RR salt (diazotized product of 4-benzoylamino-2,5-dimethoxyaniline - Zn Cl_2 in Tris-HCl buffer, pH 7.5, .01 M) as the stain to locate bands of esterase activity in the gels. Each run contained 12 tubes, one of which was a control.

Results and Discussion

Of the 15 or more esterase bands detected in the young stems, only the six bands in the center third of the gels proved consistent. They were bands #1 at Rf = 0.66, #2 at Rf = 0.62, #3 at Rf = 0.58, #4 at Rf = 0.54, #5 at Rf = 0.50, and #6 at Rf = 0.48. These bands, when present, showed either moderate or high intensity staining reflecting corresponding quantitative differences in the esterase activity of the plants assayed. Increasing quantities of the enzymes did not correlate with increasing heteroploid chromosome level which suggests that the genes producing the esterases are controlled by regulatory mechanisms that are independent of gene dosage as Sing and Brewer (1969) found in a polyploid wheat series.

The presence and intensity of the esterase bands were uniform within each population as Clegg and Allard (1972) found in *Avena* but often differed between populations. The intra-population monomorphism is striking despite the generally small sample size—5 plants for 77 populations, 24 plants for 4 populations—because it is based on so many populations. The distributions of the esterase bands within the populations depart highly significantly from Hardy-Weinberg equilibria based on the frequencies observed in the complex as a whole, except for the one universally present band (Table 1). This result suggests the action of either strong selection or rapid genetic drift leading in either case to homozygosity. This homozygosity and the concomitant lack of segregation within the populations suggest further that each esterase band is controlled by genes at separate loci rather than reflecting different homozygous and/or heterozygous genotypes of alleles from fewer loci. This suggestion is strengthened by the observation that almost all of the esterase bands co-occur in one combination or another in the diploid populations (Fig. 1).

Table 1. Gene frequencies for the six esterases based on the assumption that each band (Fig. 1) is homozygous for the strong, moderate, or silent allele (see text). The chi-square values evaluate the hypothesis that the observed phenotypes for the complex as a whole area are in Hardy-Weinberg equilibria.

	Strong allele p =	Moderate allele q =	Silent allele r =	x^2 =	P =
Esterase #1	.89	.01	.10	1181.60	>.001
Esterase #2	.01	.56	.43	495.83	>.001
Esterase #3	.27	.73	.00	478.46	>.001
Esterase #4	.00	.14	.86	592.75	>.001
Esterase #5	1.00	.00	.00	0.00	1.000
Esterase #6	.00	.10	.90	237.67	>.001

The most prominent esterase band, #5, was universally present and always at high intensity. Presumably it is necessary biochemically to the members of the complex, although in what capacity is not known.

The next most prominent esterase band, #3, was always present also, but generally at moderate intensity. The populations with high intensity band #3 occur in widely separated groups in Texas and northern Argentina (Fig. 1).

The third major esterase band, #1, is present in almost all the populations and almost always at high intensity. One exception has moderate intensity alleles instead of high intensity ones. It is the single population belonging to insular *M. glabratus* var. *externus*. The other exceptions lack the #1 esterase band altogether. They are the populations of the large, intrafertile group of aneuploid tetraploids of Chiapas and Guatemala. However, one morphologically indistinguishable, but genetically isolated population (Vickery et al., in press) in the center of that area has the #1 esterase band as do the similar n=31 populations from corresponding habitats in central Mexico. Thus, the variation in occurrence of the #1 esterase band does not suggest adaptiveness but does correspond to cytogenetic population groups.

Of the more variable esterase bands, #2 is common in populations throughout the range of the complex. Despite its absence in the Chiapas-Guatemala group of *M. glabratus* var. *glabratus* (Fig. 1), its distribution does not correlate well with any of the cytogenetic groups. However, the #2 esterase band does tend to occur in small, geographic clusters of populations particularly in *M. andicolus* and *M. glabratus* var. *fremontii* (Fig. 1). The #4 esterase band occurs throughout the complex also. The distribution of its high intensity form tends to be in clusters of populations, for example, in western Mexico and west central Argentina (Fig. 1). The #6 esterase band occurs in only 16 percent of the populations and then only in the low intensity form. Clusters of populations with it occur in the Great Basin and in Northern Argentina (Fig. 1).

Geographic clusters of populations with identical combinations of esterase bands are common (Fig. 1). Some of the characteristic combinations were restricted to one or a few clusters of cytogenetic subgroups but two of them, #1-3-5 and #1-2-3-5, were frequent in most groups and habitats throughout the range of the complex (Fig. 1).

The distribution of neither the distinctive combinations of esterase bands nor of particular individual esterase bands correlated convincingly with latitude or elevation (Hsu 1973). If detailed climatological records were available for the localities of the populations, an adaptive correlation might be found as in *Avena* (Allard 1972). However, latitude and elevation are indicative of significant environmental parameters such as temperature, day length, and length of growing season. Therefore, on the basis of the information available, there appears to be no detectable adaptive advantage to the distribution of the *Mimulus* esterases in contrast to those of *Catostomus*.

The unexpected lack of an adaptive pattern in the distribution of the esterases when taken in combination with their apparent random occurrence in individual populations or clusters of populations, or even larger groups reinforces the suggestion (Vickery 1969) of the action of genetic drift. The range in size of the population groups with the same patterns of esterase bands suggests moreover that each cluster became established at differing times in the past. Probably the founders came from similar populations but occasionally were mutants or recombinants from rare polymorphic populations. Genetic drift soon would have led to homozygosity in most of the typically small populations of the complex. Then, with time and as opportunity afforded, the new populations radiated out in their turn producing the genetic-drift-based evolutionary pattern of distinctive populations and clusters and groups of identical populations that characterize the complex now. However, none of the groups is so large as to include a whole major radiation (Fig. 1). Thus the distribution pattern of the *Mimulus* esterases confirms the predicted role of genetic drift in the complex rather than revealing adaptive values.

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in partial fulfillment of the requirements for the Ph.D. degree.

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