Differential heat-sensitivity of esterase electromorphs in natural polyploid populations of *Urginea maritima* (Liliaceae)

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Summary. The esterase electromorphs of tetra and hexaploid populations of *Urginea maritima* show differences in both heat-sensitivity and geographical distribution. Its possible adaptative significance is discussed.

Different studies have demonstrated that tetraploid plants generally express enzymes of both diploid parents[1]. Furthermore, the polyploid populations can maintain a higher proportion of individuals heterozygous at a gene locus than can the diploid populations[1]. At the molecular level this supposes the appearance in the polyploids of a great deal of enzyme multiplicity, and this provides a reasonable hypothesis to account for the wider distribution of polyploid species relative to the diploid progenitors[2–4]. The basic problem which arises is to study the consequences of the possession of multiple enzymes and demonstrate that it increases the fitness of polyploid individuals relative to diploids[1]. An approach to this problem is to investigate whether the distinct allelic forms of the same enzyme possess different biochemical properties. The biochemical differences among the electromorphs of a particular enzyme may be explored using heat-denaturation studies[5–10].

Urginea maritima (L.) Baker is a polyploid complex that is widely distributed over many different habitats since the individuals of this species inhabit the islands and coasts of the Mediterranean Sea and part of the Atlantic Ocean (Portugal, Morocco, Canary Islands) and, on the other hand, they penetrate the European and African continents through the rivers, having colonized typically continental habitats[11–14]. We report here the results from the analysis of the differential heat-sensitivity of esterase electromorphs of *U. maritima* and their geographical distribution in several natural populations from Iberian Peninsula (hexaploids, 2n = 60), Majorca, and Canary Islands (tetraploids, 2n = 40), in order to study their possible adaptative significance.

Material and techniques. The geographical distribution of different esterase electromorphs has been studied in a total of 144 individuals from 5 island and 4 continental populations of *U. maritima*. The geographical locations of these populations are shown in the table 1. The esterase isozymes studied were present in crude extracts obtained after crushing roots, leaves or flowers of each individual[15]. Horizontal starch gel electrophoresis and enzyme staining were carried out according to the methods described elsewhere[16,17]. For the study of heat-sensitivity of different esterase electromorphs we have mainly followed the methods of Bernstein et al.[5] and Pandey[8].

Results and discussion. 7 electrophoretic bands appear in the gels that can be grouped in 3 esterase activity zones (E-1, E-2, and E-3), each of which has 4,1, and 2 electromorphs respectively. The genetic analysis of these isozymes shows that the zones E-1, E-2, and E-3 are controlled by 3 genetic loci at least, so that each electromorph corresponds to an allelic variant of its respective locus. Gene dosage effects were observed, which can be explained by replication of the respective loci, since the individuals analyzed are tetra or hexaploids[15].

The geographical distribution of different esterase electromorphs and the sample size of continental and island populations are shown in table 1. The different heat-sensitivities of each electromorph are shown in table 2. It can be seen that the electromorph ESt-$1^{0.90}$ is simultaneously the most thermostable of the zone E-1 and the most frequent in continental populations. Conversely, the electromorph Est-$1^{0.80}$ is simultaneously the most thermolabile of this zone and the most frequent in the island populations. So it seems that the continental (hexaploid) populations show a bigger heat-stability, with respect to the zone E-1, than the island (tetraploid) populations. However, a similar relation of this type is not apparent in any of the remaining zones, E-2 and E-3.

The higher frequency of thermostable electromorphs of zone E-1 in the continental populations relative to the island populations could be due, perhaps, to an adaptation in response to the selection pressures of the environmental

Table 1. Geographical distribution of different esterase electromorphs in continental (a) and island (b) populations of *Urginea maritima*. (x) = Very frequent or fixed electromorph; x = Present electromorph; - = Absent electromorph

	Population*	Sample	Electromorphs E-1 1.00	0.95	0.90	0.80	E-2 0.55	E-3 0.27	0.18
a	DT	13	x	x	(x)	-	(x)	-	(x)
	IF	21	-	x	(x)	x	(x)	(x)	-
	PT	23	x	x	(x)	x	(x)	-	(x)
	VB	21	x	x	(x)	-	(x)	-	(x)
b	AD	19	x	-	x	(x)	(x)	x	x
	BI	8	-	-	-	(x)	(x)	-	(x)
	BR	8	-	-	-	(x)	(x)	-	(x)
	CA	20	-	x	x	(x)	(x)	-	(x)
	PE	11	-	-	-	(x)	(x)	-	(x)

* Geographical localities. DT, Durcal-Talara (Granada, Iberian Peninsula); IF, Ifach (Alicante, Iberian Peninsula); PT, Sierra de Cazorla (Jaén, Iberian Peninsula); VB, Vélez-Benaudalla (Granada, Iberian Peninsula); AD, Alcudia (Majorca); BI, Barranco de Igueste de San Andres (Tenerife, Canary Islands); BR, Barranco de Ruiz (Tenerife, Canary Islands); CA, Martianez (Tenerife, Canary Islands); PE, Pinar de la Esperanza (Tenerife, Canary Islands).

Table 2. The different heat-sensitivities of distinct esterase electromorphs of *Urginea maritima*

Treatment times	Electromorphs E-1 1.00	0.95	0.90	0.80	E-2 0.55	E-3 0.27	0.18
	Treatment temperature: 50 °C						
5′	*	*	*	-	*	*	*
10′	*	*	*	-	*	*	*
15′	*	*	*	-	*	*	*
	Treatment temperature: 54.5 °C						
5′	-	-	*	-	*	*	*
10′	-	-	*	-	*	*	*
15′	-	-	*	-	*	-	*
	Treatment temperature: 60 °C						
5′	-	-	-	-	*	-	-
10′	-	-	-	-	*	-	-
15′	-	-	-	-	*	-	-

*, Thermostable; -, Thermolabile.

temperatures of the continent. On this view, for the continental populations, where high temperatures are sometimes reached, the possession of the most thermostable electromorph (Est-$1^{0.90}$) may be advantageous, whereas the presence of more thermolabile electromorphs may be disadvantageous. Alternatively, in the island localities such high temperatures may not be reached and, therefore, the most thermolabile electromorph (Est-$1^{0.80}$) may reach high frequencies without decreasing the adaptation of these populations.

These differences in heat-sensitivity among the electromorphs of the same enzymatic system would have a great importance for the successs of a polyploid complex, such as *U. maritima,* in colonizing very different habitats. Efectively, if, in addition to the enzyme multiplicity characterizing the polypoloids, the distinct allelic forms of the same enzyme have different biochemical properties, as is revealed by their differential heat-sensitivity, the range of environments in which normal development can take place may be significantly extended. This type of mechanism may explain the adaptation of *U. maritima* to very different ecological conditions.

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Similar allozyme polymorphism in honeybees *(Apis mellifera)* from different continents[1]

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Summary. Honeybees *(Apis mellifera)* from Australia are polymorphic for 3 enzymes previously reported polymorphic in honeybees from South America: esterase, malate dehydrogenase and alcohol dehydrogenase. However, no genetically determined polymorphism is detected in the 4th protein system found to be variable in South America.

Many workers have predicted that the haplodiploid sex determining system found in the hymenoptera should result in low levels of genetic variability[3–6]. Although the honeybee is a commonly studied hymenopteran, there are conflicting reports about levels of genetic variability detected by electrophoresis of proteins. One study found no variation in enzymes encoded by 3 loci which have been reported polymorphic in other species[7]. Another study reported 1 locus polymorphic out of 39[8]. Other workers reported 4 enzyme and protein loci polymorphic in honeybees from South America[9–11]. The object of the present investigation was to determine whether the 4 enzyme and protein systems found to be polymorphic in South America were also polymorphic in honeybees from Australia, and whether the loci provided genetic markers for different honeybee races.

Allozyme polymorphism in *Apis mellifera* from Australia

		Malate dehydrogenase			Alcohol dehydrogenase		Esterase	
	Hive	F	M	S	F	S	F	S
Carniolan stock,	1	0.29	0.42	0.29	0.50	0.50		1.00
Meningie, South Australia	2	0.33	0.63	0.04	0.50	0.50		1.00
	3	0.42	–	0.58	0.38	0.62		1.00
	4	0.50	0.12	0.38	0.17	0.83	0.29	0.71
	5	0.67	0.25	0.08	0.75	0.25	0.04	0.96
	6	0.17	0.66	0.17	0.58	0.42		1.00
	7	0.22	0.22	0.56	0.96	0.04	0.08	0.92
	8	0.25	0.50	0.25	0.88	0.12		1.00
	9	0.50	0.08	0.42	0.29	0.71		1.00
Caucasian stock, Glenrowan, Victoria	10	0.04	0.15	0.81	0.86	0.14		1.00
	11	0.36	0.04	0.60	0.68	0.32		1.00
	12*	0.07	–	0.93	0.89	0.11		1.00
Italian stock, Melbourne, Victoria	13	0.25	0.29	0.46	0.96	0.04		1.00
	14	–	–	1.00	0.79	0.21		1.00
	15	0.07	0.14	0.79	0.50	0.50		1.00
	16	0.46	–	0.54	0.50	0.50		1.00
'Feral' swarms,	17	–	1.00	–	0.58	0.42		1.00
Southeastern South Australia	18	0.71	0.29	–	0.65	0.35		1.00
	19	0.38	0.33	0.29	0.67	0.33		1.00
	20	0.10	0.25	0.65	0.71	0.29	0.08	0.92

The racial origin of each stock was determined by the owner of the hives, and the morphology of worker bees appeared consistent with these classifications. * Imported queen, natural-mated in Canada. F, fast; M, medium; S, slow.