ISOZYME POLYMORPHISM IN SPECIES OF THE GENUS *VICIA* (LEGUMINOSAE)

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Thirty nine strains of 24 *Vicia* species representing the main sections of the genus, have been analyzed for their isozyme patterns by disc electrophoresis of leaf and cotyledon extracts. The enzyme systems studied were amylase, esterase, glutamate oxaloacetic transaminase (GOT), indo-phenol oxidase (IPO).

Comparing the zymograms obtained, one can arrive at the following conclusions: There are many bands common to species, series of sections and, in contrast, there are some bands differentiating between certain taxonomic categories. Such characterizing bands and patterns do not feature any clear general phylogenetic trend or systematic correlation. The enzymatic similarity does not necessarily correspond with the taxonomic relationships. GOT show lesser polymorphism, compared with amylase and esterase. The latter systems might be helpful in identifying species or strains.

Species which are polymorphic in many other characters reveal also enzymatic polymorphism. Other possible correlations are briefly discussed, with a special note on the evolutionary implications of such a study in *Vicia*.

INTRODUCTION

The genus *Vicia* contains over 130 species, distributed in the temperate zones of both hemispheres but centered mainly in the Mediterranean region, Western Asia and Western South America. The genus has never been monographed on a world basis. Kupicha (1976) restricted its number of species to about 140; some other consider the number to be even higher. About 10 species are of economic importance as grain or forage crops. The species are grouped into six sections (Plitmann 1970) and as many as 15 series.

The genus is rather diverse in many respects: Habitats, habitus, morphology of the reproductive organs, and karyotypes. Certain species, or groups, feature wide morphological variation as well as tremendous karyotype polymorphism (Hollings and

Stace 1974; Ladizinsky 1978; Zohary and Plitmann 1979). One of the authors (Yamamoto 1973) has studied the karyotypes of several species and re-discussed the relationships between karyotypes and other characters.

Our study of isozyme patterns in *Vicia* has been aimed to clarify or confirm some systematic relationships in the genus. It was likewise of interest to find out correlations between isozyme patterns and certain taxonomic criteria and to estimate their taxonomic value. Such kind of work has been carried out with few other genera, composed of both cultivated and wild species, as Tomatoes (Rick and Fobes 1975) and *Brassica* (Phelan and Vaughan 1976).

Besides, it may have bearings on the general problem of selective versus neutral isoenzymes as presented by Endo (1973), Johnson (1973, 1975), Zouros (1975) and many others.

MATERIALS AND METHODS

Thirty nine strains of 24 *Vicia* taxa, annuals and perennials, representing the four main sections, were used for isozyme analysis of leaves. Unfortunately, we got only very few seeds of the New World species. The species investigated, seed sources and origins are listed in Table 1. The seeds (some of which were actually second generation) were grown in experimental plots at the Faculty of Agriculture of Kagawa University.

Isozyme detection was made by the polyacrylamide disc electrophoretic method (Davis 1964). Staining methods were applied from Brewer (1970). The leaves used for examination were taken from 4th to 7th nodes. This leaf age was suitable for stable isozyme band patterns in this genus as reported previously (Yamamoto 1975).

Leaf extracts were made by grinding a mixture of 2 grams of leaflets with the same weight of 0.05 M Tris-HCl buffer solution (pH=7.54). To each column, 0.1-0.04 ml of sap were applied. Isozyme band patterns of amylase, esterase, glutamate oxaloacetic transaminase (GOT) and indo-phenol oxidase (IPO) were detected in all strains. Substrate and staining dyes used for each enzyme system are detailed in Table 2. α - and β -amylase isozyme bands were determined according to the method of Frydenberg and Nielsen (1965) and Yamamoto (1975).

Cotyledon (seedlings) extracts were used in previous preliminary experiments. Besides the 4 aforementioned enzyme systems, ADH and peroxidase (electrofocusing) were then also examined. Banding patterns in those experiments were more variable and inconstant, less comparable, thus not presented here. The cotyledons rendered generally more bands, some of which were less distinct or did not match the leaflet-bands. The clear cotyledon banding patterns have been independently taken into consideration when discussing the results. Except for a few additional annual strains, additional perennial species of which only cotyledons have been analyzed are: *V. cracca* (No. 327, 2n=28), *V. cassubica* (No. 340, 2n=12), *V. truncatula* (No. 311, 2n=?), *V. sepium* (No. 326, 2n=14), *V. amoena* (No. 334, 335, 2n=24), *V. unijuga* (No. 338, 2n=24).

The study has been carried out during the years 1975-78.

Table 1. Strains of *Vicia* species examined in this study

Section	Series	Species	2n	Seed source	Strain No.
<i>Faba</i>	<i>Fabae</i>	<i>V. bithynia</i> *	14	Lehman, 1966	129
		<i>V. narbonensis</i>	14	Plitmann, 1975	306
		"	14	Plitmann, 1975	307
	<i>Hyranicae</i>	<i>V. hyrcanica</i>	12 (14)	Lehman, 1966	127
		<i>V. esdraelonensis</i>	12	Plitmann, 1975	301
		<i>V. melanops</i>	10	Lehman, 1966	114
		"	10	Lehman, 1966	121
		"	10	Vasileva, 1967	144
	<i>Peregrinae</i>	<i>V. peregrina</i>	12?	Mettin, 1960	29
		"		Kjellqvist, 1969	195
		"	14 (12)	Plitmann, 1975	316
		<i>V. michauxii</i>	14	Lehman, 1966	128
		<i>V. persepolitana</i>	14	Plitmann, 1975	305
	<i>Luteae</i>	<i>V. lutea</i>	14	Mettin, 1960	28
<i>Vicia</i>	<i>Hybridae</i>	<i>V. pannonica</i>	12	Plitmann, 1975	302
		<i>V. hybrida</i>	12	Mettin, 1960	27
		"	12	Vasileva, 1967	156
		"	12	Kjellqvist, 1969	193
	<i>Sativae</i>	<i>V. lathyroides</i>	12	Smith, 1975	330
		<i>V. sativa</i> **	12	Vasileva, 1967	161
		"	12	Vasileva, 1967	165
		"	12	Moore, 1975	332
		"	12	Rousi, 1975	325
		<i>V. amphicarpa</i>	14	Stace, 1974	337
		<i>V. cordata</i> **	10	Cubero, 1974	261
		<i>V. incisa</i>	14	Lehman, 1966	126
<i>Ervum</i>	<i>Erviliae</i>	<i>V. ervilia</i>	14	Kjellqvist, 1969	205
		"	14	Kjellqvist, 1969	207
		<i>V. vicioides</i>	14?	Vasileva, 1967	153
	<i>Hirsutae</i>	<i>V. hirsuta</i>	14	Fac. Agr. Kagawa Univ., 1960	53
<i>Cracca</i>	<i>Villosae</i>	<i>V. villosa</i>	14	Kjellqvist, 1969	194
		"		Kjellqvist, 1969	202
		"	14	Moore, 1975	331
		<i>ssp. dasycarpa</i>	14	Shikoku Agr. Exp. sta., 1960	54
		<i>V. aff. benghalensis</i>	14	Barnard, 1963	93
		"	14	Zertova, 1968	178
		<i>V. tenuifolia</i>	24	Zertova, 1968	98
		<i>V. palaestina</i>	14	Plitmann, 1975	309
	<i>Monanthae</i>	<i>V. monantha</i>	14	Plitmann, 1975	313

* Only a few plants of this strain were obtained.

** The taxa of *V. sativa*-complex should taxonomically be considered as subspecies, though designated here as species. "*V. persepolitana*" is actually a variety of *V. michauxii*.

Table 2. Methods of isozyme detection

Enzymes	Substrate and staining dye		
Amylase	H ₂ O	49 ml	} at 37°C for 1 h
	0.02 M Phosphate buffer pH 5.7	50 ml	
	Soluble starch	1 g	
	I ₂ -KI(0.1% I ₂ in 0.5% KI sol acidified with acetic acid)		
Esterase	H ₂ O	40 ml	} at 37°C for 45 min
	0.12 M Phosphate buffer pH 6.1	60 ml	
	1% α -Naphthyl acetate	2 ml	
	Fast blue RR salt	40 mg	
GOT*	0.1 M Tris-HCl buffer pH 8	100 ml	} at 37°C for 1 h
	Pyridoxal-5-phosphate	5 mg	
	L-aspartic acid	100 mg	
	α -Ketoglutaric acid	100 mg	
	Fast blue BB salt	150 mg	
IPO**	0.03 M Tris-HCl buffer pH 8	99 ml	} at room temp. for 3 h
	1 M MgCl ₂	1 ml	
	Phenazin methosulfate	10 mg	
	MTT tetrazolium	10 mg	

* GOT: Glutamic-oxaloacetic transaminase.

** IPO: Indophenol oxidase.

RESULTS

Zymograms of amylase, esterase, GOT and IPO showing band patterns of the leaf samples examined, are presented in Fig. 1 to 4. The species detected were arranged according to their generally recognized systematic order. Considerable variation in enzyme profiles was observed among strains and species. On the other hand, as expected, no particular differences in band patterns were detected among plants of the same strain when individually analyzed. The specific isozyme patterns are as follows:

Fig. 1. Total amylase isozyme bands detected in leaf samples of *Vicia* species.

Fig. 2. Total esterase isozyme bands detected in leaf samples of *Vicia* species.

Fig. 3. Total GOT isozyme bands detected in leaf samples of *Vicia* species.

Fig. 4. Total IPO isozyme bands detected in leaf samples of *Vicia* species.

1. Amylase band pattern

The total amylase bands detected are presented in Fig. 1.

The occurrence of each band is indicated in Table 3. As shown in the table, a total of six α -amylase and four β -amylase bands were detected in *Vicia* strains. Rf values of α -amylase bands were lower than those of β . A band common to all strains was not observed, but both bands α_4 and β_3 were detected in most strains and series. Yet series *Hyrceanicae* and *Peregrinae* of sect. *Vicia* show a wider polymorphism in band patterns: Only α_2 band was detected in *V. hyrcanica*: α_2 or α_3 and β_1 or β_2 or β_3 were detected in *V. melanops* and *V. esdraelonensis*. The three strains of *V. peregrina* featured one band of α (α_1 or α_2 or α_4) and β_3 band. In *V. michauxii* and *V. persepolitana* of this series both α_4 and β_3 were present. Two bands, α_4 , β_4 and α_4 , β_3 , were observed in *V. lutea* and in *V. hybrida*, respectively. In all strains of series *Sativae*, α_4 was present but some variation was noticed in the β -amylase bands: β_4 was detected in *V. lathyloides*, *V. amphicarpa* and *V. incisa*, whereas β_3 was observed in the other taxa. In addition, α_6 was detected in *V. amphicarpa* and *V. cordata*. In section *Ervum*, the variation in band patterns was not as wide. In *V. ervilia* and *V. vicioides*, the same bands, α_4 and β_3 , were observed except for one strain of the latter, with α_3 instead of α_4 , and *V. hirsuta* with β_4 band instead of β_3 . In series *Villosae* of section *Cracca*, there were in general more bands of both α - and β -amylases compared with other sections. Polymorphism was particularly obvious in *V. villosa*. *V. monantha* has just the α_3 and β_3 bands whereas in *V. palaestina* α -amylase band was absent.

2. Esterase band pattern

The occurrence of esterase bands in *Vicia* species is summarized in Table 4. A total of 17 bands was detected. The number of bands in each strain varied from 4 to 7. Of these, e_{16} band was observed in almost all strains and one or two bands, either e_{12} or e_{13} or e_{14} , were common to all strains. In *V. narbonensis* only 4 bands e_4 , e_7 , e_{14} and e_{16} were observed. In section *Vicia*, either e_6 or e_7 were detected in all strains. Series *Hyrceanicae* and *Peregrinae* show a variation in band pattern similar to that found in amylases. In *V. lutea* only four bands were noticed; 4 or 5 bands were observed in all strains of series *Sativae* in which some polymorphism was also evident. Species

Strain No.	Species	Bands No.									
		α_1	2	3	4	5	6	β_1	2	3	4
129	<i>V. bithynica</i>										
306	<i>V. narbonensis</i>				+					+	
307	"				+					+	
127	<i>V. hyrcanica</i>		+								
301	<i>V. esdraelonensis</i>			+						+	
114	<i>V. melanops</i>			+					+		
121	"		+					+			
144	"			+						+	
29	<i>V. peregrina</i>	+									+
195	"		+								+
316	"				+						+
128	<i>V. michauxii</i>				+						+
305	<i>V. persepolitana</i>				+						+
28	<i>V. lutea</i>				+						+
302	<i>V. pannonica</i>			+						+	
27	<i>V. hybrida</i>				+					+	
156	"				+					+	
193	"				+					+	
330	<i>V. lathyroides</i>				+						+
161	<i>V. sativa</i>				+					+	
165	"				+					+	
332	"				+					+	
325-1	"				+					+	
325-2	"				+					+	
337	<i>V. amphicarpa</i>				+		+				+
261	<i>V. cordata</i>				+		+			+	
126	<i>V. incisa</i>				+						+
205	<i>V. ervilia</i>				+					+	
207	"				+					+	
153-1	<i>V. vicioides</i>			+						+	
153-2	"				+					+	
53	<i>V. hirsuta</i>				+						+
194	<i>V. villosa</i>			+	+				+	+	
202	"			+	+			+		+	
331	"					+		+	+		
54	<i>ssp. dasycarpa</i>				+			+	+		
93	<i>V. aff. benghalensis</i>			+	+			+	+		
178	"			+	+			+	+		
98	<i>V. tenuifolia</i>			+	+			+	+		
309	<i>V. palaestina</i>									+	
313	<i>V. monantha</i>			+						+	

Table 4. Occurrence of esterase isozyme bands in *Vicia* species

Strain No.	Species	Bands No.																
		e ₁	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
129	<i>V. bithynica</i>																	
306	<i>V. narbonensis</i>				+			+							+		+	
307	"				+			+							+		+	
127	<i>V. hircanica</i>		+		+			+			+	+		+				
301	<i>V. esdraelonensis</i>				+		+				+			+		+	+	
114	<i>V. melanops</i>		+		+			+			+				+		+	
121	"		+		+			+			+				+		+	
144	"	+	+		+			+			+				+		+	
29	<i>V. peregrina</i>		+					+				+		+		+		
195	"		+					+				+		+				+
316	"		+					+				+		+		+		
128	<i>V. michauxii</i>		+					+				+		+			+	
305	<i>V. persepolitana</i>				+			+			+		+		+		+	
28	<i>V. lutea</i>				+			+					+				+	
302	<i>V. pannonica</i>				+			+				+		+		+		
27	<i>V. hybrida</i>			+				+				+		+		+		
156	"		+	+				+				+		+			+	
193	"		+	+				+				+		+			+	
330	<i>V. lathyroides</i>				+		+							+			+	
161	<i>V. sativa</i>				+		+						+				+	
165	"		+				+							+			+	
332	"				+		+						+	+			+	
325-1	"				+			+						+			+	
325-2	"				+			+					+				+	
337	<i>V. amphicarpa</i>				+			+						+			+	
261	<i>V. cordata</i>				+		+							+			+	
126	<i>V. incisa</i>				+		+							+			+	
205	<i>V. ervilia</i>				+				+	+				+			+	
207	"				+				+	+				+			+	
153-1	<i>V. vicioides</i>				+				+	+				+			+	
153-2	"				+				+	+				+			+	
53	<i>V. hirsuta</i>				+	+			+	+				+			+	
194	<i>V. villosa</i>		+		+				+	+				+			+	
202	"			+	+				+	+				+			+	
331	"		+		+				+	+					+		+	
54	<i>ssp. dasycarpa</i>			+	+				+	+					+		+	
93	<i>V. aff. benghalensis</i>			+	+				+	+					+		+	
178	"			+	+				+						+		+	
98	<i>V. tenuifolia</i>			+	+				+						+	+	+	
309	<i>V. palaestina</i>					+	+							+		+	+	
313	<i>V. monantha</i>			+	+			+						+			+	

Table 5. Occurrence of GOT isozyme bands in *Vicia* species

Strain No.	Species	Bands No.						
		<i>g</i> ₁	2	3	4	5	6	7
129	<i>V. bithynica</i>							
306	<i>V. narbonensis</i>		+			+		
307	"		+			+		
127	<i>V. hyrcanica</i>		+				+	
301	<i>V. esdraelonensis</i>		+				+	
114	<i>V. melanops</i>		+		+			
121	"		+		+			
144	"		+		+			
29	<i>V. peregrina</i>		+	+				
195	"		+	+				
316	"		+	+				
128	<i>V. michauxii</i>		+	+				
305	<i>V. persepolitana</i>		+	+				
28	<i>V. lutea</i>		+			+		
302	<i>V. pannonica</i>		+			+		
27	<i>V. hybrida</i>		+			+		
156	"		+			+		
193	"		+			+		
330	<i>V. lathyroides</i>		+			+		
161	<i>V. sativa</i>		+			+		
165	"		+			+		
332	"	+				+		
325-1	"		+			+		
325-2	"		+			+		
337	<i>V. amphicarpa</i>		+			+		
261	<i>V. cordata</i>		+			+		
126	<i>V. incisa</i>	+				+		
205	<i>V. ervilia</i>		+					+
207	"		+					+
153-1	<i>V. vicioides</i>		+					+
153-2	"		+					+
53	<i>V. hirsuta</i>		+					+
194	<i>V. villosa</i>		+				+	
202	"		+				+	
331	"		+			+		
54	<i>ssp. dasycarpa</i>		+			+		
93	<i>V. aff. benghalensis</i>		+			+		
178	"		+				+	
98	<i>V. tenuifolia</i>		+				+	
309	<i>V. palaestina</i>		+			+		
313	<i>V. monantha</i>		+			+		

Table 6. Occurrence of IPO isozyme in *Vicia* species

Strain No.	Species	Bands No.								
		i_1	2	3	4	5	6	7	8	9
129	<i>V. bithynica</i>	+	+	+		+		+	+	
306	<i>V. narbonensis</i>	+	+	+		+		+	+	
307	"	+	+	+		+		+	+	
127	<i>V. hyrcanica</i>	+	+	+	+	+		+		+
301	<i>V. esdraelonensis</i>	+	+	+		+		+		+
114	<i>V. melanops</i>	+	+	+	+	+		+	+	
121	"	+	+	+	+	+		+	+	
144	"	+	+	+	+	+		+	+	
29	<i>V. peregrina</i>	+	+	+	+	+		+	+	
195	"	+	+	+	+	+		+	+	
316	"	+	+	+	+	+		+	+	
128	<i>V. michauxii</i>	+	+	+	+	+		+	+	
305	<i>V. persepolitana</i>	+	+	+	+	+		+		+
28	<i>V. lutea</i>	+	+	+		+		+	+	
302	<i>V. pannonica</i>	+	+	+		+		+		+
27	<i>V. hybrida</i>	+	+	+		+		+		+
156	"	+	+	+		+		+		+
193	"	+	+	+		+		+		+
330	<i>V. lathyroides</i>	+	+	+		+		+		
161	<i>V. sativa</i>	+	+	+		+	+	+		
165	"	+	+	+		+	+	+		
332	"	+	+	+		+	+	+		
325-1	"	+	+	+		+	+	+		
325-2	"	+	+	+		+	+	+		
337	<i>V. amphicarpa</i>	+	+	+		+	+	+		
261	<i>V. cordata</i>	+	+	+		+	+	+		
126	<i>V. incisa</i>	+	+	+		+	+	+		
205	<i>V. ervilia</i>	+	+	+		+		+	+	
207	"	+	+	+		+		+	+	
153-1	<i>V. vicioides</i>	+	+	+		+		+	+	
153-2	"	+	+	+		+		+	+	
53	<i>V. hirsuta</i>	+	+	+		+	+	+	+	
194	<i>V. villosa</i>	+	+	+		+		+	+	
202	"	+	+	+		+		+	+	
331	"	+	+	+		+		+	+	
54	<i>ssp. dasycarpa</i>	+	+	+		+		+	+	
93	<i>V. aff. benghalensis</i>	+	+	+		+		+	+	
178	"	+	+	+		+		+	+	
98	<i>V. tenuifolia</i>	+	+	+		+		+	+	
309	<i>V. palaestina</i>	+	+	+		+		+	+	
313	<i>V. monantha</i>	+	+	+		+		+	+	

of section *Ervum* have the same pattern (e_4 , e_8 , e_9 , e_{13} and e_{16}), with e_5 in *V. hirsuta*, additional to this pattern. In section *Cracca* there were averagely more bands and only e_{16} was common to all strains. Difference in band pattern (and number) was observed between the two morphological groups, one of which is *V. villosa* group including ssp. *dasycarpa*, *V. benghalensis* and *V. tenuifolia*, the other represented by *V. palaestina*. In this respect, *V. esdraelonensis* is as similar to the latter species as to members of *Hyrceanicae*.

3. GOT band pattern

The occurrence of GOT isozyme bands is summarized in Table 5. A total of seven bands was detected. In each strain two bands were observed. The g_2 band was detected in almost all strains; however, in two strains of *V. sativa* complex, g_1 band was observed instead of g_2 . Certain taxonomic entities could be characterized by the Rf values of GOT bands other than g_2 . For example, band g_6 was detected in *V. hyrcanica* and *V. esdraelonensis*, band g_4 in *V. melanops*. Strains of series *Peregrinae* have typically g_3 band; *Luteae*, *Hybridae* and *Sativae* have g_5 bands. In section *Ervum*, all strains have g_7 band; in section *Cracca* either g_5 or g_6 bands were detected. The latter section, and to some extent *V. sativa* complex (i. e., including all related taxa) were the more polymorphic.

4. IPO band pattern

The occurrence of IPO bands is summarized in Table 6. A total of nine bands was detected; in each species or strain five to seven bands were observed. Of these, five bands (i_1 , i_2 , i_3 , i_5 and i_7) were common to all strains. Other bands are characteristic to certain taxonomic entities, and might be indicative to either differences or similarities between those. In all strains of series *Fabae*, *Erviliae*, *Hirsutae* and *Villosae*, i_8 band was present, i_9 missing. In series *Hyrceanicae* and the related *Peregrinae*, i_4 band was detected. Band i_6 was observed in all strains of series *Sativae*, except *V. lathyloides*, also in *V. hirsuta* of section *Ervum*. Band i_9 was detected in *V. hyrcanica*, *V. esdraelonensis*, *V. persepolitana*, *V. hybrida* and *V. pannonica* instead of i_8 band. In accordance with the slight difference in Rf values between i_8 and i_9 bands, which substitute one another, it is presumed that both i_8 and i_9 represent two complementary alleles (as is the case of β_3 and β_4 amylase bands).

DISCUSSION

During the last decade many reports on isozyme polymorphism with bearings on taxonomic relationships have been published. Many of these works are dealing with cultivated plants and their wild relatives. Such are studies on rice cultivars (Shahi *et al.* 1969; Nakagawara 1977), *Carthamus* (Efron *et al.* 1973), *Chrysanthemum* (Krisper and Puff 1975), tomatoes (Rick and Fobes 1975), *Brassica* (Denford 1976; Denford and Vaughan 1977), *Citrus* (Esen and Scora 1977) and soybean (Broué *et al.* 1977), not to mention the many studies of this kind on wheat and barley. Most, if not all, add electrophoretic data to the taxonomic evaluation, either as additional diagnostic criteria for identification of species and strains, or in order to confirm or establish presumed systematic

relationships (and refer to Boulter *et al.* 1966; Avise 1974). Electrophoretic studies in *Vicia* have so far been carried out on proteins in general (e. g. Ladizinsky 1975; Perrino *et al.* 1977), or limited to special groups (Yamamoto 1975, 1979; Bassiri and Adams 1978).

From the present study, albeit encompassing only representative species and 4-5 enzyme systems, some indications as to specific taxonomic resemblances or distinctions and systematic affinities could already be drawn. The value of isozyme patterns in the taxonomy and evolution of *Vicia* could also be generally considered.

Certain bands (i_1 , i_2 , i_3 , i_5 , i_7) were practically common to all sections, some others (α_4 , β_3 , e_4 , e_{16} , g_2 , i_8) to most series. Four pairs were complementary bands, usually with very close Rf values: β_3 and β_4 , e_{16} and e_{17} , g_1 and g_2 , i_8 and i_9 . Presence or absence of the remaining bands was sometimes a feature typical to sections, series even species, thus helpful in taxonomic identification. For instance, α_3 and α_4 are together highly frequent in section *Cracca*, but α_3 appears just occasionally in other sections; β_1 and β_2 are quite common in series *Villosae* (characterized also by usually 4 amylase bands), β_3 in the rest. Note that α_2 and α_3 , β_1 , β_2 and β_4 feature genetic polymorphism in most series. No α - or β -amylase bands could be detected in *V. palaestina* and *V. hyrcanica*, respectively. Further, e_4 is typically absent in some species mainly of section *Vicia*, e_7 is most common in series of this section other than *Sativae*, e_8 and e_9 typical of series *Villosae*, both and e_{13} of section *Ervum*. Polymorphism is particularly shown for bands e_2 to e_4 , e_{11} and e_{12} in section *Vicia*, e_{13} to e_{15} in section *Cracca*. The advanced series *Luteae*, *Sativae* and probably *Fabae* are characterized by only 4 (instead of 5-6) esterase bands.

Band g_5 is quite common in section *Cracca*, and certain series of section *Vicia*, but absent in section *Ervum* (characterized by g_7) and the inter-related series *Hyracanicae* and *Peregrinae*. Band i_4 is typical of series *Hyracanicae* and *Peregrinae*. On the other hand, i_8 and i_9 are missing in series *Sativae*.

Some uncommon bands, like α_1 , α_6 , e_1 , i_4 or i_6 might be considered as new additions to the common patterns in series of section *Vicia* (and referred to Endo 1973).

In general, there are common vs. differentiating specific bands, yet their systematic distribution is quite haphazard and there are not overall enzymatic patterns characterizing categories higher than species.

Some systems, however, (like GOT or ADH), which are less polymorphic on intraspecific level, might be useful for taxonomic delimitation of certain species, series or sections, whereas the others (amylase, esterase) might be helpful in defining intraspecific entities.

Association coefficients have been calculated from both leaflet and cotyledon analysis. The overall similarity between strains of same species might range from 0.5 to 0.85. The similarity between species of the same group is high, but sometimes lower. Yet, partial results show that ADH and GOT are more homogenous, with many high coefficients for all species; dissimilarities when indicated are usually on higher taxonomic levels. IPO, α -esterase even amylase are more heterogenic in this sense, hence more useful for specific (or lower categories) delimitation. The resemblances between *Vicia* species, evaluated according to similarities in isozyme patterns are, again, irregular

in distribution, the enzymatic similarity does not necessarily correspond with the systematic affinity except for certain cases.

Thus, series *Hybridae* and *Luteae* considered systematically closely related show a high degree of resemblance in their GOT, amylase and IPO patterns, which is also in accordance with karyotypic similarity reported by Yamamoto (1973). They are also more associated with series *Sativae* than with any other group. Few associations of this kind could also be traced between species of a certain group. Accordingly, the subdivision of series *Villosae* is reconfirmed by amylase and esterase patterns; *V. esdraelonensis* is more related by isozyme resemblance to *V. hyrcanica* than to any other species; *V. michauxii* and *V. persepolitana* are very similar and represent two entities (populations) of a polymorphic taxon.

However, the more diverse are the taxonomic entities—the more isozyme polymorphism and the less similarity are observed.

It is quite clear that in polymorphic species or complexes—there is also more pronounced polymorphism in banding patterns. These results are compatible with the morphological, ecological and karyotypic patterns in those groups (referred to Zohary and Plitmann 1978). It is no wonder, therefore, that differences mainly in amylase and esterase systems, between strains of *V. sativa*, between strains and species of series *Villosae* and *Peregrinae* were more noticeable than in other groups. Those are probably the most variable and polymorphic taxa in the genus. Some of those are colonizers and the uniformity within and variation between populations or races, conform the situation described in *Xanthium* (Moran and Marshall 1978).

On the other hand, section *Ervum*, presumably the more primitive (according to morphology and karyotypes) is quite unique and probably the less polymorphic in isozyme patterns, which corresponds with its relative morphological homogeneity.

No direct correlation with particular morphological characters, such as colour of flower, habitus (the new perennials versus the annuals) and pubescence, was so far noticed. Bands α_3 and e_4 were significantly common in species with distinct peduncles; e_8 and e_9 (typical of section *Ervum* and series *Villosae*) were absent in the nonpeduncled species, concurrently rare in species with $2n=12$. Likewise, i_8 was common in the $2n=14$ species (sections *Cracca* and *Ervum*), rare in the others. Except for the above, no further correlations with chromosome numbers could be traced.

Another aspect of interest is the evolutionary implication of the isozyme variability in *Vicia*. At this stage we cannot point to any definite biochemical trends with phylogenetic significance. Yet, there are clear indications that certain systems are rather stable and uniform (or of very low selection rate)—whereas others are more variable, showing higher polymorphism. It might correspond with their different metabolic functions referred to Johnson (1971 and 1974).

At any rate, enzymatic polymorphism is more expressed in taxa polymorphic also in other characters. These vetches have wide and diverse ecological range and they are commonly selfers with occasional outcrossing. The actual selective value, if any, of this enzymatic polymorphism is still a matter of debate. No doubt, the present-day situation in *Vicia* reflects a complex array of evolutionary processes such as ancient divergence and recent genetic change (either 'neutral' or through direct selection),

together with the influence of biological and environmental factors, differing in different species.

The present study, though preliminary, might serve as a basis for further investigations along various lines: Detailed comparisons between species of the Old and New World in order to find out whether their evolution was parallel, or not; Correlations between habitus (and other morpho-anatomical criteria) and isozyme patterns, which might help in the taxonomy of certain groups, like section *Cracca*. Likewise, correlations between isozyme polymorphism and the breeding system (by which it might be possible to measure the amount of cross-pollination of *Vicia* species, many of which are predominantly selfers), and, even more, between such polymorphism and the ecological situation (particularly to find out the impact of disturbed and secondary habitats); Analysis of other enzyme systems, also of additional species, in order to significantly calculate genetic distances and thus to reach a better understanding of the phylogenetic relationships and evolution in *Vicia*.

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