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Enzyme polymorphism of *Pennisetum americanum* in the Ivory Coast

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ABSTRACT

Polymorphism in populations of pearl millet from the Northern Ivory Coast was studied to determine the variability of three enzymatic proteins, alcohol dehydrogenase, phosphoglucomutase and phosphoglucoseisomerase, coded by three loci. The results show significant linear variation of frequencies associated with an eastwest axis for two of the loci. No significant variability for phosphoglucoseisomerase was found. The analysis of isolated populations allowed us to examine the important effect of genic flow that might explain the appearance of a linear gradient. The adaptation of these enzymes to flooding conditions was also studied, and the environmental variation from east to west might be sufficient to cause the appearance of allelic variations and the resultant adaptation. In this paper both hypotheses are discussed.

1. INTRODUCTION

Allelic polymorphism in pearl millet (*Pennisetum americanum*) was studied by analysis of the electrophoretic patterns of 3 enzymes involved in glycolysis: alcohol dehydrogenase (ADH), phosphoglucomutase (PGM) and phosphoglucoseisomerase (PGI). Three other enzymes: glutamate dehydrogenase, malate dehydrogenase and glutamate-oxaloacetate transaminase, did not show any polymorphism in the area studied.

Pearl millet is a Sahelian cereal, primarily outbreeding, where anemophilous pollination permits an important genic flow, further than 1 km from the point of origin. The southern limit of its cultivation is around 9 degrees north. Further south heavy rainfall at the beginning of the growing season limits growth. In the north of the Ivory Coast the traditional maintenance of these cultivated populations leads to a high level of variability and offers a good opportunity for the analysis of intercultivar polymorphism. There is no human selection; the seeds are taken from stores of completly mixed seeds of the previous crop. Cultivators prefer this technique to the use of seeds

bought in the markets, because the price increases sharply at the sowing period. Usually, people buy seeds only when ecological stress such as drought dramatically reduces the previous harvest. The sowing occurs at the beginning of the rainy season (ie: July and August) and is done on longitudinal mounds one metre apart. Pocket of about 30 seeds are buried every 80 cm. Three weeks later, the plants are thinned. Three to five of the most vigourous plants per pocket are generally kept. The number of seeds sown per hectare is *ca* 4×10^5, after thinning there are *ca* 5×10^4 plants, yielding *ca* 10^8 seeds or 1000 kg.

In some areas, the cultivation of corn and rice tends to replace pearl millet. In these regions, the fields of pearl millet are often smaller and generally more isolated, and the techniques of cultivation are less careful.

2. MATERIALS AND METHODS

Field Sampling

The populations of pearl millet were collected in April 1978 when ORSTOM (Office de la Recherche Scientifique et Technique Outre-Mer) was studying the area to evaluate traditional Ivorian cultivars. The agronomic techniques of each village were investigated, and samples were taken from the January 1978 crop, which had been stored in barns. Samples were chosen to be representative of the intracultivar polymorphism and we consider that a sample of 100–200 grams will reflect the total heterogeneity of the population. Subsamples of seeds were taken for electrophoretic analysis of two allelic forms of enzyme locus. Figure 1 shows where pearl millet is cultivated in the Ivory Coast and the origins of the samples.

Electrophoresis Procedures

Electrophoretic analyses were made on individual dry seeds, which were crushed in 20 μl of Tris-HCl buffer (0.05 M, pH 8.0) containing dithiothreitol (5 mM), and the crude extract was absorbed onto Whatman n°3 paper discs (4 mm in diameter). The electrophoretic techniques and enzymatic assays used were modifications of procedures described by Brewer (1970). The 14% starch gel was buffered with histidine, (pH 6.0, 5 mM) containing NaCl (2.5 mM) and a bridge buffer of citrate (0.41 M, pH 6.0) was used. The samples were included in the ($8 \times 23 \times 0.6$ cm) gel and run for 6 hours at 8.5 V/cm at 4°C, (Second and Trouslot 1980).

The isozymes of ADH were detected on a horizontal slice of the gel using a solution containing nicotinamide adenine dinucleotide (20 mg), nitrobluetetrazolium (20 mg), phenazine methosulfate (2 mg) and ethanol (0.5 ml) in 100 ml of Tris HCl (pH 8.5, 0.005 M). Another slice was examined for PGM and

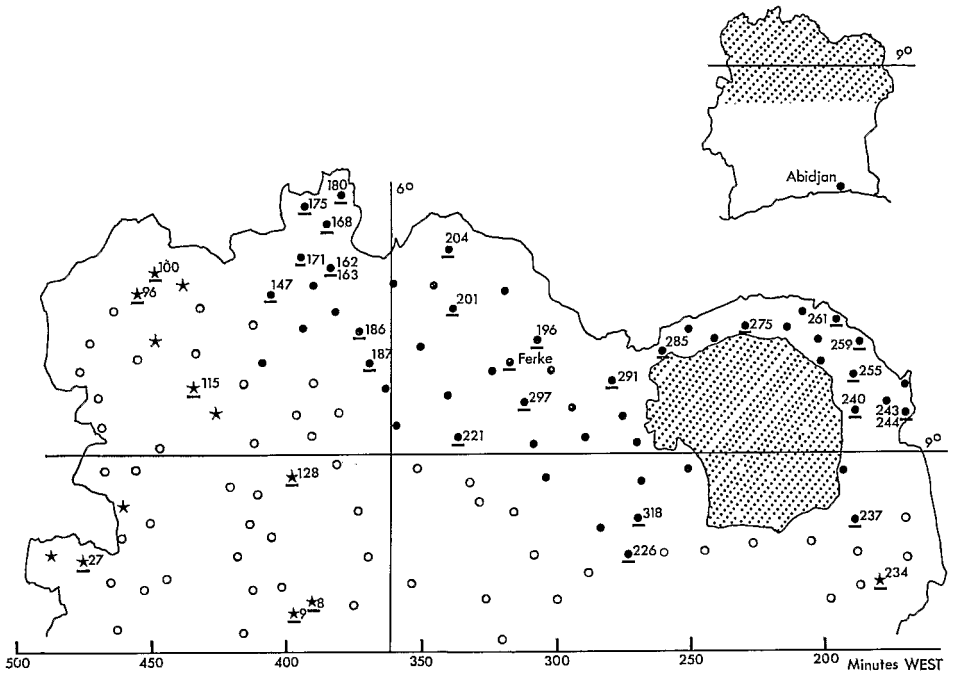

Fig. 1. Localities surveyed for pearl millet in northern Ivory Coast in April 1978. (○–pearl millet not cultivated; ●–sample collected; ●–sample analyzed by electrophoresis; ★–marginal population; the number corresponds to the samples listed in Tables 2 and 3.

PGI in a 2% agar solution containing glucose-1-phosphate (100 mg), fructose-6-phosphate (100 mg), $MgCl_2$ (4 mM), nicotinamide adenine dinucleotide phosphate (10 mg), nitrobluetetrazolium (20 mg), phenazinemethosulfate (2 mg) and glucose-6-phosphate dehydrogenase (15 I.U) in 100 ml of Tris-HCl buffer (pH 8.5, 0.25 M).

3. RESULTS

First, we studied the monogenic inheritance of the three enzymatic systems. The segregrations of the descendants of hybrids for these systems (Table 1) are in good agreement with the hypothesis of control by one gene with two alleles. That enzymes coded by the genes of ADH and PGM are dimeric proteins is indicated by the appearance of a line of intermediate migration on the zymograms of the heterozygotes (Fig. 2).

The ADH of pearl millet seems to be similar to those present in numerous other plants (Schwartz *et al.* 1966; Torres *et al.* 1974; Marshall *et al.* 1974). In dry seed, most of the ADH is coded by 1 locus (ADH_1). The present analysis considers only the two allelic forms (Slow and Fast) coded by this single locus

Table 1. *Segregation of seeds for different allozymes and goodness of fit to a 1:2:1 ratio*

	Number of phenotypes in F_2 seeds			Total	χ^2 1:2:1
ADH-1	47 S S	105 S F	49 F F	201	0.44
PGM	25 R R	37 R L	22 L L	84	1.41
PGI	36 R R	59 R L	23 L L	118	2.86

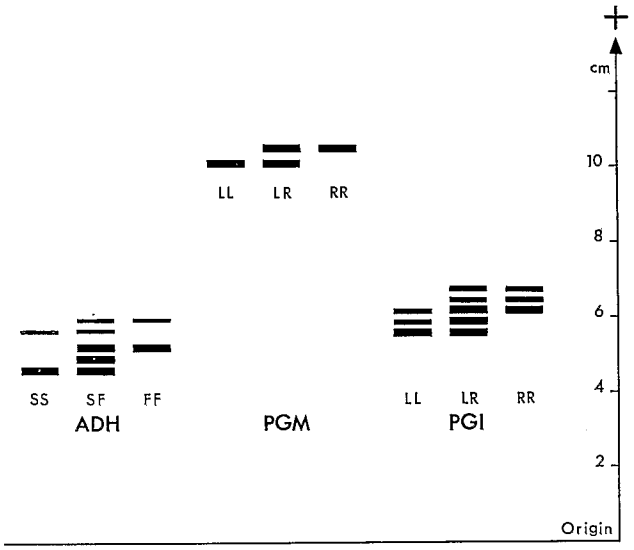

Fig. 2. Zymograms of the three phenotypes analyzed—(ADH: alcoholdehydrogenase, PGM: phosphoglucomutase, PGI: phosphoglucoseisomerase.)

(Leblanc 1978; Banuett-Bourillon *et al.* 1979).

The zymograms of PGI, obtained from dry seeds, are complicated by thin and faster bands which are absent in extracts of young leaves. On the other hand, the electrophoretic patterns of PGM show evidence of only two bands in zymograms of heterozygotes. This is compatible with the fact that PGM is a monomeric protein (Scandalios 1969).

4. ANALYSIS OF POPULATIONS

For this study, 36 populations have been selected out of all the populations collected, more or less at random, except that the average distance between samples does not exceed 50 kms. Samples from 36 sites were investigated for

Table 2. *Populations analyzed and their relation to panmixy. "N" is the size of samples.*

Population	ADH					PGM					PGI				
	S S	S F	F F	N	χ^2	R R	R L	L L	N	χ^2	R R	R L	L L	N	χ^2
8	57	0	0	57	—	0	0	57	57	—					
9	52	6	2	60	—										
27	0	0	60	60	—	0	0	60	60	—	0	0	60	60	—
96	16	20	14	50	1.97										
100	2	26	36	64	1.12										
115	2	18	40	60	0.01	7	23	12	42	0.52					
128	1	7	48	56	0.05	1	7	23	31	—					
147	15	28	17	60	2.26	19	32	14	65	0.01					
162	9	35	22	66	0.71	7	40	24	70	2.75	0	18	50	68	1.58
163	18	29	13	60	0.04	13	32	16	60	0.19	2	7	51	60	5.50
168	16	35	13	64	0.60	11	36	13	60	2.51	4	25	31	60	0.09
171	21	55	44	120	0.28	47	60	20	127	0.01	0	44	80	124	5.76
175	6	30	24	60	0.59	36	8	4	48	5.49					
180	11	36	19	66	0.76	17	24	17	58	1.72	5	29	34	68	0.13
186	12	24	25	61	1.96	16	27	13	56	0.07	3	23	30	56	1.08
187	13	33	28	74	0.37	9	38	15	57	1.61					
196	20	35	17	72	0.05	12	25	21	58	0.79	2	27	40	69	1.10
201	13	28	19	60	0.20	15	20	10	45	0.49					
204	16	35	9	60	1.98	21	23	14	58	2.21	12	34	18	64	0.34
221	12	38	10	60	4.32	16	27	14	57	0.15					
226	16	57	50	123	0.01	15	35	14	64	0.57	3	31	30	64	2.03
234	6	24	30	60	0.14	1	6	48	55	2.17					
240	3	17	47	67	0.79	1	22	45	68	0.85	4	28	36	68	0.25
243	0	6	66	72	0.16	3	24	42	69	0.04	8	31	29	68	0.02
244	6	20	39	65	1.90	3	19	36	58	0.07	1	28	36	65	2.98
255	7	18	35	60	3.22	1	5	32	38	1.80					
259	7	27	35	69	0.27	2	13	51	66	0.96	0	25	44	69	3.41
261	8	19	33	60	3.30	3	17	57	77	1.32					
275	13	25	33	71	3.92	4	30	35	69	0.55	2	27	43	72	0.86
285	13	31	16	60	0.09	6	14	24	44	2.41					
291	12	33	15	60	0.65	4	14	6	24	0.72					
297	12	30	18	60	0.01	13	25	21	59	1.06					
298	22	33	12	67	0.02	15	33	19	67	0.01	5	31	33	69	0.36
Ferké	58	103	39	200	0.31	82	83	35	200	3.25	89	86	25	200	0.35
237	11	17	51	79	14.02										

the allelic frequencies for ADH, 31 samples for PGM, and 17 samples for PGI. The populations from all sites except 237 (which might be a mixture of several populations) are in a Hardy-Weinberg equilibrium, demonstrating that

Table 3. *Allelic frequencies of three allozymes in the different populations studied.*

Populations		ADH		PGM		PGI		Longitude (mm)
		p (S)	q (F)	p (R)	q (L)	p (R)	q (L)	
	147	0.48	0.52	0.54	0.56	—	—	405
	162	0.40	0.60	0.37	0.63	0.13	0.87	384
	163	0.54	0.46	0.48	0.52	0.09	0.91	384
	168	0.51	0.49	0.48	0.52	0.28	0.72	385
	171	0.40	0.60	0.62	0.38	0.17	0.83	395
	175	0.35	0.65	0.83	0.17	—	—	392
	180	0.44	0.56	0.50	0.50	0.29	0.71	377
	186	0.39	0.61	0.53	0.47	0.30	0.70	375
	187	0.40	0.60	0.45	0.55	—	—	369
	196	0.52	0.48	0.42	0.58	0.22	0.78	310
	201	0.45	0.55	0.56	0.44	—	—	336
	204	0.56	0.44	0.56	0.44	0.45	0.55	337
	221	0.52	0.48	0.52	0.48	—	—	344
	226	0.31	0.69	0.51	0.49	0.29	0.71	271
	240	0.17	0.83	0.18	0.82	0.26	0.74	185
	243	0.04	0.96	0.22	0.78	0.35	0.65	165
	244	0.25	0.75	0.22	0.78	0.23	0.77	165
	255	0.27	0.73	0.09	0.91	—	—	186
	259	0.30	0.70	0.13	0.87	0.18	0.82	184
	261	0.29	0.71	0.15	0.85	—	—	189
	275	0.36	0.64	0.28	0.72	0.22	0.78	229
	285	0.47	0.53	0.30	0.70	—	—	259
	291	0.47	0.53	0.46	0.54	—	—	283
	297	0.45	0.55	0.43	0.57	—	—	315
	298	0.57	0.43	0.47	0.53	0.30	0.70	303
	318	0.34	0.66	—	—	—	—	264
	Ferké	0.55	0.45	0.62	0.38	0.34	0.66	315
Marginal populations	8	1.00	0.00	0.00	1.00	—	—	387
	9	0.92	0.08	—	—	—	—	393
	27	0.00	1.00	0.00	1.00	0.00	1.00	468
	96	0.52	0.48	—	—	—	—	456
	100	0.23	0.77	—	—	—	—	453
	115	0.18	0.82	0.44	0.56	—	—	435
	128	0.08	0.92	—	—	—	—	388
	234	0.30	0.70	0.07	0.93	—	—	176

there is no sampling bias (Table 2). Population 237 has been eliminated from the following analysis.

The allelic frequencies calculated for each locus are recorded in Table 3.

Table 4. *Correlation of the three enzymatic loci and the longitude of the Ivory Coast pearl millet populations (marginals excluded). The number of samples studied in each regression is given in the last line.* $**=p<0.01$

	Frequency of ADH (F)	Frequency of PGM (R)	Frequency of PGI (R)
Longitude	−0.656**	0.835**	−0.136
Frequency of ADH (F)		−0.588**	0.011
Frequency of PGM (R)			0.234
No. of populations	27	26	16

Table 5. *Segregation of seed allozymes in selfed* ADH_F/ADH_S-PGM_R/PGM_L *hybrids of pearl millet. The number of observed seeds is given in the upper figures and the number of "expected" seeds in the figures below in parentheses.*

ADH / PGM		Number of seeds S S	S F	F F	Total
Number of seeds	L L	4 (4.38)	10 (9.59)	6 (6.03)	20
	R L	7 (6.58)	16 (14.38)	7 (9.04)	30
	R R	5 (5.04)	9 (11.03)	9 (6.93)	23
Total		16	35	21	73

$\chi^2=1.713$

The populations found in areas where pearl millet culture is disappearing or infrequent are categorized as "marginal populations" and will not be considered them in analyzing regressions in relation to east-west gradients. The matrix of correlations (Table 4) is calculated on the basis of populations sampled in the belt of continuous cultivation of pearl millet, as indicated in Fig. 1. The correlation matrix shows two statistically significant interrelationships. The frequency of ADH(F) is correlated with longitude ($r=-0.6560$, $P<0.001$) and a strong correlation for PGM(R) ($r=0.8354$, $P<0.001$) is also evident. Similar results have been described for these two differentiated loci in *Hordeum spontaneum* (Brown *et al.* 1978). However, the frequency of PGI(R) is not correlated with longitude. Since a significant interrelationship may exist between PGM and ADH, the multiple correlation has been calculated for these two loci and the minutes of longitude. The value of this correlation ($R=0.8681$) adds little to the regression of allelic frequency on longitude,

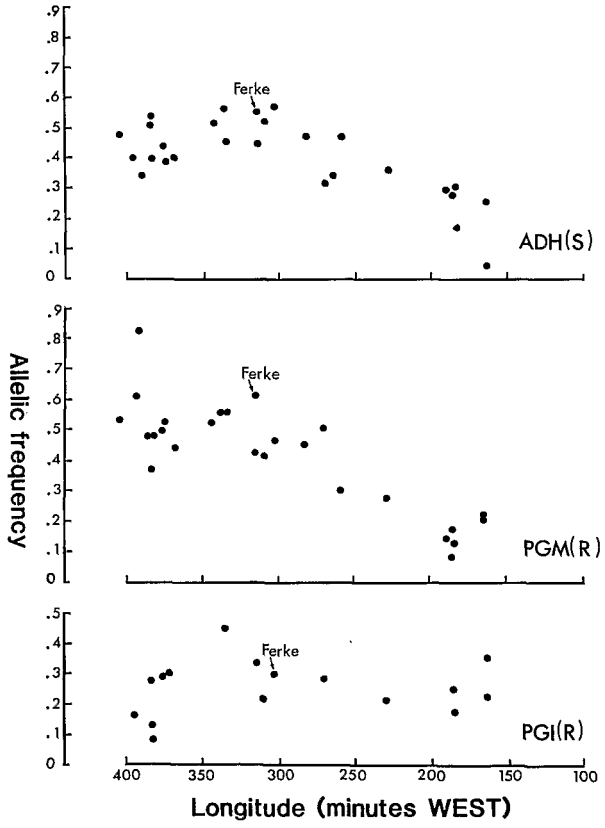

Fig. 3. Allelic frequencies of seed allozymes of pearl millet in relation to longitude.

perhaps because the interloci interrelationship is relatively weak. This correlation cannot be due to a strong genetic link between the two loci, as demonstrated by the analysis of the descendants of a double-hybrid (Table 5). Fig. 3 presents the value of allelic frequencies in relation to the longitude at the site of each sample. We note, particularly for ADH, a flattening of the slope when the sample originates further west than 350 minutes. It is important to associate these values with the northern origin of these samples, in that the influence of the Sahelian climate brings more dryness.

5. DISCUSSION

Each population is described by the allelic frequencies of three allelic systems; we have demonstrated that samples were in accordance with the Hardy-Weinberg equilibrium.

Our results provide evidence for two important points:

1) Most of the isolated populations tend to fix their enzymatic characters. Populations in areas habitually cropped for pearl millet are more polymorphic

than isolated populations. The latter tend to fix an allele independent of longitude. For example populations 8 and 9 fix ADH(S) while population 27 fixes ADH(F). In the last 30 years or so the culture of pearl millet has been progressively abandonned from north to south. For example in 1974, the marginal population 115 was in an area where pearl millet culture was widespread. We observe a stronger tendency towards inbreeding where isolation has lasted longer (compare Table 4 with Fig. 1). In these localities, the number of plants grown is very small (from 10 to 100) and the lack of care shown for this cereal can produce environmental stress. Due to isolation without genic flow, the variations of frequencies are unalterable and little by little genetic variability is lost. This is a confirmation of the results obtained in *Hordeum jubatum* (Shumacker *et al.* 1980) showing an allelic fixation in distinct marginal regions.

2) In the belt of widespread pearl millet culture, there is an east to west orientated cline of allelic frequencies of two genes without any significant genetic linkage. In contrast, the third locus does not present any significant cline in the whole area studied. The observed linear variation of frequency is consistent with the hypothesis of conservation of polymorphism but does not discriminate between the various genetic models. The importance of the zone of efficient pollination and the limited intermarket exchanges of seeds are sufficient to explain the appearance of such a gradient by genic exchanges between two populations with distinct allelic profiles (Kimura and Maruyama, 1971; Christiansen *et al.* 1974). The lack of genic flow will lead to inbreeding in isolated populations. But passive gene flow is not a sufficient explanation of certain phenomena observed in our results. Thinning tends to encourage heterosis but we did not notice that an excess of heterozygotes modified panmictic stability (Brown, 1979). Furthermore, the area of intensive culture around Ferkessedougou is the zone where heterogeneity is maximal. According to the hypothesis of Christiansen (1974), the relative flattening of the frequency-longitude slope would be the sign of the progressive disappearance of two older populations. There is historical evidence of an East-West migration of cultivated pearl millet, associated with human migrations, and our results show a current homogeneity of the polymorphism of African pearl millet.

The linear gradient that we observed can also be explained by adaptative variations along the East-West axis (Clarke, 1975). Along this geographic gradient, there are important climatic variations able to modify the moisture regime of these plants. In the Eastern Ivory Coast, seeds are sown in slopping fields to impede drainage, in sandy soils, and furthermore rainfall is not heavy. The two enzymatic systems showing a linear variation in these different conditions are both included in the glycolytic pathway. This is important because the plants can be in anoxic, flooded conditions before thinning

(Johnson, 1974). Although the role of PGM in flooding conditions is still not known, ADH shows functional variation linked to the allelic type (Schwartz, 1969; Marshall *et al.* 1973; Brown *et al.* 1976). Similar variations have been shown in pearl millet (Leblanc, 1978). We have also demonstrated the presence of a coefficient of gametic selection against ADH(S), maintained at about 0.5 in a survey of the western zone (Leblanc, 1978). These observations are in opposition to previously reported data for bromus and maize (Brown *et al.* 1974; Schwartz, 1969). In the light of the observed segregations, we suggest the existence of a counterbalancing adaptative zygotic advantage for the allelic type S. If this is the case, the lack of variation of PGI, even though it is an enzyme of glycolysis, can be due to a important role of this enzyme in the regulation of flow through the glycolytic pathway, or to a low selection pressure in the observed geographical region.

The analysis of the evolution of the East-West measured gradient should lead to the confirmation of one or other of the major hypotheses. If evolution is due only to the effect of gene flow, the slope of the gradient should decline. If on the other hand the evolution is due to adaptative variation, the gradient should be stable, provided the selective pressures remain constant.

REFERENCES

BANUETT-BOURILLON, F. and HAGUE, D. R. (1979) Genetic analysis of alcohol dehydrogenase isoenzymes in pearl millet (*Pennisetum typhoides*). *Biochemical Genetics* **17**, 537–552.

BREWER, G. J. and SING, C. F. (1970) *An introduction to isoenzymes techniques*. Academic Press. NY.

BROWN, A. D. H., MARSHALL, D. R. and ALBRECHT, L. (1974) The maintenance of alcohol dehydrogenase polymorphism in *Bromus mollis L.* *Aust. J. Biol. Sci.* **27**, 545–559.

BROWN, A. D. H., MARSHALL, D. R. and MUNDAY, J. (1976) Adaptedness of variants at an alcohol dehydrogenase locus in *Bromus mollis L.* (Soft Bromegrass). *Aust. J. Biol. Sci.* **29**, 389–396.

BROWN, A. D. H., NEVO, E., ZOHARY, D. and DAGAN, O. (1978) Genetic variation in natural populations of wild barley (*Hordeum spontaneum*). *Genetica* **49**, 97–108.

BROWN, A. D. H. (1979) Enzyme polymorphism in plant populations. *Theoret. Pop. Biol.* **15**, 1–42.

CHRISTIANSEN, F. B. and FRYDENBERG, O. (1974) Geographical patterns of four polymorphisms in *Zoarces viviparus* as evidence of selection. *Genetics* **77**, 765–770.

CLARKE, B. (1975) The contribution of ecological genetics to evolutionary theory: detecting the direct effects of natural selection on particular polymorphic loci. *Genetics* **79**, 101–103.

JOHNSON, G. B. (1974) Enzyme polymorphism and metabolism. *Science* **184**, 28–37.

KIMURA, M. and MARUYAMA, T. (1971) Pattern of neutral polymorphism in a geographically structured population. *Genet. Res.* **18**, 125–131.

LEBLANC, J. M. (1978) Etudes sur le systeme des alcooldeshydrogenase de mil: *Pennisetum typhoïdeum* (*americanum*), *Thesis*, Paris XI-ORSAY.

MARSHALL, D. R., BROUE, P. and PRYOR, A. J. (1973) Adaptative significance of alcohol dehydrogenase isoenzymes in maïze. *Nat. New Biol.* **244**, 17–18.

MARSHALL, D. R., BROUE, P. and ORAM, R. N. (1974) Genetic control of alcohol dehydrogenase isoenzymes in narrow leafed lupins. *J. Hered.* **65**, 198–203.

SCANDALIOS, J. G. (1969) Genetic control of multiple forms of enzymes in plants: A review. *Biochem. Genet.* **3**, 37–79.

SCHWARTZ, D. and ENDO, T. (1966) Alcohol dehydrogenase polymorphism in maize-Simple and compound loci. *Genetics* **53**, 709–715.

SCHWARTZ, D. (1969) An example of gene fixation resulting from selective advantage in suboptimal conditions. *Am. Nat.* **103**, 479–481.

SECOND, G. and TROUSLOT, P. (1980) Electrophorese d'enzymes de riz (*Oryza* sp.). *Travaux et Documents de l'ORSTOM*. Vol. 120. ORSTOM Paris.

SHUMAKER, K. M. and BABBLE, G. R. (1980) Patterns of allozymic similarity in ecologically central and marginal populations of *Hordeum jubatum* in UTAH. *Evolution* **34**, 110–116.