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Patterns of Genetic Differentiation in Perennial Ryegrass (*Lolium perenne* L.) Populations Sampled from Different Geographical Areas in Europe

W.M.U. Fernando, M.D. Hayward[1] and M.J. Kearsey[2]

Coconut Research Institute
Bandirippuwa Estate
Lunuwila.

ABSTRACT: *Genetic differentiation of perennial ryegrass populations collected in different parts of Europe was studied using isozyme polymorphisms. Allozyme frequencies at seven enzyme loci were determined for twenty Lolium perenne populations. Highly significant differences were detected in the allele frequencies of populations at all loci analysed. A high percentage of loci were polymorphic in the populations ranging from 71 – 100% and the gene diversity varied from 0.234 – 0.410. The presence of GOT – 1 'a' allele was found to be associated with the early flowering nature of the populations. No strict relationship was found between the genetic distance and the geographical distribution of populations. Nei's genetic distance statistics indicated that the German accession BA10998 was clearly distinct from the rest. Principal component analysis of the allozyme frequencies resulted in certain clusters with the accession BA10998 being furthermost from the rest. As indicated by the principal component analyses, ACO – 2 and GOT – 1 loci revealed their importance in differentiating the populations.*

INTRODUCTION

The genus *Lolium* has its origin around the northern and eastern Mediterranean but is now widely distributed throughout the temperate areas of the world. Within the genus a range of life forms may be encountered from the short lived inbreeding annuals such as *L. temulentum* to the long lived outbreeders as *L. rigidium* and *L. multiflorum* or the extreme perennials such as *L. perenne*. These latter

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1 AFRC Institute of Grassland and Environmental Research. Aberystwyth. U.K.

2 University of Birmingham. Birmingham. U.K.

forms are well adapted to outbreeding being anemophilous and possessing a two locus gametophytically determined incompatibility system (Cornish *et. al.*, 1979). *L. perenne* is specifically adapted to survive in association with the grazing animal and the range of phenotypic expression as a response to grazing could vary with the grazing intensity. In addition there is a superimposed wealth of ecotypic adaptation to a wide range of climatic and edaphic conditions.

Extensive collections of *L. perenne* in Europe have now resulted in valuable gene bank material, and studies of genetic variability of these populations are important requisites for their use as successful breeding material in future programmes. In general, most plant species possess large stores of variability in genes specifying isozymes and isozyme analysis has been one of the most appropriate techniques for measuring variation readily and yet as close as possible to the DNA level. By quantifying the range of available genetic diversity within and between populations, one can adjust the collection, evaluation and breeding strategies to obtain the maximum variation from any given population. In this study we have investigated the within and between population variations in 4 isozyme systems revealed by starch gel electrophoresis.

MATERIALS AND METHODS

Twenty populations originally sampled from different parts of Europe were used in the study. The provenance of the populations are presented in Table 1. A maximum of 40 plants raised from seed were used from each population. Young actively growing leaf material from each plant was assayed electrophoretically.

Genetic variation was described by mean number of alleles per locus (A), proportion of polymorphic loci (P) and gene diversity (average heterozygosity). The standard genetic distance and gene diversities were calculated utilizing the methods of Nei (1972, 1973). Heterogeneity X^2 analysis was used to test significant differences between the allele frequencies of populations for individual loci. Principal component analysis was used to ordinate the isozymic differences between populations. The analysis used all alleles as active variables and the weights of the main principal components indicate the contribution of each allele in differentiating the populations.

Table 1. Provenance of populations.

Pop No.	Accession	Geo. region	Country
1	BA10998	W.Europe	Germany
2	BA11015	W.Europe	Germany
3	BA10292	W.Europe	U.K.
4	BA9960	W.Europe	U.K.
5	BA9977	C.Europe	Rumania
6	BA10276	W.Europe	Switzerland
7	BA9983	C.Europe	Rumania
8	BA10286	W.Europe	Switzerland
9	BA11005	W.Europe	Germany
10	BA9968	C.Europe	Rumania
11	BA10990	C.Europe	Austria
12	BA10103	N.Europe	Norway
13	BA10278	W.Europe	Switzerland
14	BA9982	C.Europe	Rumania
15	BA10995	C.Europe	Austria
16	BA11151	W.Europe	U.K.
17	BA9812	W.Europe	U.K.
18	BA10832	W.Europe	U.K.
19	BA10989	C.Europe	Austria
20	BA10992	C.Europe	Austria

Electrophoretic procedure

A horizontal starch gel was employed to separate the electrophoretic variants of the enzyme systems phosphoglucoisomerase (PGI), glutamate oxaloacetate transaminase (GOT), acid phosphate (ACP), and aconitic hydrates (ACO). The electrophoresis was carried out according to Hayward and McAdam (1977) for the systems PGI, GOT and ACP and Wendal and Weeden (1989) for the ACO system with slight modifications. The 3 enzymes PGI. GOT and ACP exist in dineric forms in *Lolium perenne* (Hayward and McAdam, 1977). Although the subunit structure of aconitase enzyme has not been observed in ryegrass

it has been shown by Brouquisse *et al.*, (1987) that higher plant aconitase exist in monomeric form.

RESULTS

Interpretation of banding

PGI: Assays reveal two regions of activity which are believed to be controlled by two loci coding for the subcellular forms cytosolic and plastid (Jones, 1984), but only the slower migrating region (PGI – 2) was clear enough to be scored under the electrophoretic conditions used.

GOT: Three regions of activity were detected *viz.* GOT – 1, GOT – 2 and GOT – 3. According to Hayward and McAdam (1977) these isozymes are under the control of three separate loci.

ACP: The allelic variants observed are reported to be under the control of a single locus (ACP – 1) (Hayward and McAdam, 1977).

ACO: Assays reveal two regions of activity. It was assumed that aconitase exist in monomeric form in perennial ryegrass as no differences were detected in the level of intensity of the banding where double bands were present in either of the areas and the maximum number of bands observed within a single track was 4. Therefore we could assume that this enzyme is monomeric in *Lolium perenne* and that 2 isozymes (ACO – 1 and ACO – 2) are present in the populations under these electrophoretic conditions.

Allele frequencies

The parameters of genetic variability are presented in Table 2. For each population the 95% criterion for polymorphic loci was employed (Gottlieb, 1981). A maximum of 23 alleles were revealed in all populations and population 10 (BA 9968) had the highest number of alleles present in a single population. The average number of alleles per locus ranges from 2.28 to 3.00 with an inter – populational mean of 2.60. A high proportion of polymorphic loci (0.71 to 1.00 with a mean of 0.85) was observed in the populations analyzed. A local polymorphism was observed at the GOT – 1 locus in 4 populations out of the 20

(populations 1, 6, 12 and 13) with the presence of a fast migrating 'a' allele (Table 2). The average gene diversities were calculated according to Nei (1973). The highest and lowest gene diversities were observed in populations 1 and 2. Highly significant differences were detected in the allele frequencies among populations (X^2 prob < 0.001) at all loci analyzed.

Genetic distances

Genetic distances were calculated according to Nei (1972) for paired comparisons of the 20 populations based on the normalised identity of all loci. The mean genetic distances are given in Table 3. The mean genetic distance ranges from 0.282 (population 1) to 0.103 (population 14).

Structure of genetic diversity

The results of the principal component analysis are presented in Table 4 and Figure 1. The first 3 principal components explained 54% of the total variation and the plot of the first 3 components gave certain clusters with population 1 being furthermost from all others. Principal component (PC) 1 which explained the highest variation categorised populations giving higher weights for alleles of ACO – 2 and GOT – 1 loci whereas 'a' and 'b' alleles of PGI – 2 while, GOT – 2 loci revealed their importance on PC 2.

DISCUSSION

The hypotheses of genetic control of variation observed are discussed in the light of the reproductive system, distribution of populations and the place of origin of these populations. In this study high amounts of genetic variation were found in *Lolium perenne* populations as measured by electrophoresis of isoenzymes. Excluding the rare alleles the extent of polymorphism shown by populations were primarily due to differences in allele frequencies rather than the allelic polymorphism itself. This has been shown to be true in outbreeding species, at least for alleles in moderate to high frequencies, according to Gottlieb (1981) a single population has the potential of capturing most

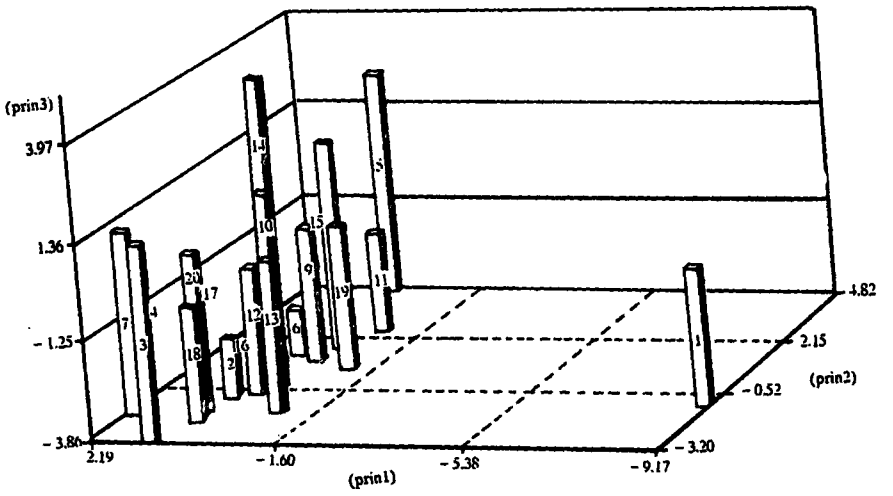

Figure 1. A 3-dimensional representation of population scores for the first three principal components derived from the variance-covariance matrix of allele frequencies of 7 loci of the 20 populations.

of the isozyme variation encountered in the species as a whole. According to the significance levels of X^2 values marked differences occur between populations in their allele frequencies. It is important to note that all populations show the same common alleles for the loci analyzed in spite of their broad eco-geographic range of origin. The estimated gene diversity values (0.234 - 0.410) were in agreement with the diversity values obtained by Hayward and McAdam (1985) for U.K.

Table 2. Genetic summary of the populations. (A = average number of alleles per locus, P = proportion of polymorphic loci, N = number of samples analysed per population.

Pop No.	N	A	P	gene diversity	freq. of 'a' alleles at GOT-1
1	34	2.57	1.00	0.410	0.22
2	34	2.28	0.71	0.234	0.00
3	37	2.71	0.85	0.348	0.00
4	35	2.71	0.85	0.350	0.00
5	35	2.42	0.71	0.365	0.00
6	38	2.57	0.85	0.289	0.02
7	39	2.50	0.85	0.366	0.00
8	37	2.50	0.85	0.339	0.00
9	38	2.70	0.85	0.375	0.00
10	37	3.00	0.85	0.406	0.00
11	36	2.70	0.85	0.323	0.00
12	38	2.57	1.00	0.314	0.03
13	37	2.57	1.00	0.324	0.08
14	37	2.57	0.71	0.335	0.00
15	35	2.42	0.71	0.322	0.00
16	39	2.28	0.71	0.274	0.00
17	38	2.57	0.85	0.318	0.00
18	38	2.57	0.85	0.315	0.00
19	36	2.85	0.85	0.330	0.00
20	38	2.57	0.85	0.369	0.00

Table 3. Mean genetic distance and range (estimated according to Nei (1972).

Population	mean genetic distance	range
1	0.282	(0.208 – 0.353)
2	0.074	(0.013 – 0.295)
3	0.073	(0.020 – 0.293)
4	0.069	(0.016 – 0.335)
5	0.082	(0.020 – 0.323)
6	0.059	(0.011 – 0.294)
7	0.071	(0.020 – 0.353)
8	0.072	(0.025 – 0.245)
9	0.051	(0.009 – 0.219)
10	0.048	(0.009 – 0.251)
11	0.062	(0.011 – 0.268)
12	0.054	(0.013 – 0.235)
13	0.055	(0.018 – 0.253)
14	0.103	(0.053 – 0.336)
15	0.067	(0.020 – 0.313)
16	0.079	(0.030 – 0.308)
17	0.056	(0.014 – 0.266)
18	0.051	(0.017 – 0.267)
19	0.075	(0.017 – 0.208)
20	0.052	(0.016 – 0.306)

Table 4. Percentage variance, weights and eigen values of the first 3 principal components of isozyme data.

Locus	Allele	Prin 1	Prin 2	Prin 3
PGI – 2	a	– 0.147	0.419	0.047
	b	0.221	– 0.379	0.005
	c	– 0.208	– 0.023	– 0.139
	d	0.062	– 0.049	0.086
GOT – 1	a	– 0.397	– 0.087	– 0.031
	b	0.397	0.087	– 0.031
GOT – 2	a	– 0.029	– 0.390	0.116
	b	0.029	0.390	– 0.116
GOT – 3	a	0.183	0.093	0.038
	b	0.045	– 0.260	– 0.334
	c	– 0.128	0.203	0.421
	d	– 0.234	0.124	– 0.051
ACP – 1	a	– 0.092	0.105	– 0.407
	b	0.095	– 0.164	0.246
	c	0.037	– 0.004	0.409
	d	0.032	0.197	0.256
ACO – 1	a	– 0.236	– 0.211	0.132
	b	0.116	0.012	0.152
	c	0.165	0.224	– 0.276
ACO – 2	a	– 0.407	– 0.050	0.005
	b	0.393	0.005	0.021
	c	0.051	0.217	0.077
	d	0.124	– 0.018	– 0.254
Eigen value		5.339	4.04	3.122
% variance		23.21	17.56	12.57

populations (0.372) and for Italian populations (0.424). In parallel with the results of proportion of polymorphic loci and number of alleles/locus obtained in the present study the highest and lowest diversities were obtained in the near-Mediterranean populations while populations from U.K. and Norway showed intermediate values.

An association between the GOT-1 polymorphism and earliness in flowering was observed, in 4 populations out of 20 and population 1, which had the highest `a' allele frequency was the earliest to flower (Fernando, 1992). Introgression of this material with late flowering groups could be advantageous as it provides a marker for screening material if this relationship persists.

The relative genetic distance values (Table 3) show that population 1 (BA 10998) is quite distinct from the other set of populations. But with the exception of population 1 all other populations tend to be similar in their genetic distance values indicating a low interpopulation differentiation.

Principal component analysis identifies the alleles of GOT-1 and ACO-2 loci as the most active variables in discriminating the populations (Table 4). In agreement with the results of the genetic distance values, population 1 showed the widest separation in allelic diversity from the rest of the populations. Although some clustering was observed, none of the clusters consisted of populations exclusively from one geographical region, indicating that the allelic similarity is not restricted to a specific region.

The intensity of population differentiation found in this study for allozyme variants in *L. perenne* has important implications in the optimising of sampling strategies in future expeditions. According to Marshall and Brown (1983) the most efficient sampling strategy would be to concentrate on the most variable populations at the expense of the less variable or monomorphic populations. Furthermore along with the common and widespread alleles, rare alleles (frequencies less than 0.05, Marshall and Brown, 1983) were found at all loci thus satisfying the prime objective of plant exploration which is to collect at least one copy of each of the different variants in a target species (Marshall and Brown, 1975). Moreover population 1 could be used as a putative parent in interpopulation crosses with the other accessions analysed (on the basis

of genetic distance values) as maximum heterosis is expected when the parents are genetically diverse (Humphreys, 1991).

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