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Inheritance and genetic diversity of some enzymes in the sexual and diploid pool of the agamic complex of *Maximae* (*Panicum maximum* Jacq., *P. infestum* Anders. and *P. trichocladum* K.Schum.)

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Summary

The tribe *Maximae* (*Panicum maximum* Jacq., *P. infestum* Anders., *P. trichocladum* K.Schum.) includes two sympatric pools with different modes of reproduction and ploidy levels: an apomictic and tetraploid pool on the one hand, and a smaller, sexual and diploid pool on the other.

In the latter, the sexuality allowed crosses and hence it was possible to gather evidence of the structure (monomeric or dimeric) of nine enzymatic systems and of their inheritance (16 loci). Some segregation distortion was observed in the malatedehydrogenase and esterase systems.

The genetic diversity of the diploid pool is large, in agreement with its mode of reproduction (allogamy and anemophily). The diversity is divided into three groups corresponding to the geographical origin of populations. This organisation is very similar to that observed from morphological traits.

Introduction

Starch-gel electrophoresis is an easy and cheap technique for analyzing enzymatic polymorphism and its use in higher plants has been extensive (Tanksley & Orton, 1983), principally in the search of genetic markers (Hart et al., 1980) and the estimation of genotypic polymorphisms (Marshall & Allard, 1969).

The tribe *Maximae* (*Panicoideae*) includes three botanical species (*Panicum maximum* Jacq., *P. infestum* Anders., *P. trichocladum* K.Schum.), but shows all intermediate types in the wild. In fact, this tribe is an agamic complex with a centre of diversity probably located in Tanzania and in Kenya (Pernès, 1975). Two sympatric pools constitute the complex: an apomictic and tetraploid pool (2n = 32) and a sexual and diploid pool (2n = 16) (Pernès, 1975). The latter pool is small, accounts for less than 7% of the genotypes collected in the centre of diversity[1], and is split into small populations. Despite reciprocal gene flow between the two pools either by spontaneous haploidization or by recurrent tetraploidization (Savidan & Pernès, 1982), the diploid pool shows much less morphological variation than the tetraploid pool (Pernès, 1975). With respect to modes of reproduction, this situation constitutes a paradox whose understanding requires the quantification of gene flow, using enzymatic markers for example. In this case, the study of isozyme inher-

[1] 0% elsewhere.

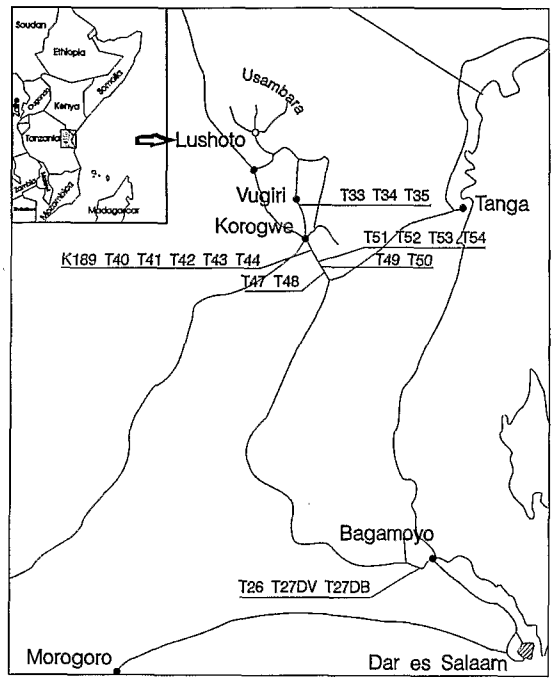

Fig. 1. Map showing collection sites for the diploid accessions (according to Pernès, 1975).

itance and the estimation of enzymatic polymorphism in the two pools constitutes an initial step. For obvious reasons, such a study begins with the sexual and diploid pool.

In this paper, the structure (monomeric or dimeric) and the inheritance of nine enzymatic systems, including sixteen loci, are described. The presence of distortions of segregation and their possible origin are also discussed. Lastly, the marked enzymatic polymorphism and the organization of the sexual pool are emphasized and compared with our knowledge on morphological variation.

Materials and methods

Plant material

Twenty sexual diploids of *Panicum maximum* were collected in Tanzania (Combes, 1975; Pernès, 1975). These perennial clones were maintained as a living collection at the research station of Adiopodoumé (Côte-d'Ivoire) (Fig. 1). Despite their strong self-incompatibility, four of them (T26, T33, T53, T54) gave selfings (61 plants from about 3,000,000 flowers for the T26 accession, for example). Fifteen crosses were also made (Table 1). The progeny size varied from 10 to 73 according to the difficulties encountered during flowering.

Starch electrophoresis

The technique described by Second & Trouslot (1980) for rice was used. Its main characteristics are the following:

- starch gel (14%);
- histidine/citric buffer pH 6.0 or pH 8.0;
- 20 mA for each gel;
- 4° C;
- run for 5 to 6 hours.

The recommended colloquial name (Wendel & Weeden, 1989) and the enzyme commission number were used to describe the enzymes:

- Aspartate aminotransferase (AAT) E.C.2.6.1.1,
- Malate dehydrogenase (MDH) E.C.1.1.1.37,
- Phosphogluconate dehydrogenase (PGD) E.C.1.1.1.44,
- Shikimate dehydrogenase (SKD) E.C.1.1.1.25,
- Glucose-6-phosphate isomerase (GPI) E.C.5.3.1.9,
- Phosphoglucose mutase (PGM) E.C.5.4.2.2,
- Aminopeptidase (AMP) E.C.3.4.11.1,
- Endopeptidase (ENP) E.C.3.4.–.–,
- Esterase (EST) E.C.3.1.1.–.

All enzymes were run in pH 6.0 gels except for PGD and SKD (pH 8.0). The green part of the leav-

Table 1. Size of self-fertilisations and crosses

Cross	Progeny size	Cross	Progeny size
T26 SF	61	T34 · T33	43
T33 SF	15	T34 · T35	13
T53 SF	42	T34 · T49	21
T54 SF	73	T35 · T34	47
T26 · T35	10	T40 · T33	46
T26 · T40	10	T43 · T47	23
T26 · T49	10	T43 · T48	12
T33 · T34	68	T49 · T34	24
T33 · T35	46	T50 · T43	26
T33 · T40	58		

es was used for AAT, GPI, PGM and EST. For all dehydrogenases (MDH, PGD, SKD) and peptidases (AMP, ENP), samples were taken from the white part of young leaves.

Three types of difficulties were encountered during the identification and the interpretation of zymograms:

- for EST and AAT, the fluctuations near the migration front limited our interpretation to the electromorphs located in the lower half of the migration zone;
- for PGD, the existence of two unstable bands was confirmed by running electrophorezing several samples of the same clone on the same starch gel. These two bands were not used in zymogram identification;
- for PGI, the presence of secondary isozymes was recognized from the decreased intensity of bands.

The enzyme structure (monomeric, dimeric, etc.) was inferred from the different zymogram patterns recorded among diploids and their progenies. For example, AAT patterns (Fig. 2a) suggested a dimeric structure.

Genetic parameters of diversity

Four parameters were computed:

- the % P of polymorphic loci;
- the mean number A of alleles per locus;
- the mean genetic diversity $H = \Sigma p_i^2$ (Marshall & Allard, 1970; Nei, 1973);
- the effective number of alleles $A_e = 1/(1-H)$.

Statistical methods

Evidence of inheritance

For each enzyme, a Mendelian assumption concerning its inheritance was expressed by the different segregation observed among progenies. The assumption was tested (χ^2 test for goodness of fit) for each cross, except for some selfings that gave obvious results (100% of the parental type). In order to simplify Table 2, the segregations of phenotypically

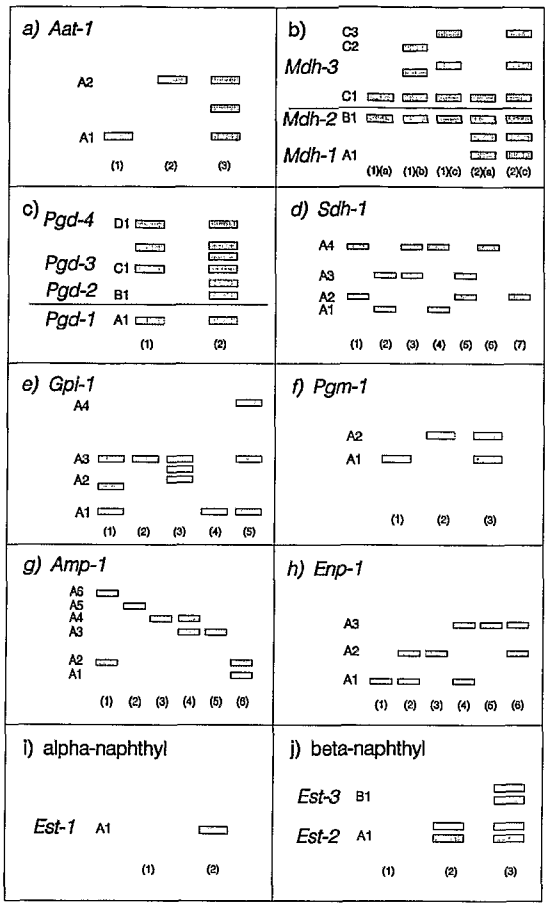

Fig. 2. Diagrams of zymograms.
a) AAT: (1) = phenotype [A1]; (2) = phenotype [A2]; (3) = phenotype [A1A2]
b) MDH thermosensitive: (a) = phenotype [C1]; (b) = phenotype [C1C2]; (c) = phenotype [C1C3]
MDH Non-thermosensitive: (1) = phenotype [A0B1]; (2) = phenotype [A1B1]
c) PGD: (1) = phenotype [A1B0C1D1]; (2) = phenotype [A1B1C1D1]
d) SKD: (1) = phenotype [A2A4]; (2) = phenotype [A1A3]; (3) = phenotype [A3A4]; 4 = phenotype [A1A4]; (5) = phenotype [A2A3]; (6) = phenotype [A4]; (7) = phenotype [A2]
e) GPI: (1) = phenotype [A1A3]; (2) = phenotype [A3]; (3) = phenotype [A2A3]; (4) = phenotype [A1A1]; (5) = phenotype [A1A4]
f) PGM: (1) = phenotype [A1]; (2) = phenotype [A2]; (3) = phenotype [A1A2]
g) AMP: (1) = phenotype [A2A6]; (2) = phenotype [A5]; (3) = phenotype [A4]; (4) = phenotype [A3A4]; (5) = phenotype [A3]; (6) = phenotype [A1A2]
h) ENP: (1) = phenotype [A1]; (2) = phenotype [A1A2]; (3) = phenotype [A2]; (4) = phenotype [A1A3]; (5) = phenotype [A3]; (6) = phenotype [A2A3]
i) α-Naphthyl-EST: (1) = phenotype [A0]; (2) = phenotype [A1]
j) β-Naphthyl-EST: (1) = phenotype [A0B0]; (2) = phenotype [A1B0]; (3) = phenotype [A1B1]

Table 2. Observed segregations for enzymatic systems

Isozymes	Crosses	Results	χ^2 tests
AAT	[A2/A2]·[A2/A2]	61 [A2]	
	[A1/A1]·[A1/A1]	25 [A1]	
	[A1/A1]·[A1/A2]	54 [A1], 46 [A1A2]	0.64 N.S.
	[A1A2]·[A1/A1]	30 [A1A2]	
MDH thermosensitive	[C1C1]·[C1/C1]	61 [C1]	
	[C1/C2]·[C1/C2]	37 [C1], 36 [C1C2], 0 [C2]	37.5 ***
	[C1/C3]·[C1/C3]	56 [C1], 53 [C1C3], 16 [C3]	28.5 ***
	[C1/C3]·[C1/C3]	8 [C1], 15 [C1C3], 12 [C3]	1.63 N.S.
	[C1/C1]·[C1/C3]	2 [C1], 8 [C1C3]	3.60 N.S.
	[C1/C3]·[C1/C1]	2 [C1], 10 [C1C3]	5.30 *
MDH non-thermosensitive	·A1B1/A1B1]·[A1B1/A1B1]	120 [A1B1]	
	[A0B1/A0B1]·[A1B1/A1B1]	10 [A1B1]	
	[A0B1/A0B1]·[A0B1/A1B1]	6 [A1B1] and 6 [A0B1]	0.00 N.S.
	[A1B1/A0B1]·[A0B1/A0B1]	15 [A1B1], 11 [A0B1]	0.62 N.S.
SKD	[A2A3]·[A2A3]	7 [A2], 13 [A2A3], 4 [A3]	0.92 N.S.
PGD	[B0C1D1/B0C1D1]·[B0C1D1/B0C1D1]	76 [B0C1D1]	
	[B1C1D1/B0C1D1·[B1C1D1/B1C1D1]	14 [B0C1D1], 28 [B1C1D1]	1.56 N.S.
GPI	[A3/A3]·[A3/A3]	61 [A3]	
	[A3/A3]·[A1/A1]	58 [A1A3]	
	[A1/A4]·[A3/A4]	18 [A3A4], 25 [A1A3]	1.13 N.S.
	[A2/A3]·[A1/A4]	11 [A1A3], 8 [A3A4], 8 [A2A4], 9 [A1A2]	0.66 N.S.
PGM	[A2/A2]·[A2/A2]	40 [A2]	
	[A1A1]·[A1/A1]	24 [A1]	
	[A1A2]·[A1/A2]	5 [A1], 7 [A2], 13 [A1A2]	0.20 N.S.
AMP	[A1/A2]·[A1/A2]	15 [A1], 13 [A2], 31 [A1A2]	0.23 N.S.
	·A5/A5]·[A5/A5]	25 [A5]	
	[A4/A4]·[A4/A4]	24 [A4]	
ENP	[A2/A3]·[A2/A3]	12 [A2], 11 [A3], 25 [A2A3]	0.125 N.S.
	[A3/A3]·[A3/A3]	18 [A3]	
α EST	[A0/A0]·[A0/A0]	121 [A0]	
	[A0/A0]·[A1/A0]	19 [A0], 21 [A1]	0.10 N.S.
	[A1/A0]·[A0/A0]	38 [A0], 8 [A1]	19.6 ***
	[A1/A0]·[A0/A0]	9 [A0], 15 [A1]	1.5 N.S.
β EST	[A1B0/A1B0]·[A1B0/A1B0]	85 [A1B0]	
	[A0B0/A0B0]·[A1B0/A0B0]	11]A0B0], 31 [A1B0]	9.52 **
	[A0B0/A0B0]·[A1B0/A0B0]	10 [A0B0], 3 [A1B0]	3.77 N.S.
	[A1B0/A0B0]·[A0B0/A0B0]	26 [A0B0], 11 [A1B0]	6.08 *
	[A1B0/A0B0]·[A1B0/A0B0]	5 [A0B0], 41 [A1B0]	4.89 *
	[A0B0/A0B0]·[A1B1/A1B0]	11 [A0B0], 7 [A1B0], 3 [A0B1]	13.10 **
	[A1B1/A1B0]·[A0B0/A0B0]	10 [A0B0], 8 [A1B0], 6 [A0B1]	19.6 ***

The different assumptions are:

- AAT. Dimeric enzyme; one locus *Aat-1* (2 alleles A1 and A2).
- MDH thermosensitive. Dimeric enzyme; one locus *Mdh-3* (3 alleles C1, C2 and C3).
- MDH non-thermosensitive. Dimeric enzyme; two loci *Mdh-1* (2 alleles A1 and A0) and *Mdh-2* (1 allele B1).
- SKD. Monomeric enzyme, one locus *Skd-1* (4 alleles A1, A2, A3 and A4).
- PGD. Dimeric enzyme; three loci *Pgd-2, Pgd-3* and *Pgd-4* (2, 1 and 1 alleles respectively).
- GPI. Dimeric enzyme; one locus *Gpi-1* (4 alleles A1, A2, A3 and A4).
- PGM. Monomeric enzyme; one locus *Pgm-1* (2 alleles A1 and A2).
- AMP. Monomeric enzyme; one locus *Amp-1* (6 alleles A1 to A6).
- ENP. Monomeric enzyme; one locus *Enp-1* (3 alleles A1, A2 and A3).
- α-naphthyl EST. Monomeric enzyme; one locus *Est-1* (2 alleles of which one nil A0).
- β-naphthyl EST. Monomeric enzymes; each being coded for by one locus *Est-2* and *Est-3* with 2 alleles, of which a nil.

identical crosses were pooled when they were similar by the χ^2 test.

Analysis of the organisation of genetic diversity

To study the structure of the genetic diversity of the diploid pool, we used hierarchical clustering (method of the reciprocal neighbour) (Juan, 1982) and metric χ^2 (Benzécri, 1973), which is especially adapted to qualitative data. The distance $d(i,i')$ between two individuals i and i' is then calculated as follows:

$$d(i,i') = \sum_{j=1}^{p} \frac{1}{f_j} \left(\frac{f_{ij}}{f_{i.}} - \frac{F_{i'j}}{f_{i'.}} \right)^2,$$

where $f_{ij} = 1$ if individuals i have the allele j, else $f_{ij} = 0$ (presence/absence). Note that p is the total number of alleles, that $f_{i.}$ (weight of individual i) and $f_{i'.}$ (weight of individual i') are numbers of alleles of individuals i and i' respectively, and that $f_{.j}$ (weight of allele j) is the number of individuals that have the allele j. When two individuals (or classes) i and i' were detected as being the nearest ones, they were included in a new class q according to the criterion of variance, also called Ward's critierion (1963).

Correspondence analysis (Benzécri, 1973), similar to principal component analysis, is especially adapted to qualitative data tables. It differs in the use of the χ^2 distance (cf. above) instead of the Euclidian distance. Like P.C.A., this method indicates:

- how many independent factors explain the diversity;
- what part of diversity is explained;
- the meaning of factors (the interpretation of factors, from eigenvectors, was one of our main aims).

In these two types of analysis, the alleles whose frequency was less than or equal to 5% were considered as rare and were not taken into account.

Results

Structure of enzymes

Two types of enzyme structure were inferred from the different zymograms encountered in the diploids (Fig. 2) and their progenies:

- monomeric: SKD, PGM, AMP, ENP and EST;
- dimeric: AAT, MDH, PGD and PGI.

Inheritance

Table 2 recapitulates results concerning enzyme inheritance. Inheritance was particularly simple for some enzyme systems: one isozyme – one locus – two alleles. This was the case for AAT (one locus *Aat-1* with two alleles A1 and A2) and PGM (one locus *Pgm-1* with two alleles A1 and A2). Other enzyme systems were also represented by one isozyme and one locus, but the number of alleles increased: ENP (one locus *Enp-1* with three alleles A1, A2 and A3), SKD (one locus *Skd-1* with four alleles A1, . . ., A4), GPI (one locus *Gpi-1* with four alleles A1, . . ., A4) and AMP (one locus *Amp-1* with six alleles A1, . . ., A6). Lastly, other enzyme systems (MDH, PGD and EST) showed the presence of several isozymes, some of them being coded by several loci.

For MDH, a thermosensitivity test at 60° C defined two zones corresponding to two different isozymes (absence of heterodimers):

- an insensitive zone (the slowest bands), where the isozyme was coded by two loci (*Mdh-1* and *Mdh-2*) with two alleles ($A0^2$ and A1) and one allele (B1), respectively.
- a sensitive zone, where the isozyme was coded by one locus *Mdh-3* with three alleles C1, C2 and C3.

For PGD, the absence of heterodimers between the bands A1 and B1 (Fig. 2c) suggested the presence of two isozymes:

- the first isozyme (coded by the locus *Pgd-1*) showed no allelic variation;
- by contrast, the second was coded by three loci *Pgd-2* (two alleles $B0^3$ and B1, *Pgd-3* (one allele C1) and *Pgd-4* (one allele D1).

Lastly, two types of esterases could be distinguished according to band colour, brown or red, corres-

[2] A0 is either a null allele or an allele of which the electromorph is indistinguishable from the band B1.

[3] B0 is either a null allele or an allele of which the electromorph is undistinguishalbe from one of the other bands.

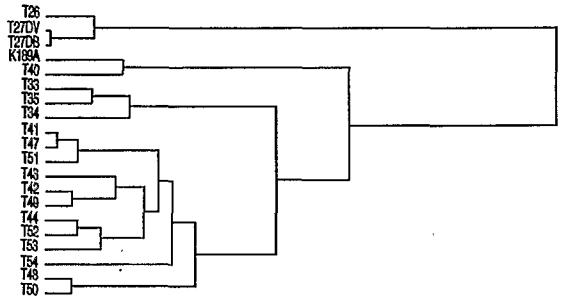

Fig. 3. Clustering of the 20 diploid accessions.

ponding to the decomposition of the α-naphthyl-acetate or the β-naphthyl-acetate, respectively. For the α-napththyl-esterase, only one isozyme was emphasized, coded by one locus *Est-1* with two alleles, one of which was null. For the β-naphthyl-esterases, which were characterized by double-bands, two isozymes, each coded by one locus (*Est-2* and *Est-3*) with null alleles, were observed.

Segregation distortion

Most studied enzyme systems did not show distortion of segregation. This was so for AAT, PGD, SKD, PGI, PGM, AMP and ENP (Table 2). By contrast, the thermosensitive isozyme of MDH and the esterases presented some more or less large distortions.

The locus *Mdh-3* exhibited two segregation distortions (Table 2). The first, peculiar to the clone T33, led to a lower frequency of the allele C3, of which the selective disadvantages existed in both the homozygous and heterozygous forms (genotypes C3C3 and C3C1). The second concerned the allele C2 with the complete absence of the genotype C2C2 (segregation 37:36:0) in the selfings of the heterozygous clone T54.

The two types of esterases presented some segregation distortions. For the α-naphthyl-esterase (locus *Est-1*), the distortion concerned the cross T40 · T33 ($\chi^2 = 19.56$). For the β-naphthyl-esterases, it was observed in the majority of crosses and varied both in direction and size depending on the parents. Nevertheless, the comparison between the cross T34 · T49 and its reciprocal one emphasized the absence of maternal effects, allowing the pooling of the results. The average segregation was then 30 [A0], 15 [A1] for the locus *Est-2,* and 36 [B0], 9 [B1] for the locus *Est-3.* A test of independence between the two loci was performed taking these distortions into account. The expected frequencies of phenotypes were 23.76 [A0B0], 11.88 [A1B0], 5.94 [A0B1] and 2.97 [A1B1]. They fitted well with the observed values 21 [A0B0], 15 [A1B0], 9 [A0B1] and 0 [A1B1] ($\chi^2 = 5.68$; $P > 0.05$).

Genetic diversity

Estimation of genetic diversity of the diploid pool

The percentage P of polymorphic loci and the number A of alleles per locus were estimated from all 16 loci. The two other parameters (mean genetic diversity H and effective number of alleles A_e) were calculated without the loci *Est-1, Est-2, Est-3* (several genotypes are unknown due to the presence of null alleles).

The four parameters showed a high enzymatic polymorphism in the diploid pool:

- % P of polymorphic loci = 75.0%;
- number A of alleles per locus = 2.38;
- mean genetic diversity H = 0.284;
- effective number of alleles $A_e = 1.40$.

Nevertheless, the values of the percentage of polymorphic loci and of the number of alleles per locus were strongly influenced by the presence of the rare alleles, as the corrected values show:

- % P of polymorphic loci = 62.5%;
- number A of alleles per locus = 1.94.

By contrast, the estimations of the mean genetic diversity, but especially of the effective number of alleles, were little modified:

- mean genetic diversity H = 0.272;
- effective number of alleles $A_e = 1.37$.

Organisation of genetic diversity

Two major groups corresponding to the two main geographical zones with diploids were emphasized by cluster analysis:

- Bagamoyo, with the clones T26, T27DV and T27DB;
- and Korogwe for the others (Fig. 3).

Table 3. Intralocus genetic diversity (H) in three nested populations

Locus	All 20 diploids	Korogwe *s.l.*	Korogwe *s.s.*
Aat-1	0.2888	0.0571	0
Mdh-1	0.3488	0.3893	0.3299
Mdh-2	0	0	0
Mdh-3	0.22375	0.25779	0.15625
Pgd-1	0	0	0
Pgd-2	0.0488	0.0571	0.0799
Pgd-3	0	0	0
Pgd-4	0	0	0
Skd-1	0.7488	0.7457	0.6736
Gpi-1	0.5238	0.5796	0.4965
Pgm-1	0.4200	0.4568	0.4688
Amp-1	0.5888	0.4491	0.4479
Enp-1	0.5050	0.4240	0.2257

The latter group was itself structured into three differentiated sub-groups:

- clones T33, T34, T35 from the population of Vugiri located 30 km N of Korogwe;
- clones T40 and K189 from the south of Korogwe;
- clones T41, T42, T44, T47, T48, T49, T50, T51. T52, T53, T54 also from the south of Korogwe (Fig. 3).

The first three factors of the correspondence analysis represent, in the statistical sense, 60% of the enzymatic variation and explain well the above structure. The first factor concerned three loci *Aat-1, Enp-1* and *Amp-1,* and contrasted the Bagamoyo clones and the others. The second factor affected four loci *Enp-1, Gpi-1, Amp-1* and *Skd-1* and allowed clones T40 and K189 to be distinguished from the others. Lastly, the third characterized the population of Vugiri (T33, T34 and T35) for four loci *Amp-1, Skd-1, Mdh-1* and *Mdh-3.*

Population differentiation and intra-locus genetic diversity

Owing to the small size of the groups (Bagamoyo, Vugiri, Korogwe), classical Nei's statistics (1973) were not used. Nevertheless, for studying the between- and within-population variation for each locus, three nested populations corresponding to smaller and smaller geographical distributions were defined:

- the first (pop. A) was constituted by all 20 diploids;
- the second (pop. B) was obtained by removing Bagamoyo clones from population A;
- the third (pop. C) by removing the population of Vugiri and the clones T40 and K189A from population B.

Table 3 shows changes in the index of intra-locus diversity as populations became geographically smaller and smaller (from pop. A to pop. C). The magnitude of these changes depended on the locus:

- the loci *Mdh-1, Pgd-2, Gpi-1, Skd-1* and *Pgm-1* had approximately the same diversity whatever the size of the geographical distribution;
- for the other studied loci, diversity decreased with the size of the geographical distribution, especially for *Aat-1* when Bagamoyo clones were removed. This locus was also the one that showed a significant deficit of heterozygotes ($\chi^2 = 3.94$).

Discussion

Structure of enzymes and distortions of segregations

Results for the nine enzymatic systems are summarized in Table 4. The nature of enzymes (dimeric or

Table 4. Principal traits of the enzymatic loci

Enzymatic system	Protein nature	Locus	Allele number	Distortion
AAT	Dimer	*Aat-1*	2	No
MDH	Dimer	*Mdh-1*	2	No
		Mdh-2	1	No
		Mdh-3	3	Yes
PGD	Dimer	*Pgd-1*	1	No
		Pgd-2	1	No
		Pgd-3	1	No
		Pgd-4	2	No
SKD	Monomer	*Skd-1*	4	No
GPI	Dimer	*Gpi-1*	4	No
PGM	Monomer	*Pgm-1*	2	No
AMP	Monomer	*Amp-1*	6	No
ENP	Monomer	*Enp-1*	3	No
EST	Monomer	*Est-1*	2	Yes
		Est-2	2	Yes
		Est-3	2	Yes

monomeric), the number of loci, the number of alleles, but also the presence or absence of distortions of segregations, are listed. Three points will now be discussed: 1) the similarities between our results on enzyme structure and those of the literature; 2) the presence of distortion of segregation for the *Mdh* loci; and 3) the presence of distortion of segregation for the *Est* loci.

The isozymes structure that we emphasized in *P. maximum* corresponded well to those reviewed by Weeden & Wendel (1989). Thus, the dimeric structure of AAT, MDH, GPI and PGD, and the monomeric structure of PGM, EST, AMP, ENP and SKD, were observed in *Oryza sativa* (Second & Trouslot, 1980; Glazmann et al., 1988). These enzymatic structures were also recorded by other authors (Kahler & Allard, 1981; Benito, 1983; Price & Kahler, 1983; de Cherisey et al., 1985) in other monocotyledons (*Triticum, Hordeum, Avena, Setaria*). The presence of a thermosensitive MDH with fast electromorphs and of EST with null alleles constitutes another similarity with *O. sativa* (Second & Trouslot, 1980).

The locus *Mdh-3* with three alleles C1, C2, C3 showed strong distortions of segregation with parental effects. Alleles C2 and C3 were highly counterselected, even at the heterozygous level. A linkage with genes for self-incompatibility could explain the differences between crosses. However, a negative relationship, which was recorded between the vigour and the heterozygosity of this locus in a tetraploid progeny 'sexual × apomict' (Gnagne, unpublished), would suggest post-zygotic selection. The maintenance of such alleles is possible only if their presence is favoured under other conditions. All these facts suggest cytoplasmic interaction.

All esterase loci also showed distortions of segregation with parental effects. By contrast to *Mdh-3,* distortions could be pre-zygotic. A linkage with genes of self-incompatibility is an assumption which could explain these results (Cornish et al., 1980; Wendel & Parks, 1984; Manganaris & Alston, 1987) and should be studied.

Isozyme polymorphism: richness and structure

Papers on the isozyme polymorphism of *Panicum maximum* are scarce. Usberti & Jain (1978) compared sexual and asexual populations, but the mode of reproduction (true sexuality or hypersexual apomixis) and the ploidy level (diploid or tetraploid) of their three highly sexual lines are unknown. In addition, these lines were not wild accessions.

Two main results were highlighted by our study: 1) the large degree of enzymatic polymorphism of the diploid pool; 2) the organisation of the pool revealed by the enzymatic polymorphism.

The isozyme polymorphism of the diploid pool is high with 75% polymorphic loci and 2.37 alleles per locus of which 19% are rare. These values are in agreement with the allogamous and anemophilous mode of reproduction of this pool (Hamrick & Godt, 1989) and are known in other self-incompatible Gramineae such as *Pennisetum glaucum* (Tostain et al., 1987).

The isozymic variation is principally structured in two geographically distant populations. This is particularly true for the locus Aat-1 which differentiates the populations of Bagamoyo and Korogwe. For this locus, frequencies of the alleles A1 and A2 in each of these two populations are almost inverted. According to the principle of Wahlund, this organisation leads to a deficit of heterozygotes (Lucotte, 1983). Although of minor importance and concerning other loci, a geographical differentiation of allele frequencies also exists within the diploid zone of Korogwe. A particular feature of this organisation of enzymatic polymorphism is that it corresponds to the one described by Pernès (1975) using morphological traits.

Our next step will be to study the relations between the structures of the two pools and to compare their enzymatic polymorphism. Indeed, two arguments favour the existence of gene flow between the two pools: 1) in the diploid zone, sexual plants are closely mixed to the tetraploid apomicts (Combes, 1975; Pernès, 1975) and pollen from the two pools fall on each diploid stigma; 2) the presence of spontaneous haploidisation and of recurrent tetraploidisation has been demonstrated (Savidan & Pernès, 1982). This study should also improve

our understanding of the respective roles of the modes of reproduction on the polymorphism of this agamic complex.

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