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Isozyme polymorphism and organization of the agamic complex of the *Maximae* (*Panicum maximum* Jacq., *P. infestum* Anders, and *P. trichocladum* K. Schum.) in Tanzania

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Abstract 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. From an analysis of isozyme polymorphism five main results were evident. First, overall polymorphism is considerable showing that apomixis does not lead to a reduction in diversity. Second, the isozyme polymorphism of the two pools is similar, and this may be explained by reciprocal gene flow (low, but continuous) between these two pools. Third, maximum local polymorphism is due to the simultaneous presence of *P. maximum* sexuals and *P. infestum* apomicts. A continuum exists between the two species. Fourth, a high proportion of rare alleles, arising from introgression from *P. infestum*, characterized the isozyme polymorphism. These rare alleles, strongly counter-selected at the diploid level, are maintained by apomixis; the frequency of triplex or quadruplex genotypes was nevertheless low. Fifth, the heterozygosity level within apomicts is not higher than that of sexuals, showing that the apomixis-polyploidy combination does not lead to a higher frequency of very heterozygous individuals.

Key words *Panicum maximum* · Isozyme polymorphism · Gene flow · Sexuality · Apomixis

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Introduction

The evolutionary potentialities of agamic plants have long been considered to be low owing to the absence of sexuality (Darlington 1939; Stebbins 1941; see Marshall and Brown 1981). Nevertheless, as early as 1954, Clausen noticed that apomixis is more often than not facultative[1], and attributes important evolutionary possibilities to the residual sexuality. This is the "T-model hypothesis" where sexuality yields a new well-adapted genotype, while apomixis allows its multiplication. More recently, studies on agamic complexes showed the presence in each of an apomictic pool, a sexual pool, with gene flow between the two pools (de Wet 1968; Savidan and Pernès 1982).

The agamic complex of the *Maximae* (*Panicoideae*), which includes three botanical species (*Panicum maximum* Jacq., *P. infestum* Anders., *P. trichocladum* K. Schum.), has its centre of diversity in Tanzania and in Kenya (Pernes 1975). The apomictic pool (93% of genotypes in the centre of diversity, and 100% elsewhere) is distributed throughout the tropical world. This pool includes the three botanical species as well as intermediate types, the C types, morphologically hybrid between *P. maximum* and *P. infestum* (Combes 1975; Pernès 1975). Apomicts are always polyploid, especially tetraploid (2n = 32). The sexual pool is split into small populations present in some areas of Tanzania. In these populations, sexuals (2n = 16) are still mixed with apomicts. Sexuals belong to *P. maximum*, and in particular to the large, erect morphotype with broad leaves.

The high isozyme polymorphism of diploids, evaluated from nine enzymatic systems and including 16 loci (Assienan et al. 1993), corresponds to that given in the literature for allogamous and anemophilous plants (Hamrick and Godt 1989). Three groups were identified, in relation to their geographical origin, and were similar

[1] Some embryo sacs are of meiotic origin

to the groups observed by Pernès (1975) from morphological traits (Assienan et al. 1993).

In the present paper, we compare, qualitatively and quantitatively, the isozyme polymorphism of Tanzanian sexuals and apomicts. We also study the typology and the geographical distribution of this polymorphism, as well as the relationship of *P. maximum* with *P. infestum*. The discussion addresses: (1) the origin of C types in relation to the known existence of gene flow; (2) the similarity and differences of polymorphism in the two pools; (3) the polymorphism and the frequency of rare alleles, and lastly (4) the heterozygosity of individuals in relation to their ploidy level.

Materials and methods

Plant material

Twenty diploid sexuals of *P. maximum* and 313 tetraploid apomicts were collected in Tanzania and in Kenya during two field trips in 1967 and 1969 (Combes 1975; Pernès 1975). These perennial clones were maintained as a living collection at the research station of Adiopodoumé (Côte-d'Ivoire).

Our sample includes all 94 clones from Tanzania. It covers the two geographical zones where the diploids were found and thus represents the heart of the centre of diversity. It comprises 20 sexual clones, 63 clones of *P. maximum sensu stricto*, nine clones of C types, and two clones of *P. infestum*.

The meiotic behaviour of these clones was studied by Combes (1975). The *P. maximum* apomicts are autotetraploids. Only the C types show variable meiotic behavior, and some present a regular allopolyploid meiosis. Nevertheless, Combes emphasizes that these C types with regular meiosis spontaneously yielded dihaploids with meiosis as regular as that of their parents, thus showing that the genomes of *P. maximum* and *P. infestum* are not really differentiated. We can therefore consider our genotypes as autotetraploids, with a number of loci equal to that of diploids.

Starch electrophoresis

The technique described by Second and Trouslot (1980) for rice was used. Its main characteristics include the following: startch gel (14%); histidine/citric buffer pH 6.0 or pH 8.0; 20 mA for each gel; 4 °C; run for 5–6 h.

Six enzymatic systems were studied. These were: 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, glucose-6-phosphate isomerase (GPI) E.C.5.3.1.9, phosphoglucose mutase (PGM) E.C.5.4.2.2, and esterase (EST) E.C.3.1.1.

All enzymes were run in pH 6.0 gels except for PGD (pH 8.0). The green part of the leaves was used for AAT, GPI, PGM and EST. For both dehydrogenases (MDH, PGD), samples were taken from the white part of young leaves.

Counting and interpretation of the zymograms

Our previous results on sexual and diploid plants (Assienan et al. 1993) provided information that has been used here. Thus, for EST and AAT, the interpretation of bands was limited to those located in the lower half of the migration zone. For PGD, two unstable bands were not taken into account. For GPI, secondary isozymes were also not interpreted. The enzyme structure is known to be either monomeric (PGM, EST) or dimeric (AAT, MDH, PGD, GPI). Two MDH enzymes, one thermosensitive at 60 °C, do not give a heterodimer. Two types of esterase are distinguished according to band colour, brown or red, corresponding to the decomposition of α-naphthyl acetate (αEST) or β-naphtyl acetate (βEST), respectively. In the diploid plants, the βEST bands are doublets. Nevertheless, some previous studies on apomicts showed the existence of single bands of βEST, particularly in *P. infestum*, as well as in the C types.

Several loci of the enzyme systems studied here were encountered in diploids. These are *Aat-1* (two alleles A1 and A2), *Mdh1* (two alleles A0 and A1) and *Mdh-2* (one allele B1) – these loci give a non-thermosensitive enzyme with heterodimers, *Mdh-3* (three alleles C1, C2 and C3), *Pgd-1* (one allele A1), *Pgd-2* (two alleles B0 and B1), *Pgd-3* (one allele C1), *Pgd-4* (one allele D1), *Gpi-1* (four alleles A1 to A4), *Pgm-1* (two alleles A1 and A2), *Est-1* (two alleles A0 and A1), *Est-2* (two alleles A0 and A1) and *Est-3* (two alleles B0 and B1). The last two esterase loci coded for the β-naphthyl esterases.

Notations

For the monmeric enzymes, all bands were noted. For the dimeric enzymes, only the bands corresponding to a homodimer were taken into account.

Each band is identified by a name (for example, AAT-A2), where the first part indicates the enzyme system (AAT). When inheritance was already known, the second part of the name specifies the locus (A) and the allele (2). These known loci are represented by the letters A, B, C and D. The letter X indicates an unknown inheritance (for the bands specific to the apomicts).

The bands were sorted into six classes, according to their frequency: always present bands ($f_j = 100\%$); very frequent bands ($100\% > f_j > 90\%$); frequent bands ($90\% > f_j > 60\%$); fairly frequent bands ($60\% > f_j > 40\%$); infrequent bands ($40\% > f_j > 10\%$); and lastly, absent or rare bands ($f_j < 10\%$).

The allelic structure of most polyploid clones (simplex, duplex, etc.) is still unknown. Consequently, we were not able to estimate allele frequencies and the classical diversity parameters of Nei, as in the study on diploids (Assienan et al. 1993). The within-group diversity was measured by two other well-known parameters of diversity: the %P of polymorphic loci and the number A of alleles per locus (Hamrick and Godt 1989). A second estimation of these parameters (%P′ and A′) was made without including rare bands. Lastly, the allelic richness of individuals was measured by the number of bands per individual (NBI).

Statistical methods

Categorical splitting

The sample was split into three groups according to the mode of reproduction or the morphological affinity with *P. infestum*: group S, including the 20 collected diploids; group A, including all types of *P. maximum sensu stricto*; and group C, with nine C types and two *P. infestum*.

The sample was independently divided into three other groups according to geographical origin (Fig. 1). The three regions are Korogwe-Vugiri, Kilosa-Morogoro, and Bagamoyo-Dar es Salaam. The first and the third are zones with diploids. The Bagamoyo-Das es Salaam zone contrasts with the others in its proximity to the coast.

Comparison of groups

For each band, the equality of frequencies of groups A, S and C was tested using the Fisher test and the procedure of multiple comparisons of Ryan (1960): the proportions p were sorted by increasing order: $p_1, p_2, \ldots, p_p$; the farthest proportions p_1 and p_p are taken into account [the group $(p_1; p_p)$ includes $k = p$ means; the Fisher test give a risk α, which is corrected according to the formula $\alpha_k = \alpha p(k-1)/2$]; if α_k is higher than 5%, the two proportions p_1 and p_p are considered as equal and the procedure stops; otherwise the difference is significant, and the procedure continues with the next step, i.e. comparison of p_2 to p_p and p_1 to p_{p-1} (in these two cases, we have $k = p-1$), etc.

The same procedure was applied to a comparison of the proportion of the geographical groups.

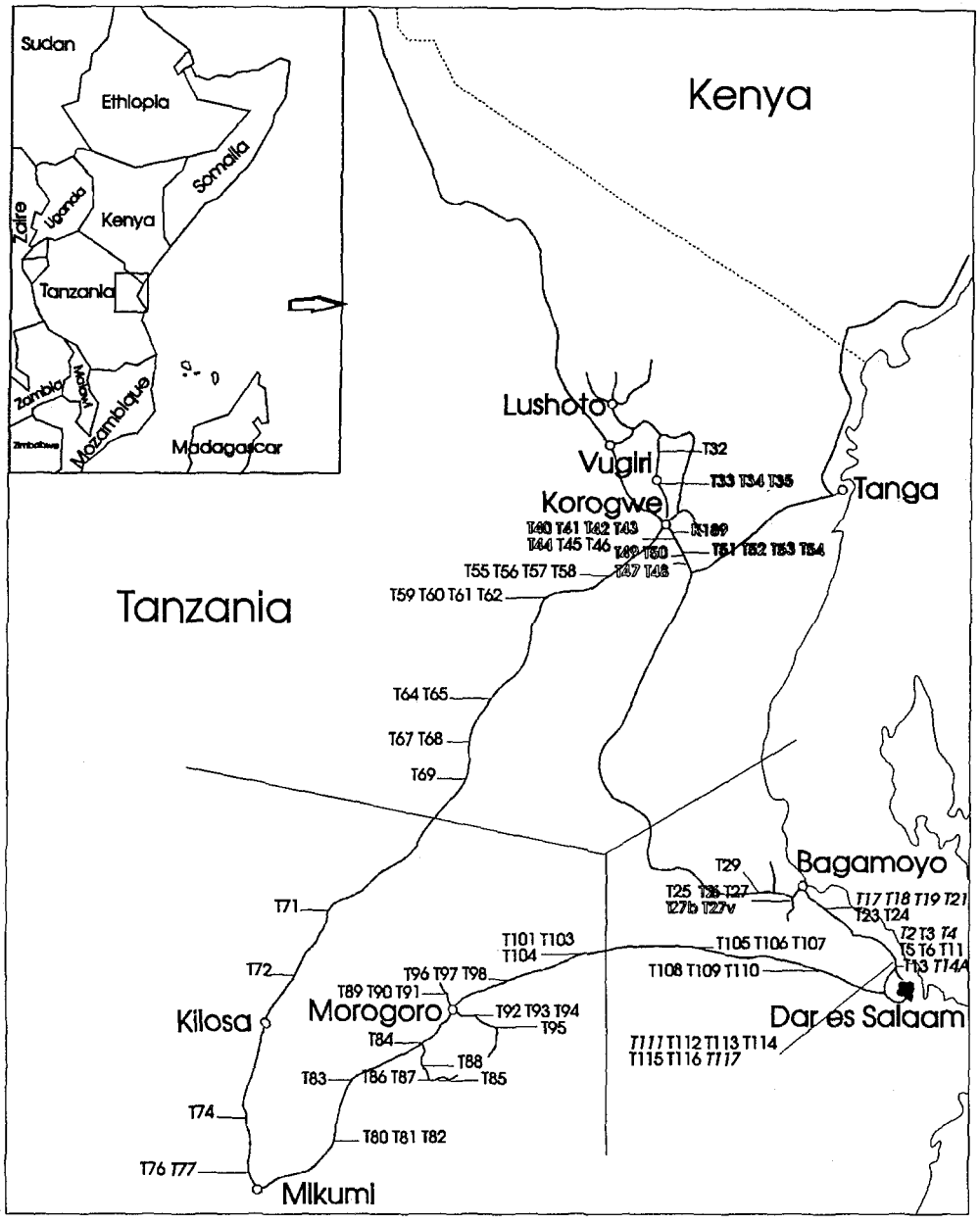


Fig. 1 Geographical distribution of clones. Clones of groups C, S and A are represented in *italic*, ***bold*** and *normal*, respectively. The three geographical zones (Korogwe-Vugiri, Kilosa-Morogoro and Bagamoyo-Dar es Salaam) were arbitrarily defined

The NBI was submitted to a two-way ANOVA with fixed effects (unbalanced and non-orthogonal design). The two tested effects were the type of plant (three categories: A, S and C) and the region (three categories: Bagamoyo, Korogwe and Kilosa). The expected (adjusted) means of types of plant or region were computed and, when the corresponding effect was significant, these means were compared using L.S.D. corrected by Ryan's method.

Analysis of the organisation of within- and between-group diversity

To study the structure of the genetic diversity, we used hierarchical clustering (the method of reciprocal neighbours) (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:

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

where $f_{ij} = 1$ if individual i has the band j, otherwise $f_{ij} = 0$ (presence/absence). Note that p is the total number of bands, that $f_{i.}$ (weight of individual i) and $f_{i'.}$ (weight of individual i') are the numbers of bands of individuals i and i', respectively, and that $f_{.j}$ (weight of band j) is the number of individuals with the band 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 Ward's (1963) linkage rule.

Correspondence analysis (Benzécri 1973), which is similar to principal component analysis (P.C.A.), is especially adapted to qualitative data. Like P.C.A., this method indicates (1) how many independent factors explain the diversity, (2) what fraction of the diversity is explained, and (3) the meaning of factors (factor loading). It differs principally from P.C.A. (1) by the use of the χ^2 distance instead of the Euclidian distance, and (2) by the possibility of representing the projection of variables and individuals on the same scatter plot.

Clustering and correspondence analysis were applied to complete disjunctive tables. In such tabulated data, each band is represented by two variables: presence and absence. The main advantage of this data

Table 1 Band frequencies (1) in all studied geotypes, (2) in the three groups A, S and C, and (3) in the three regions Bagamoyo, Korogwe et Kilosa. For each band, Fisher's test combined with Ryan's correction allowed multiple comparisons of proportions. Indices (a, b, c for the types of plants, and m, n, o for the regions) indicate the result of comparisons, e.g. for MDH-C2, S is not different from A, while C is different from S and A

Band		Types of plants				Geographical distribution		
		All clones	S	C	A	Bagamoyo	Korogwe	Kilosa
AAT	A1	0.95	0.85^{a}	0.82^{a}	1.00^{a}	0.85^{m}	1.00^{m}	1.00^{m}
	A2	0.59	0.20^{a}	1.00^{c}	0.64^{b}	0.91^{m}	0.33^{n}	0.48^{n}
	X1	0.05	0.00^{a}	0.00^{a}	0.08^{a}	0.03^{m}	0.00^{m}	0.15^{m}
MDH	C1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
	C2	0.21	0.05^{a}	0.91^{b}	0.14^{a}	0.47^{m}	0.06^{n}	0.07^{n}
	C3	0.10	0.20^{b}	0.09^{a}	0.06^{a}	0.03^{m}	0.24^{n}	0.00^{m}
	B1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
	A1	0.66	0.35^{a}	1.00^{c}	0.70^{b}	0.88^{m}	0.58^{n}	0.48^{n}
	X1	0.16	0.00^{a}	0.00^{a}	0.24^{b}	0.09^{m}	0.03^{m}	0.41^{n}
PGD	A1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
	B1	0.03	0.05^{a}	0.00^{a}	0.03^{a}	0.00^{m}	0.09^{m}	0.00^{m}
	C1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
	D1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
GPI	A1	0.07	0.35^{b}	0.00^{a}	0.00^{a}	0.00^{m}	0.21^{n}	0.00^{m}
	A2	0.46	0.25^{a}	0.36^{ab}	0.54^{b}	0.47^{m}	0.33^{m}	0.59^{m}
	A3	0.98	0.90^{a}	1.00^{a}	1.00^{a}	1.00^{m}	0.94^{m}	1.00^{m}
	A4	0.01	0.05^{a}	0.00^{a}	0.00^{a}	0.00^{m}	0.03^{m}	0.00^{m}
PGM	A1	0.17	0.55^{b}	0.36^{ab}	0.02^{a}	0.12^{mm}	0.33^{n}	0.04^{m}
	A2	0.98	0.95^{a}	0.91^{a}	1.00^{a}	1.00^{m}	0.97^{m}	0.96^{m}
	X1	0.03	0.00^{a}	0.27^{b}	0.00^{a}	0.09^{m}	0.00^{m}	0.00^{m}
	X2	0.04	0.00^{a}	0.00^{a}	0.06^{a}	0.09^{m}	0.03^{m}	0.00^{m}
β-EST	A1	0.71	0.85^{a}	0.73^{a}	0.67^{a}	0.77^{m}	0.79^{m}	0.56^{m}
	B1	0.11	0.10^{a}	0.18^{a}	0.10^{a}	0.15^{m}	0.06^{m}	0.11^{m}
	X1	0.02	0.00^{a}	0.00^{a}	0.03^{a}	0.03^{m}	0.03^{m}	0.00^{m}
	X2	0.01	0.00^{a}	0.09^{a}	0.00^{a}	0.03^{m}	0.00^{m}	0.00^{m}
	X3	0.03	0.00^{a}	0.18^{a}	0.03^{a}	0.09^{m}	0.00^{m}	0.04^{m}
	X4	0.07	0.00^{a}	0.45^{b}	0.03^{a}	0.21^{m}	0.00^{n}	0.00^{n}
	X5	0.06	0.00^{a}	0.36^{b}	0.03^{a}	0.18^{m}	0.00^{n}	0.00^{n}
α-EST	A1	0.28	0.30^{a}	0.18^{a}	0.30^{a}	0.27^{m}	0.33^{m}	0.26^{m}

re-coding is that it gives the same weight to all individuals, whatever their number of bands.

For the correspondence analysis and the clustering based upon χ^2 distance, it is recommended to use variables with relatively similar weights. As a general rule, we retained infrequent to frequent bands. Nevertheless, for the study of the small groups C and S we were more restrictive, and only took into account bands with a strength of between 3 and $n_i - 3$. For the analysis of all genotypes, we considered bands of frequency higher than 10% in at least one of the groups, and lower than 100% overall.

Results

Thirty seven zymograms were analyzed. Of the 29 observed bands (Table 1), five (MDH-C1, MDH-B1, PGD-A1, PGD-C1 and PGD-D1) did not show any diversity, and the rare bands were particularly numerous (41%). In fact, only nine bands (AAT-A2, MDH-A1, MDH-X1, MDH-C2, GPI-C2, PGM-A1, βEST-A1, βEST-B1 and αEST-A1) had a frequency of between 10% and 90%.

Genetic interpretation of new bands

Some system (PGD, GPI and αEST) did not show any new diversity in addition to the known diversity in sexuals. Other systems presented some bands unknown at the diploid level. These were:

AAT-X1, which should correspond to a new allele of the locus *Aat-1*;

MDH-X1, which according to correspondence analysis[2] and zymograms, should be an allele of the locus *Mdh-1*;

PGM-X1 and PGM-X2, which should be alleles of the locus *Pgm-1*;

the β-esterases. For these enzymes, the known presence of null alleles leads to two opposite assumptions.

(1) assumption H1, the number of loci (13) does not change, and all new bands should correspond to new alleles. (2) assumption H2, all new bands correspond to added loci. The number of loci is then 16. This implies

[2] Two alleles at a given locus contrasted on a factorial axis

that, in diploids, the three added loci were homozygous with null alleles. All intermediate assumptions may exist, but only some crosses at the tetraploid level using sexual colchiploidised plants as female (obligate apomixis) will be able to provide a definite answer.

Quantitative comparison of enzymatic diversity between sexuals and apomicts

The percentage of polymorphic loci in apomicts (groups A and C) was estimated for each of these assumptions, H1 (%P = 69) and H2 (%P = 75). In the same way, the number of alleles per locus, A, varied from 2.46 (H1) to 2.25 (H2). Note the very high enzymatic diversity, on the one hand, and the relatively low discrepancy between the two estimations (< 10%) for each parameter, on the other. Because of the weak influence of the underlying genetic assumption, only the estimation for H1 will be considered in what follows.

For the diploids, the percentage of polymorphic loci was 69, and this value was not different from that of the apomicts. By contrast, the number of alleles per locus was considerably lower (1.92, which represents a 22% difference). When rare bands were not taken into account, the number of alleles per locus, A′ became equal (1.62) for the two pools, while the percentage of polymorphic loci for diploids and apomicts was still equal (%P′ = 62). Thus, the quantitative difference in diversity between pools is due to the rare bands.

Qualitative comparison of enzymatic diversity in groups A, S and C

Table 1 gives within-group frequencies and the results of statistical tests. Only the PGD system did not show between-group differences. For other systems, AAT-A2, MDH-C2, MDH-C3, MDH-A1, MDH-X1, GPI-A1, GPI-A2, PGM-A1, PGM-X1, βEST-X4 and βEST-X5, 42% of the bands showed between-group differences. Nevertheless, none of these bands was completely specific to a group.

Some bands had similar frequencies in groups S and C, different from that of group A. This was the case for band MDH-X1, which was absent from groups S and C and present at low frequency in group A (24%). This was also the case for band PGM-A1, which was rare in group A (2%) and fairly frequent (48%) in groups S and C. This indicates some affinities between sexuals and C types.

The following bands were more frequent in group C, bearing out its specificity: AAT-A2, MDH-A1, MDH-C2, PGM-X1, βEST-X4 and βEST-X5. Their lower frequency in other groups also shows the existence of introgressions in apomictic or sexual plants with a phenotype nevertheless typical of *P. maximum*. Note also that the presence or absence of a doublet in β-nahthyl esterases characterizes diploids of *P. maximum* and *P. infestum*, respectively.

One hand (GPI-A1) existed only in diploids, and at moderate frequency (35%).

Genetic organisation in groups A, S and C

Comparison of groups for the heterozygosity of individuals

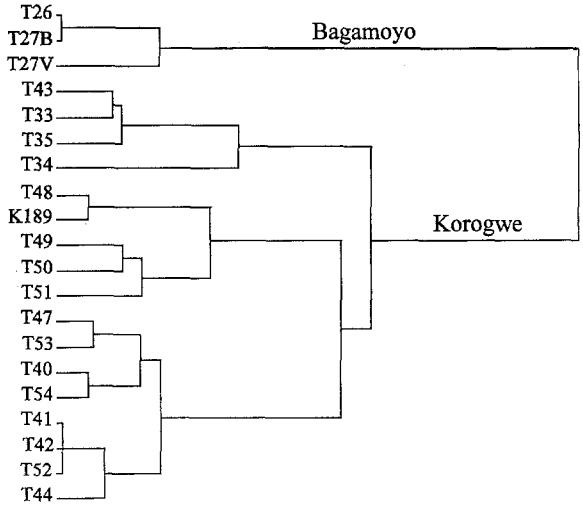
The band richness of individuals (NBI) reflects the level of heterozygosity in diploids and in tetraploids. For all individuals, NBI varied from 8 to 16. Nevertheless, some very highly significant differences existed between the group ($F_{2;90} = 11.00$). The C group, with 13.3 bands per individual (expected mean), contrasted significantly with the two other groups, S and A (10.6 and 11.6 bands, respectively), and thus showed a higher level of heterozygosity. More surprising was the similarity between groups S and A, despite their different ploidy levels.

Organisation of the within-group polymorphism

Hierarchical clustering allowed a study of the organisation of within-group polymorphism. The choice of bands was determined by their frequencies. (see Materials and methods). For the C group, the bands used were GPI-A2, PGM-A1, PGM-X1, βEST-X4, βEST-X5 and βEST-A1. For the S group, they were AAT-A1, AAT-A2, MDH-A1, MDH-C3, GPI-A1, GPI-A2, PGM-A1, βEST-A1 and αEST-A1. For the A group, they were AAT-A2, MDH-A1, MDH-C2, MDH-X1, βEST-A1 and αEST-A1.

C types cannot be distinguished from the two clones of *P. infestum* (T14A and TT1), and no biologically interpretable sub-group can be highlighted within the C group. This was not the case for the S group, for which a geographical differentiation appeared between the clones of Bagamoyo and those of Korogwe (Fig. 2). The presence of sub-groups also characterized the A group. The first (A1) included genotypes with bands MDH-C2 and MDH-A1, and also rare clones with bands βEST-X5 and βEST-X4. These plants were clearly classified as typical *P. maximum* according to their morphology, but

Fig. 2 Diversity structure of the sexual *P. maximum* from a clustering analysis



having numerous isozymes introgressed from *P. infestum*. Sub-groups A2 and A3 differed from the the latter by the absence of the band MDH-C2, and the presence of the band αEST-A1 characterising the A2 sub-group. The last sub-group (A4) was characterised not only by the absence of MDH-C2, but also by the quasi-absence of αEST-A1 (5.3%) and MDH-A1 (10.5%). This sub-group was relatively rich in MDH-X1 (47.4%), a band unknown in C types and in sexuals. All sub-groups represented different levels of introgression by *P. infestum*.

Relationships between the three diversity groups

Sixteen monomers were used in the analysis of total diversity: AAT-A1, AAT-A2, MDH-X1, MDH-A1, MDH-C2, MDH-C3, GPI-A1, GPI-A2, PGM-X1, PGM-A1, βEST-X3, βEST-X4, βEST-X5, βEST-A1, βEST-B1 and αEST-A1. The first factorial scatter plot (factors 1 and 2) showed an L-shaped structure of diversity (Fig. 3). The first factor associated the bands βEST-X4, βEST-X5 and MDH-C2, characteristic of group C, and highlighted a continuum between *P. maximum* and *P. infestum*. The second factor was characterised essentially by GPI-A2 and PGM-A1, two bands which existed only in diploid sexuals. We noted that the diversity of sexuals and that of the apomicts only partially overlapped, but that in the overlap zone sexuals were located in the prolongation of the *P. maximum* -*P. infestum* continuum.

Geographical organisation of the diversity

Graphical representations of the enzymatic diversity of each region (Korogwe-Vugiri, Bagamoyo-Dar es Salaam, and Mikumi-Kilosa) were constructed from the last analysis (Fig. 4).

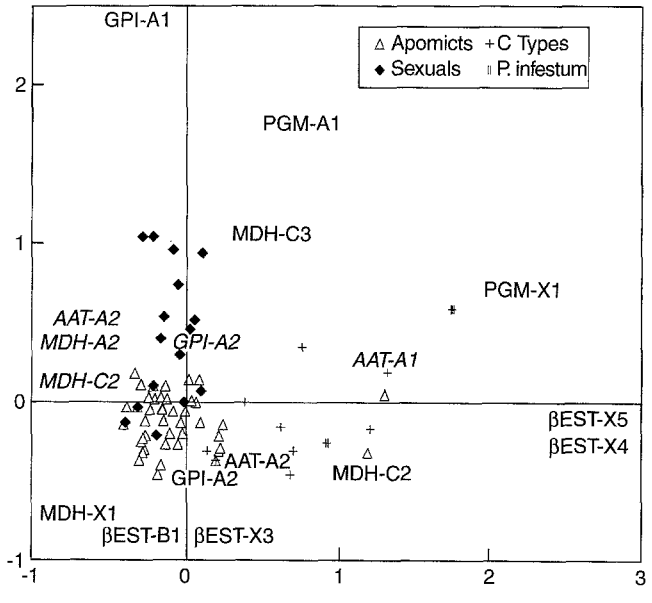


Fig. 3 Scatter diagram representing the organization of isozyme diversity in the main groups (from the two first factors of the correspondence analysis)

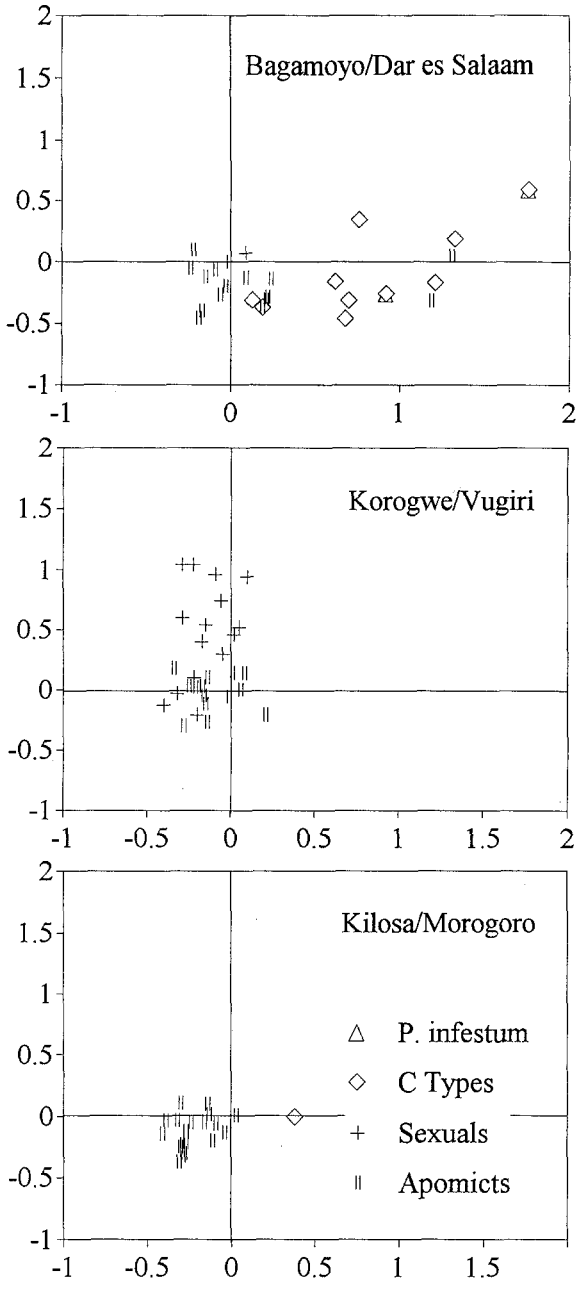


Fig. 4 Scatter diagrams representing the geographical organization of isozyme diversity (from the two first factors of the correspondence analysis)

The Bagamoyo-Dar es Salaam region appeared very polymorphic, as shown by the number of alleles per locus (A = 2.39), but this diversity is partially explained by the presence of numerous rare alleles (A′ = 1.77). The presence of the *P. maximum* - *P. infestum* continuum led to a higher frequency of bands characteristic of C types (AAT-A2, MDH-C2, MDH-A1, βEST-X4 and βEST-X5). Note that the diversity of the diploid sexuals of Bagamoyo is low and is characterised by their near location to the scatter plot origin. Their enzymatic

Table 2 Monomer frequencies in the three geographical zones. For each monomer, a χ^2 test was used to verify the frequency equality of the monomer in the three groups. When the test lead to rejection of the null hypothesis, a 2-by-2 comparison of groups was applied

Band		Frequencies			Test results			
		Bagamoyo	Korogwe	Kilosa	Global	S vs C	S vs A	C vs A
AAT	A1	0.85	1.00	1.00	**	N.S.	*	*
	A2	0.91	0.33	0.48	***	***	***	*
	X1	0.03	0.00	0.15	N.S.	–	–	–
MDH	C1	1.00	1.00	1.00	N.S.	–	–	–
	C2	0.47	0.06	0.07	***	***	N.S.	***
	C3	0.03	0.24	0.00	*	N.S.	N.S.	N.S.
	B1	1.00	1.00	1.00	N.S.	–	–	–
	A1	0.88	0.58	0.48	***	***	**	*
	X1	0.09	0.03	0.41	**	N.S.	**	N.S.
PGD	A1	1.00	1.00	1.00	N.S.	–	–	–
	B1	0.00	0.09	0.00	N.S.	–	–	–
	C1	1.00	1.00	1.00	N.S.	–	–	–
	D1	1.00	1.00	1.00	N.S.	–	–	–
GPI	A1	0.00	0.21	0.00	***	*	***	N.S.
	A2	0.47	0.33	0.59	***	N.S.	*	N.S.
	A3	1.00	0.94	1.00	*	N.S.	*	N.S.
	A4	0.00	0.03	0.00	N.S.	–	–	–
PGM	A1	0.12	0.33	0.04	***	N.S.	***	**
	A2	1.00	0.97	0.96	N.S.	–	–	–
	X1	0.09	0.00	0.00	**	*	N.S.	**
	X2	0.09	0.03	0.00	N.S.	–	–	–
β-EST	A1	0.77	0.79	0.56	*	N.S.	N.S.	N.S.
	B1	0.15	0.06	0.11	N.S.	–	–	–
	X1	0.03	0.03	0.00	N.S.	–	–	–
	X2	0.03	0.00	0.00	N.S.			
	X3	0.09	0.00	0.04	*	*	N.S.	*
	X4	0.21	0.00	0.00	***	**	N.S.	***
	X5	0.18	0.00	0.00	***	**	N.S.	**
α-EST	A1	0.27	0.33	0.26	N.S.	–	–	–

pattern can be considered as representative of the mean enzymatic pattern of all clones.

The enzymatic diversity of the Korogwe-Vugiri region was less marked, with 2.15 alleles per locus, Nevertheless, this estimator was still strongly influenced by rate alleles ($A' = 1.62$). The enzymatic polymorphism of sexuals was higher here than that of the sympatric apomicts, and was characterised by a higher frequency of bands MDH-C3, GPI-A1 and PGM-A1. The diversity of apomicts and that of sexuals overlapped only partially.

The third region, Kilosa-Morogoro, appeared as the lowest polymorphic zone. Except for one C type, this region included only apomictic genotypes, *P. maximum* s.s. The number of alleles per locus was lower ($A = 1.85$), but was less influenced by rare alleles ($A' = 1.62$). Only one band (MDH-X1) was more frequent.

The decreasing regional sorting, represented by Bagamoyo, Korogwe, and Kilosa, corresponded to "sexuality with C types", "sexuality without C types", and "non-sexuality with very rate C types". Note that without the rare alleles, the diversity in Korogwe and Kilosa became similar.

A highly significant regional effect, which was independent of the frequencies of types of plants (A, S or C), existed for NBI ($F_{2;90} = 5.99$). Once again, the Bagamoyo region (NBI = 12.36) contrasted with that of Kilosa (NBI = 11.15). This was due essentially to the existence in the first region of clones of *P. maximum* introgressed by *P. infestum* (sub-group A1). Note also the intermediate location of Korogwe (NBI = 11.90).

Only one trait was practically invariant between regions. This was the percentage of polymorphic loci, for which the slight superiority of the Korogwe region (%P = 0.692) over the regions of Bagamoyo and Kilosa (%P = 0.62) is, in fact, due only to the presence of a rare allele, B1, at the *Pgd-2* locus.

Discussion

Mutual gene flow between the two pools of the agamic complex of the *Maximae* has been previously emphasized (Savidan and Pernès 1982). The emergence of tetraploid sexuals in between-diploid crosses (about 0.5% of progenies), or after pollination of a diploid

sexual plant by an apomict, allows gene flow from the sexual pool to the apomictic pool (Savidan and Pernès 1982). Theoretically, these tetraploid sexuals disappear rapidly (Pernès 1975; Williams 1975) explaining their absence among collected accessions (Pernès 1975). Their role is nonetheless active, because they are fertilizable, without limit, by the neighbouring apomictic pollen, and only one tetraploid sexual is sufficient to generate polymorphism.

Gene flow also occurs from the apomictic pool to the sexual pool. Some dihaploids appear at low frequency (0.5%) in between-tetraploid crosses, sexuals × apomicts or apomicts × apomicts[3] (Savidan and Pernès 1982). Dihaploids are of two types, differing by the nature of their embryo sacs. Dihaploids with embryo sacs of aposporic origin are sterile (Savidan and Perès 1982), thus avoiding the overunning of the diploid pool by apomixis (Noirot 1993). By contrast, dihaploids with embryo sacs of meiotic origin can be crossed with other diploids, and indeed can even recreate a diploid population when there are at least two. Note that such gene flow exists also in other agamic complexes, such as *Botriochloa-Dichantium* (De Wet 1968).

The origin of C types: relationship between *P. maximum* and *P. infestum*

The C types are plants with sharp leaves and tillers, often hairy, and morphologically intermediate between *P. maximum* and *P. infestum* (Combes 1975; Pernès 1975). As in *P. infestum*, the inflorescence does not have verticils but, as in *P. maximum*, the length of branchings decreases from the panicle base towards the panicle top. In field experiments, these C types are easily crossed with tetraploid sexuals of *P. maximum* giving plants with verticilled panicles. The frequent presence of C types in wild populations where the two species live together (Bagamoyo-Dar es Salaam) is an additional argument in favour of their hybrid origin (Pernès 1975), as is their production from crosses between *P. maximum* (2n=4x sexual) and *P. infestum* (Noirot personal observation).

Our studies on enzymatic diversity confirm the hybrid nature of C types with the presence of an introgressive continuum between the two species. In addition, it is most probable that a large majority of C types are F_1-hybrids. Nevertheless, the presence of verticilled plants (sub-group A1) among *P. maximum*, but having en-zymatic alleles typical of *P. infestum*, shows the existence of wild *P. maximum* × C type backcrosses. The presence of diploid sexuals in the region of Bagamoyo at one of the poles of the introgressive continuum suggests the participation of the diploids in the origin of the C types.

[3] Some reduced embryo sacs (average 5%) are seen in apomictic plants. There are hypersexual apomicts, i.e. with a high percentage of reduced embryo sacs.

Inheritance studies of enzymatic traits in diploid sexuals (Assienan et al. 1993) showed the existence of segregation distortion for alleles C1, C2 and C3 of the *Mdh-3* locus (corresponding to the bands MDH-C1, MDH-C2, MDH-C3, respectively). In particular, homozygotes (C2C2) were totally absent from (C1C2) × (C1C2) crosses, whereas homozygotes (C3C3), as well as heterozygotes (C1C3), were counter-selected in (C1C3) × (C1C3) crosses. Maintenance of these alleles at the diploid level was unexplained (Assienan et al. 1993). In fact, these alleles belong to one or other of the two plants of *P. infestum* and, for the C2 allele, to all C types. This confirms regular gene flow from *P. infestum* to the diploid pool; but, mainly, the existence of segregation distortion emphasized the quite regular elimination of the introgressed alleles. The process of distortion was also observed for esterases (Assienan et al. 1993). This is reminiscent of the existence of morphological-viability linkage systems highlighted by Grant (1971) in interspecific crosses.

Similarities and differences in enzymatic polymorphism in sexuals and apomicts

The first characteristic of the apomictic pool is a polymorphism similar to that of the diploid pool. This similarity has already been observed in other agamic complexes, such as those of *Antennaria* (Bayer 1991) and *Eupatorium altissimum* (Yahara et al. 1991). Note also that, in first case, polymorphism of the two pools is high, whereas it is low in *E. altissimum*. This similarity is well explained by the presence of gene flow.

This gene flow accounts for similarities, but not differences. Some of these differences may be due to geographical distance (absence of MDH-X1 in sexuals) or to the distortion of segregations (some alleles of *P. infestum*). In contrast, other differences exist even in the sympatric region of Korogwe, as in the case of the two bands GPI-A1 and PGM-A1, which are absent, or virtually absent, in apomicts. A relationship to the mode or reproduction seems to constitute an explanatory hypothesis. Nevertheless, if alleles corresponding to bands GPI-A1 and PGM-A1 were linked to sexuality (inheritance of apomixis in *P. maximum* is monogenic with a dominant allele *A*), wild apomicts, which are genetically *Aaaa* (Savidan 1982), should have these alleles too. Enzymatic analysis of progenies between colchitetraploids and apomicts should be undertaken in order to resolve this problem.

Polymorphism in the *Maximae* species complex

The enzymatic polymorphism of the sexual pool appears high: 69% of polymorphic loci, and 2.46 alleles per locus. Nevertheless, such polymorphism is known in other Gramineae, e.g. *Pennisetum glaucum* (Tostain et al.

1987), as well as in other allogamous and anemophilous plants (Hamrick and Godt 1989).

The presence of gene flow explains the similarities between sexual and apomictic pools (see discussion above), but is not sufficient to explain the maintenance of marked polymorphism in the whole complex. By contrast, this maintenance is theoretically expected in a model developed by Marshall and Weir (1979). These authors showed that the proportion of apomicts does not change the genotypic composition at equilibrium in species where apomixis and allogamy co-exist. They also showed that the equilibrium is reached all the more slowly when the proportion of apomicts is high.

This situation is precisely observed in *P. maximum*, where one of the main sources of fluctuations in the genotypic composition of populations is the introgression of alleles from *P. infestum*. This process is particularly evident geographically: zones where *P. maximum* sexuals and *P. infestum* live together are more polymorphic. In addition, polymorphism is due in a large part to the existence of many rare alleles (41% of encountered alleles have a frequency less than 10%), which are also known to be mostly subject to distortion of segregation in diploids. Note that this distortion is a second source of fluctuations in the allelic, and thus genotypic, composition of the complex. Apomixis acts as a buffer to fluctuations and thus allows maintenance, at low frequency, of alleles which should be virtually removed the moment they were introgressed.

Polyploidy and heterozygosity

The existence of three or four alleles at many loci leads us to assume *a priori* the presence of a high level of heterozygosity in apomicts by the maintenance of quadruplex or triplex structures. In fact, no quadruplex types were observed in our study, and apomict zymograms are essentially constituted by duplex or simplex structures. In addition, the number of bands per apomictic individual does not differ from the number of bands observed on average in a sexual individual. In fact, the probability of an individual having several alleles at the same locus is low owing to the large number of rare alleles (41%). This situation clearly shows that the advantage of the polyploidy-apomixis relationship is not to maintain the within-individual heterozygosity level of an apomict higher than that of a diploid.

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