



DIPLOID-TETRAPLOID-DIHAPLOID CYCLES AND THE EVOLUTION OF *PANICUM MAXIMUM* JACQ.

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Recent research interests in plant evolution focus more and more on compartmentalization of gene pools at different levels of ploidy, which allow for coexistence of different modes of reproduction. Equilibria between tetraploid-apomictic and diploid-sexual compartments have been discussed in *Parthenium argentatum* (Rollins et al., 1947), in the *Bothriochloa-Dichanthium* complex (de Wet, 1968), and in *Ranunculus auricomus* (Nogler, 1978). The inverse, i.e., tetraploid-sexual and diploid-apomictic, although a rare event, may have played a part in the evolution of the genus *Potentilla* (Asker, 1978, 1979). Mendiburu et al. (1974), on the other hand, demonstrated a different kind of compartmentalization of gene pools, with tetraploid-self fertilization and diploid-self incompatibility systems.

The *Panicum maximum* agamic complex (*Panicaceae*), like the *Bothriochloa-Dichanthium* complex (*Andropogoneae*), belongs to the subfamily *Panicoideae* of the Gramineae. Accessions from Kenya and Tanzania studied by Combes and Pernès (1970) were shown to be either tetraploid ($2n = 32$) and apomictic, or diploid ($2n = 16$) and fully sexual. Analyses of natural populations (Pernès, 1972) demonstrated three main population types: monomorphic; polymorphic, discontinuous (i.e., the different biotypes are morphologically well differentiated); and intermediate but still highly polymorphic, continuous. This class includes mixed populations of diploid

sexual and tetraploid apomictic biotypes.

The major objective of this study is to discuss the possible relations between diploid-sexual and tetraploid-apomictic gene pools in *Panicum maximum*, in the light of cytogenetical and embryological analyses, in relation to previous data from biology and plant evolution.

MATERIALS AND METHODS

Five hundred accessions of *Panicum maximum* have been established in a field collection, either from seeds or from tillers, in the southern part of Ivory Coast. They were primarily introduced from two trips made in 1967 and 1969, across Kenya and Tanzania. Twenty two of these accessions proved to be diploid ($2n = 16$) and entirely sexual.

Most other accessions were shown to be tetraploid ($2n = 32$) with an apomictic reproductive behavior. Biotypes of *Panicum infestum* Anders. were also collected in Tanzania, as well as natural interspecific hybrids between *P. maximum* and *P. infestum*. The sexual potential of the apomictic accessions, as detected by embryological analyses, appeared to be generally low, with an average of 8.2% sexuality for 8,355 ovules observed (Savidan, 1982a). One apomictic interspecific hybrid between *P. maximum* and *P. infestum* (T19) differed from the others, as an interesting exception: 67% of its ovules showed sexual behavior, and produced about 40% "off types" in its offspring. This allowed us to use this apomict as a female in crossing experiments.

Sexual diploids were treated with colchicine, and the tetraploids thus obtained remained fully sexual. Sexuality at both

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the diploid and tetraploid levels appeared to be associated with high self-sterility. This trait has been used in crossing experiments, one inflorescence from a sexual tetraploid progenitor being put in a paper bag together with one or several inflorescences from the apomict used as male. Pollen from apomicts is usually functional, as a result of a regular meiosis (Combes, 1972).

Reproductive behavior was determined either from progeny tests or from embryological studies. The latter method was preferred since a cytological technique is available that is convenient and rapid. It involves clearing (Herr, 1971), and the use of phase contrast microscopy. Gametophytic apomixis in *Panicum maximum* is characterized by 4-nucleate embryo sacs, with one egg, two synergids and a single polar nucleus. Sexual biotypes show only ovules with a single 8-nucleate embryo sac. Progeny tests, used as controls, have proved that the correlation between embryological observations and the reproductive behavior is absolute.

RESULTS

Spontaneous Tetraploidization and Evolution of Sexuality at the Tetraploid Level

Crossing experiments using diploid sexual *Panicum maximum* as pistillate parents revealed the production of some functional gametes without chromosome reduction (Table 1). Since sexual diploids and apomictic tetraploids are found together in mixed populations in Tanzania, $2x \times 4x$ crosses appeared to be of special interest. They yielded only small progenies that included diploids from self-fertilization, triploids ($x + 2x$), and tetraploids coming from fertilization of unreduced gametes ($2x + 2x$).

Six of these tetraploid hybrids have been examined cytologically. Three were apomictic, and three were sexual. Thus, spontaneous tetraploidization is possible in nature by means of $2x \times 4x$ crosses, that lead to some sexual tetraploids. A similar pattern has been shown in studies

on *Dichanthium* (Harlan et al., 1964; de Wet and Harlan, 1970) and a general discussion on such a polyploidization process was presented by Harlan and de Wet (1975).

Apomixis in *Panicum maximum* behaves genetically as a single gene, dominant over sexuality (Savidan, 1980, 1982b). Sexual diploids have the genotype *aa*, while the apomicts have at least one dominant allele. At a given generation, *n*, three genotypes could be encountered in the population as follows (cf. Pernès, 1971):

Phenotypes	Genotypes	Frequencies
Apomictic	<i>AAaa</i>	P_n
Apomictic	<i>Aaaa</i>	$2Q_n$
Sexual	<i>aaaa</i>	R_n

with $P_n + 2Q_n + R_n = 1$, and P_n low in frequency as a result of apomictic \times apomictic crosses. Then if the next generation, *n* + 1, is the result of a random association of the following gametes:

male frequencies	female frequencies
<i>AA</i> $P_n/6$	<i>AAaa</i> P_n
<i>Aa</i> $2P_n/3 + Q_n$	<i>Aaaa</i> $2Q_n$
<i>aa</i> $P_n/6 + Q_n + R_n$	<i>aa</i> R_n

the three possible genotypes have the frequencies shown below:

$$\begin{aligned}
 AAaa: & 1/6P_nR_n + P_n \\
 Aaaa: & 2Q_n + R_n(2/3P_n + Q_n) \\
 aaaa: & R_n(1/6P_n + Q_n + R_n).
 \end{aligned}$$

The frequency of genotype *AAaa*, which has been assumed to be low at generation *n* due to infrequent apomictic \times apomictic hybrids, cannot increase in the population. Almost all apomictic accessions would thus be *Aaaa*.

The frequency of the sexual plants with the *aaaa* genotype becomes:

$$\begin{aligned}
 R_{n+1} &= R_n(1/6P_n + Q_n + R_n) \\
 &= R_n(Q_n + R_n) \text{ as } 1/6P_n \text{ is close to zero} \\
 &= R_n \frac{(1 - P_n + R_n)}{2} \\
 \text{as } Q_n &= \frac{1 - P_n - R_n}{2}. \tag{1}
 \end{aligned}$$

The formula (1) shows two possible points of equilibrium:

$R = 0$, stable equilibrium for which the population is entirely apomictic (P genotypes $AAaa$; $1 - P$, close to 1, genotypes $Aaaa$),

$R = 1$, unstable equilibrium for which the population is entirely sexual (genotype $aaaa$).

Thus if the apomictic allele A appears in a given sexual population, the latter will become entirely apomictic, almost all the genotypes being $Aaaa$, although no special selective advantage has been postulated for the apomictic phenotype (Pernès, 1971). In a mixed diploid-tetraploid population, regular appearance of a few sexual tetraploids would not have been followed by any increase of sexuality at the tetraploid level. This can explain the absence of sexual tetraploids among the biotypes we collected in nature.

Increase of Genetic Variation among Tetraploids by Means of Interspecific Hybridization

The reorganization and subsequent evolution of variability at the tetraploid level could simultaneously take place in several sites where genetically distinct biotypes are sympatric (Pernès, 1972). This can be illustrated by Tanzanian populations in which *P. maximum* is mixed with the related species *P. infestum*.

The degree of sexuality of facultative apomicts of *Panicum* is usually low (see Materials and Methods), but some natural interspecific hybrids proved to be highly facultatively sexual, as exemplified by the T19 accession. Thus the means of temporarily reacquiring sexuality do not necessarily occur through a direct return to diploidy. Apomictic interspecific hybrids such as T19 could release some new phenotypes following open pollination by apomictic neighbors. Such crosses, using T19 as female, were attempted. Of 585 plants analyzed, 291 were of the maternal type and 294 were "off-types." These consisted of 111 selfed ($4x$), 118 hybrid ($4x$), 34 hybrid ($6x$), and 31 dihaploids ($2x$).

Numerous dihaploids, which arose from

the parthenogenetic development of $2x$ gametes (i.e., haploids from a tetraploid), were thus observed.

Analysis of Dihaploids Arising from Interspecific Hybrids

The cytogenetical analysis of interspecific hybrid offspring has led to the discovery of 60 dihaploids. They were all male sterile, as no viable pollen could be observed, and had specially low vigor. As a consequence they were lost, year after year, in spite of careful attempts to conserve them: only five could be maintained in our field collection, eight years after they were obtained.

Embryogenesis has been studied in one-third of these dihaploids. Almost all of them were female sterile as the flowers failed to open and/or as the ovules appeared to be empty. Two of them exhibited a low percentage of well differentiated ovules, with 4-nucleate, i.e., apomictic, embryo sacs. These dihaploids gave no seed, either from self-fertilization or from cross-pollination by sexual male fertile accessions. As in the case of most dihaploids of *Dichanthium annulatum* which arose from tetraploid apomicts (de Wet, 1968), our dihaploids were thus poorly adapted for survival in nature.

Analysis of Dihaploids Arising from Autotetraploids

Systematic crosses were performed between seven sexual and eight apomictic tetraploids (Savidan, 1980, 1982b). The chromosome numbers of the progenies are summarized in Table 1. Two dihaploids were obtained among the F_1 hybrids. These two plants were highly vigorous, with few tillers but broad leaves. They closely resembled the sexual parents as well as the natural diploids of our collection. These two plants showed regular meiosis, with eight bivalents. They were fertile, with good seed production, and the embryological observation of 41 and 53 ovules respectively, showed them to be entirely sexual.

Later two other sexual dihaploids were obtained in the offspring of an apomictic

TABLE 1. Failure of meiotic reduction in *Panicum maximum* crossing experiments. Hybrids from fertilization of unreduced gametes are underlined.

Cross combinations	No plants with 2n =			
	16	24	32	48 64
2x sexual × 2x sexual	212	<u>3</u>	<u>1</u>	
2x sexual × 4x apom.	12 ¹	5	<u>7</u>	
4x sexual × 4x apom.	2		281	<u>45</u> <u>5</u>

¹ Result of self fertilization.

hybrid that exhibited a high percentage of residual sexuality (Savidan, 1982a). Like the others, they proved to be sexual and vigorous.

DISCUSSION

Dihaploids of *Panicum maximum* can be classified in two types according to their origin. Type I corresponds to the dihaploids obtained in the F₁, i.e., directly following hybridization. Type II corresponds to those obtained in the progeny of these F₁ hybrids (following a similar classification presented by Harlan and de Wet, 1975, for polyploids). Two sexual dihaploids of *P. maximum* are of type I, while the two others, and all sterile dihaploids, are of type II. Each facultative apomict, as stated by de Wet and Harlan (1970), could produce tetraploid "off-types," a lower proportion of hexaploid "off-types" (respectively 2/3 and 1/3 in *Panicum maximum* var. Common guinea) and some rare dihaploids. The high percentage of sexuality which could be observed in a few apomictic hybrids could thus be an important factor for the production of new type II dihaploids.

The sexual dihaploids could play a role in the evolution of the agamic complex, especially if one considers their vigor, fertility and resemblance to the natural diploids found in East Africa. Such a resemblance has also been noted between diploids and dihaploids of the *Bothriochloa-Dichanthium* complex (de Wet and Harlan, 1970). According to de Wet (1968) a few of the diploids collected in these genera could have a recent dihaploid origin, a statement mainly based on similarities observed in cytological analyses.

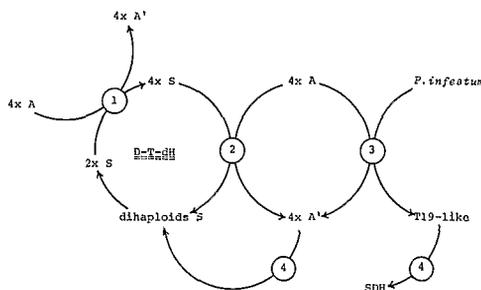


FIG. 1. Diploid-tetraploid-dihaploid cycle and recombination-inducing mechanisms involved in *Panicum maximum* evolution.

1. 2x sexual × 4x apom. intraspecific hybridizations
→ sexuality at the tetraploid level
→ new apomictic 4x
2. 4x sexual × 4x apom. intraspecific hybridizations
→ sexual dihaploids
→ new apomictic 4x, some with a high percentage of sexuality
3. Interspecific hybridization with *P. infestum*
→ new apomictic 4x
→ T19-like plants, with a high percentage of sexuality
4. Increase of sexuality in some apomictic hybrids
 - a. autotetraploids → sexual dihaploids
 - b. allotetraploids → sterile dihaploids (SDH)

Thus the *Panicum maximum* complex we studied seems to be in an active phase of evolution, characterized by the presence of recombination-inducing mechanisms. Among them, an essential role could be played by tetraploidization, the temporarily tetraploid phase of sexuality, and haploidization, all processes which enable the diploid-tetraploid-dihaploid cycles to function (Fig. 1). Within the diversity of *Panicum maximum*, the resemblance between diploids and neighboring apomictic tetraploids noted by Pernès et al. (1975) could be considered a natural result of a permanent genetic mixing which could be perpetuated by such cycles.

The diploid-tetraploid-dihaploid cycles seem to be a major process for the evolution of the *Bothriochloa-Dichanthium* agamic complex (de Wet, 1968, 1971). They may play a similar role in *Parthenium* and *Ranunculus*. They could be also at the origin of variability in *P. maximum*. The analogy between this complex and

that which has been studied by Harlan and de Wet must be pointed out. The success of such plants, and the success of apomixis in general, could be attributed to the continued contact between sexual reproduction and the apomictic process itself. Stebbins (1980) has recently reaffirmed that "polyploid phylogeny can still be regarded as usually unidirectional, from lower to higher levels." The sexual dihaploids of *Panicum maximum* bring new evidence to sustain the opposite thesis: haploidy plays a role in maintaining and increasing variability at the diploid level (de Wet, 1980).

SUMMARY

Natural populations of *Panicum maximum* are characterized by diploid-sexual and tetraploid-apomictic gene pools. Genetic exchanges between the two compartments are enabled by the following mechanisms: (1) tetraploidization by means of $2x \times 4x$ crosses, (2) temporary phase of sexuality and hybridization at the tetraploid level, and (3) haploidization and sexual return to the diploid level.

Thus variability in *P. maximum* could be perpetuated as the result of a continued contact between sexual and apomictic modes of reproduction through diploid-tetraploid-dihaploid cycles.

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