

## Allelic ratios and sterility in the agamic complex of the *Maximae* (*Panicoidae*): evolutionary role of the residual sexuality

M. Noirot

*Orstom, BP 5045, 34032 Montpellier Cedex 1, France*

*Key words:* *Panicum maximum*; apomixis; sterility; allelic ratio; residual sexuality.

### Abstract

The agamic complex of the tribe *Maximae* is constituted by two pools characterized by their own breeding system (sexuality on the one hand, facultative apomixis on the other hand). Facultative apomicts shows two types of embryo-sacs (aposporic or meiotic). Presence of aposporic embryo-sacs is genetically controlled by the dominant allele A (all apomictic plants are Aaaa). Meiotic embryo-sacs, constituting residual sexuality, can allow crosses between apomicts and theoretically the generation of other genotypes than Aaaa. Additionally, some cases of sterile apomicts appears in a breeding system known to bypass it.

This led us to study these two paradoxes. In fact, the sterility would exist when the ratio A/a is greater than 0.25. This would prevent the total overrunning of an apomictic population by AAAA genotypes. The role of residual sexuality is then to allow generation of true sexuals (aaaa) from apomicts (Aaaa).

### Introduction

Gametophytic apomixis is a frequent mode of reproduction in higher plants. According to Stebbins (1950), apomixis is often associated with polyploidy and interspecific hybridization. The apomictic and polyploid species are frequently related to a diploid and sexual species (Asker, 1979) to form a complex according to Harlan's definition (1970). Diploid-tetraploid-dihaploid cycles allow gene flow between the apomictic and sexual gene pools (De Wet and Harlan, 1970; Savidan and Pernès, 1982). When apomixis is facultative, an apomictic plant produces two types of embryo-sacs: aposporic embryo-sacs and meiotic embryo-sacs. Meiotic embryo-sacs must undergo double-fertilization to yield an embryo and the

proportion of meiotic embryo-sacs produced by an apomict constitutes its rate of residual sexuality (Warmke, 1954). Apomixis also can be pseudogamous, in which case pollen is necessary for albumen development and parthenogenetic release.

The tribe *Maximae* (*Panicoideae*) in the Graminae is a good example of an agamic complex where apomixis is both facultative and pseudogamous. The complex is characterized by two pools. The first is tetraploid ( $2n = 32$ ) and apomictic (facultative apomixis) and includes three botanical species (*Panicum maximum* Jacq., *P. infestum* Anders. and *P. trichocladum* K. Schum.) and their intermediate phenotypes (Combes, 1975; Pernès, 1975). In the natural populations of Kenya and Tanzania, where the centre of diversity is located, most accessions belong to the polyploid pool (Pernès, 1975). The second pool, containing only *P. maximum*, is diploid ( $2n = 16$ ) and its plants are entirely sexual and relatively rare (5%) (Combes, 1975; Pernès, 1975). Their lower morphological variability is included in that of the apomictic pool (Combes, 1975; Pernès, 1975) despite gene flow between these two pools (Savidan and Pernès, 1982).

The presence of an aposporic embryo-sac is genetically controlled by one *locus* with two alleles A and a (Savidan, 1982). The dominant allele A characterizes the apomictic plants. Experimental crosses between colchitetraploid sexuals (aaaa) and natural apomicts show that all the natural apomicts have an Aaaa genotype (Savidan, 1982). The rate of residual sexuality is a quantitative trait controlled by the genotype, but it can also be influenced by environmental conditions (Savidan, 1982). In the great majority of natural apomicts, the residual sexuality rate is between 2 and 10%, but can reach 90% in very rare genotypes: the hypersexual apomicts (Savidan, 1982). Residual sexuality should allow some crosses between apomicts, and therefore should give genotypes with more than one A allele (AAaa, AAAa and AAAA). In the wild, these genotypes are unknown.

According to Stebbins (1950), the great advantage of apomixis is that it bypasses sterility problems. Nevertheless, sterility is encountered in plants with aposporic embryo-sacs, especially in spontaneous dihaploids supposed to be Aa or in a trihaploid presumed to be Aaa (Combes, 1975). Such cases of sterility have also been encountered in the *Botrichloa-Dichantium* complex (De Wet and Harlan, 1970). In the *Maximae*, sterility is characterized by closed glumes, empty pollen and inhibition of anther and stigma dehiscence (pers. obs.). This sterility also affects the embryo-sacs (Savidan, 1982).

In this paper, a model is used to simulate the evolution of a population consisting of sexuals (aaaa) and facultative apomicts (Aaaa) and the results are compared with field observations. We show why an hypothesis of sterility, owing to some allelic ratios A/a, is necessary to explain the absence of any genotype different from Aaaa among the natural apomicts on the one hand, and the existence of sterile apomicts on the other hand. The effect of this sterility on the evolution of the apomictic population is discussed in relation with the role of residual sexuality.

**Model**

Consider a population, produced by an  $aaaa \times Aaaa$  cross, where 50% of the individuals are sexual  $aaaa$  and 50% are apomict  $Aaaa$ . It is assumed that: 1) all the genotypes have the same pollen production, 2) the pollen is randomly distributed through the population, and 3) all genotypes have the same fitness.

At a given generation, the population can include five different genotypes:  $aaaa$ ,  $Aaaa$ ,  $AAaa$ ,  $AAAa$  and  $AAAA$ . The first is sexual, the others are apomictic. Their relative frequencies are respectively  $x_1$ ,  $x_2$ ,  $x_3$ ,  $x_4$  and  $x_5$ , with  $x_1 + x_2 + x_3 + x_4 + x_5 = 1$ . Such a population gives pollen with three types of gametes,  $aa$ ,  $Aa$  and  $AA$ . Their relative frequencies are  $m_1$ ,  $m_2$  and  $m_3$ , with  $m_1 + m_2 + m_3 = 1$ . Thus, we have:

$$aa \quad m_1 = x_1 + x_2/2 + x_3/6$$

$$Aa \quad m_2 = x_2/2 + 4x_3/6 + x_4/2$$

$$AA \quad m_3 = x_3/6 + x_4/2 + x_5$$

The population of the female gametes is more complex. The embryo-sacs are produced by meiosis or by apospory. The former are  $aa$  or  $Aa$  or  $AA$ , like the pollen; the latter have the parental genotype ( $Aaaa$  or  $AAaa$  or  $AAAa$  or  $AAAA$ ). The relative frequencies  $f_1$ ,  $f_2$ , etc. of the different embryo-sacs depends on the residual sexuality rate ( $\alpha$ ):

\*sexuality,

$$aa \quad f_1 = x_1 + \alpha x_2/2 + \alpha x_3/6$$

$$Aa \quad f_2 = \alpha x_2/2 + 4\alpha x_3/6 + \alpha x_4/2$$

$$AA \quad f_3 = \alpha x_3/6 + \alpha x_4/2 + \alpha x_5$$

\*apomixis (rate =  $1 - \alpha$ )

$$Aaaa \quad f_4 = (1 - \alpha)x_2$$

$$AAaa \quad f_5 = (1 - \alpha)x_3$$

$$AAAa \quad f_6 = (1 - \alpha)x_4$$

$$AAAA \quad f_7 = (1 - \alpha)x_5$$

At the next generation, we have the following genotypic frequencies:

$$aaaa \quad x_1 = m_1 f_1$$

$$Aaaa \quad x_2 = m_2 f_1 + m_1 f_2 + f_4$$

$$AAaa \quad x_3 = m_3 f_1 + m_2 f_2 + m_1 f_3 + f_5$$

$$AAAa \quad x_4 = m_3 f_2 + m_2 f_3 + f_6$$

$$AAAA \quad x_5 = m_3 f_3 + f_7$$

## Results and discussion

The genotypes of 30 apomicts native to Kenya and Tanzania were determined by crosses with sexual colchitetraploids *aaaa*. All the *Aaaa* (Savidan, 1982). In our simulations, we have set the residual sexuality rate at 5% corresponding to a frequent situation in the complex (Savidan, 1982). Table 1 shows the simulated evolution of the population from the first to the thousandth generation. After the hundredth generation, the sexuals are practically absent (0.6%) and the genotype *Aaaa* represents less than 50% of the individuals. This frequency is significantly different from the observed value (100% of genotype *Aaaa* in the natural apomicts) despite the small sample (30). A higher rate accelerates the effect of this residual sexuality. A lower, but non-zero, rate decelerates it.

In a second simulation, total sterility of the *AAAA* genotypes was assumed. Before the hundredth generation, the results (see Table 2) do not differ greatly from those of the first simulation: the *Aaaa* genotypes become more frequent. However, this is still an insufficient fit with the observations. A third simulation, with sterility extended to the *AAaa* genotypes, also gave a poor fit with the observed values.

**Table 1.** Evolution of a population initially composed of 50% *Aaaa* (apomictic) and 50% *aaaa* (sexual) genotypes. Model without sterility.

Generations	GENOTYPES				
	<i>aaaa</i>	<i>Aaaa</i>	<i>AAaa</i>	<i>AAAa</i>	<i>AAAA</i>
1	38.40	61.30	0.30	0.00	0.00
10	2.40	89.20	8.20	0.20	0.00
50	1.10	61.20	31.50	5.80	0.40
100	0.60	43.40	39.90	14.40	1.70
500	0.08	12.60	33.40	38.10	15.80
1000	0.03	6.40	25.00	42.30	26.30

**Table 2.** Evolution of a population initially composed of 50% *Aaaa* (apomictic) and 50% *aaaa* (sexual) genotypes. Model with sterility for genotype *AAAA*.

Generations	GENOTYPES				
	<i>aaaa</i>	<i>Aaaa</i>	<i>AAaa</i>	<i>AAAa</i>	<i>AAAA</i>
1	38.40	61.30	0.30	0.00	0.00
10	2.40	89.10	8.20	0.20	0.00
50	1.10	61.80	31.50	5.60	0.03
100	0.70	46.20	40.40	12.70	0.08
500	0.50	36.30	43.90	19.30	0.14
1000	0.40	33.50	44.50	21.50	0.17

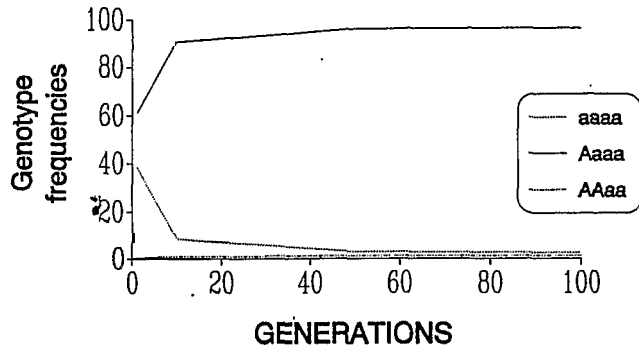


Fig. 1. Evolution of the genotypic frequencies of aaaa, Aaaa and AAaa (genotypes AAAa and AAAA are always missing) from the first to the hundredth generation (beyond this, there is no evolution).

To obtain a good fit, complete sterility when the genotype contains more than one A allele must be supposed (see Fig. 1). In this case, the genotypic frequencies are stabilized at the twentieth generation. The frequency of the sexuals aaaa is 2.6%, and the frequencies of the apomicts Aaaa and AAaa are respectively 96.2% and 1.2%. The other genotypes, AAAa and AAAA, are missing.

A sterility hypothesis, arising when the allelic ratio  $A/a$  is greater than 0.25, explains some other observations:

- five sterile dihaploids, taken at random from 70 for embryo-sac analysis, show aposporic embryo-sacs (Savidan 1982). The genotype of these dihaploids is supposed to be Aa;
- the colchicopolyloidization of one of these sterile dihaploids gave a sterile plant, with aposporic embryo-sacs, and its genotype is presumed to be AAaa;
- a trihaploid, issued from a hexaploid and fertile apomict, had aposporic embryo-sacs and was sterile. It was assumed to be Aaa.

The sterility of the Aa genotypes prevents overrunning of the diploid pool by apomixis, as expected for models in which sexuality and apomixis are in competition (Pernès 1975; Williams 1975; Marshall and Brown 1981). Also, some sterile dihaploids have been found in the agamic complex *Botriochloa-Dichantium* (De Wet and Harlan, 1970), known for its functional similarity to the *Maximae* complex.

In a population of AAAA genotypes, residual sexuality gives 100% of AAAA genotypes, and without mutations it is impossible to reobtain aaaa sexuals. With Aaaa genotypes, however, selfings and crosses can give aaaa sexuals at a rate of  $a/4$ . In fact, under natural conditions, the probability of emergence of a sexual off-type from sowing is near zero for two reasons. Firstly, the perenniality and the high density of the individuals in an old-established population limits plantlet survival. Secondly, the vigour of plants arising from sexuality is strongly variable, and the vigour of a descendant is very often less than that of the parents (Noirot, 1990).

In contrast, when the fitness of the apomictic population is strongly decreased, by factors such as disease, the residual sexuality provides a rapid process of adapta

tion. In this case, 25% of sexual off-types can provide a new genetic diversity in the population. The considerable morphological polymorphism of the apomictic pool could only be the result of the sexuality of the diploid pool through recurrent tetraploidization (Savidan and Pernès, 1982). Some polymorphic populations, such as the M'Gwakaethé population, geographically distant from the sexual pool (Pernès, 1975; Combes, 1975), may be the result of spontaneous explosion of sexuality (emergence of genotype aaaa), quickly overrun by apomixis. This theory corresponds to the "Model T" of Heslop-Harrison described by Marshall and Brown (1981). In the *Maximae*, as in other agamic species (Knox, 1967; Heslop-Harrison, 1961; Saran and De Wet, 1970), the residual sexuality rate of an apomict is influenced by the environment (Savidan, 1982). Thus, some environmental conditions can temporarily increase the rate  $\alpha$  and favour the probability of emergence of aaaa sexuals.

Lastly, it is probable that the allele A governs many functions of reproduction, as an initiator gene (Savidan, 1982). In the *Maximae*, apomixis acts like an inhibitor of sexuality traits. At a low dose, it allows parthenogenesis and interferes with the incompatibility system. Autosterility, frequent in diploid or tetraploid sexuals (Savidan and Pernès, 1982), is unknown among our apomictic accessions (pers. obs). At higher doses, the allele A represses some other functions such as male fertility, anther dehiscence and glume opening. For these reasons, gametophytic apomixis is very interesting for the functional study of sexuality.

### Acknowledgements

We thank Mr Pham for early contributions to this study and Drs. Hamon, Charrier, Gouyon, Lechowicz and Mrs Till-Bottraud and the referees for their helpful comments.

### References

- Asker, S. 1979. Progress in apomixis research. *Hereditas* 91: 231–240.
- Combes, D. 1975. Polymorphisme et modes de reproduction dans la section des *Maximae* du genre *Panicum* (Graminées) en Afrique. Mém. ORSTOM 77: 99 pp., ORSTOM, Paris.
- De Wet, J. M. J., and J. R. Harlan. 1970. Apomixis, polyploidy and speciation in *Dichantium*. *Evolution* 24: 270–277.
- Harlan, J. R. 1970. The evolution of cultivated plants. In *Genetic resources in plants. Their exploration and conservation*. Ed. Frankel and Benett, Blackwell Scientific Publ., Oxford.
- Heslop-Harrison, J. 1961. Apomixis, environment and adaptation. In: *Recent Adv. Bot.* 1: 891–895. Proc. 9th. Int. Cong., University of Toronto Press.
- Knox, R. B. 1967. Apomixis. Seasonal and population differences in a grass. *Science* 157: 325–326.
- Marshall, D. R., and A. H. D. Brown. 1981. The evolution of apomixis. *Heredity* 47(1): 1–15.
- Noirot, M. 1990. Diversité des mises en place des structures reproductives chez *Panicum maximum*. Logique d'une réponse optimale à des contraintes. Conséquences pour l'amélioration de la production semencière. Etudes et thèses: 145 pp., ORSTOM, Paris.
- Pernès, J. 1975. Organisation évolutive d'un groupe agamique: la section des *Maximae* du genre *Panicum* (Graminées). Mém. ORSTOM 75: 106 pp., ORSTOM, Paris.

- Saran, S., and J. M. J. De Wet. 1970. The mode of reproduction in *Dichantium intermedium* (Gramineae). Bull. Torr. Bot. Club 97: 6-13.
- Savidan, Y. 1982. Nature et hérédité de l'apomixie chez *Panicum maximum* Jacq. Trav. & Doc. ORSTOM 153: 160 pp., ORSTOM, Paris.
- Savidan, Y., and J. Pernès. 1982. Diploid-tetraploid-dihaploid cycles and the evolution of *Panicum maximum* Jacq. Evolution 36(3): 596-600.
- Stebbins, G. L. 1950. Variation and evolution in plants. 643 pp. Columbia University Press, New York.
- Warmke, H. E. 1951. Cytotaxonomy investigations of some varieties of *Panicum maximum* and *P. purpurascens* in Puerto Rico. Agron. J. 43: 143-149.
- Warmke, H. E. 1954. Apomixis in *Panicum maximum*. Amer. J. Bot. 41: 5-11.
- Williams, G. C. 1977. Sex and evolution. 200 pp. Princeton University Press, Princeton.

Received 25 September 1991;

accepted 15 April 1992.

Corresponding Editor: M. Lechowicz