Chapter 2

Apomixis and the Management of Genetic Diversity

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Introduction

Apomixis is a mode of reproduction (asexual propagation through seeds) that exists in many plants from different botanical families (review in Asker and Jerling 1992; Carman 1997). It is most frequent in the dicots Rosaceae and Asteraceae and in the moncot Poaceae. Some of these Poaceae genera are tropical forages with wide colonizing ability, e.g., *Panicum maximum*. From its center of origin in East Africa, through human activities it has expanded to West Africa, where it can be found colonizing roadsides, and to tropical regions of the Americas and Asia.

Apomixis attracts considerable theoretical interest as it may help us better understand the sexual mode of reproduction. It is also of practical interest to breeders as a means of genetic fixation, potentially offering the capability of indefinite multiplication of heterotic genetic combinations. In the case of apomictic tropical forages (see Valle and Miles, Chap. 10), the problem faced by breeders is how to overcome apomixis to take advantage of genetic recombination in order to create new genetic combinations to be maintained through apomixis. Another challenge is to transfer apomixis into crops in which heterosis has been well documented. Research projects focused on this goal are underway for pearl millet, *Pennisetum glaucum* (Hanna et al. 1993) maize (see Savidan, Chap. 11), and wheat (Carman 1992). Rice breeders are also interested as F_1 hybrids in rice show heterosis (see Toenniessen, Chap. 1).

Some scientists have solely pursued the simplest model of apomixis, that with a complete lack of sexuality, i.e., no possibility of recombination and evolution. In this case, population genetics models show a diffusion of apomixis genes into natural populations without a need for some form of selective advantage (Pernes 1971; Marshall and Brown 1981). If this holds true, transferring apomixis to crops could ultimately decrease genetic diversity in those crops and pose a threat to the environment. From modern apomictic varieties, the apomixis gene could move to landraces and wild ancestors in their center of origin. In a recent review of apomictic risk, van Dijk and van Damme (2000) based their discussion almost entirely on this model. However, before overstating this possibility, one should know more precisely how apomixis functions, what diversity is conserved in wild populations where apomixis is the dominant mode of reproduction, and how apomixis could be transferred to landraces.

To address these issues, this chapter discusses (*i*) genetic variation observed in progeny of apomicts, (*ii*) diversity observed in wild apomictic populations, (*iii*) evolution—processes of agamic complexes, and (*iv*) the possibility of transferring apomixis from synthetic apomictic crops to landraces and wild relatives.

Progeny of Apomictic Plants

In most apomicts, the apomictic mode of reproduction is linked to pseudogamy, whereby the endosperm develops only after fertilization while the embryo develops parthenogenetically (Nogler 1984; see Crane, Chap. 3). Apomixis can be split into two logical stages, which does not necessarily imply two different genetic controls (see Sherwood, Chap. 5): (*i*) development of an embryo sac without reduction and (*ii*) parthenogenetic development of the embryo without fertilization. This results in an embryo with $2n + 0$ chromosomes that is genetically identical to the maternal plant. However, in some cases, the embryo sac is reduced while in others fertilization occurs. It is therefore possible to distinguish four categories in the progeny of apomictic plants, with respective frequencies dependent on success rates of the different stages (Table 2.1).

The four categories can be identified through the use of chromosome counts (or flow cytometry) and isozymes or molecular markers. The IRD-CIMMYT Apomixis team used isozymes to score *Tripsacum* progeny (Berthaud et al. 1993). In *Poa*, isozymes and random amplified polymorphic DNA (RAPDs) have been used (Huff and Bara 1993: Barcaccia et al. 1994). It is difficult to find detailed results of progeny analyses in the literature. Frequently, only morphological distinctions between true (maternal) and off-type progeny are reported.

Data on *Panicum maximum* (Combes 1975) are presented in Table 2.2. In the F_2 generation of a *P. infestum* x *P. maximum* cross (T19 progeny), frequencies of plants produced through sexuality and of haploid plant production were high. In one case (progeny from T19-36-5), 40% of a 177-plant progeny were off-types, including seven haploids. F_2 progeny from other crosses involving accession T19 were less variable; only four haploids ($n + 0$) were found out of 1,500 observed. In *P. maximum*, the proportion of off-types, including $2n + n$ and $n + n$ was 3%, based on a total of 2,100 progeny observed. We can therefore conclude apomixis in *P. maximum* is facultative.

For the *Parthenium* (Asteraceae) species, Guayule and Mariola, frequencies of the four categories of progeny (Table 2.3) were extracted from Powers and Rollins (1945). Haploid plants were produced at a low rate. Most plants were produced from unfertilized unreduced female gametes, however, the

Table 2.1 Genetic constitution of progeny from apomictic plants

Female meiosis	Fertilization	
	yes	no
Yes	$n+n$*	$n+0$
No	$2n+n$**	$2n+0$

* also called 8_{II} (Rutishauser 1948)
** also called 8_{III} (Rutishauser 1948)

Table 2.2 Size of four categories defined in Table 2.1 for two *Panicum maximum* clones (from Combes 1975)

Clone	Progeny size	$n+0$	$n+n$	$2n+n$	$2n+0$
256	551	0	16	6	529
H267,1*	238	4**	27	2	205

* hexaploid plant, from $2n + n$ progeny of "267"
** 3 plants with $2n = 24$ and 1 plant with $2n = 23$

Table 2.3 Size of four categories defined in Table 2.1 for three types of progeny involving two *Parthenium* species. Adapted from Powers and Rollins (1945)

Combination	Progeny size	$n+0$	$n+n$ (%)	$2n+n$ (%)	$2n+0$ (%)
P. argentatum x *P. argentatum*	342	0	14 (4)	5 (1.5)	323 (94.5)
P. argentatum x *P. incanum*	888	2	48 (5.4)	78 (8.8)	760 (85.6)
P. incanum x *P. argentatum*	567	0	76 (13.4)	66 (11.7)	425 (75.0)

categories $n + n$ and $2n + n$ appeared at significant rates. Stebbins and Kodani (1944) showed a frequency of occurrence of $2n+n$ progeny of 5.6%, ranging from 0.14% to 49%. Thus, apomixis in *Parthenium* is largely facultative.

In *Tripsacum* we found an average 2.7% ($n + n$) progeny, 8.1% ($2n + n$), and 89.2% ($2n + 0$) progeny (Table 2.4). From seeds collected in wild populations, we analyzed the occurrence of $2n + n$ progeny (it is difficult to test for $n + n$ progeny in this situation because clones are distributed in small niches of land and interpollination occurs from identical genotypes, making detection of new isozyme patterns difficult). The frequencies for three wild populations of *Tripsacum* we observed are presented in Table 2.5. According to the table, it appears that within one species, but in various populations, the rate of $2n + n$ progeny is variable and significant, being quite high in the case of population #39 "La Toma." Experiments are in progress to analyze the effect of the environment (flowering and pollination) on the stability of these parameters.

For *Dichanthium* and *Bothriochloa* (Poaceae), Harlan et al. (1964) reported rates of $2n+n$ progeny from crosses between tetraploid species. These combinations, however, are interspecific and therefore are difficult to compare with the former examples. Bashaw et al. (1992) showed that in crosses between

Table 2.4 Estimation of apomixis rate and categories of progeny from chromosome counts and isozyme analyses of *Tripsacum* populations (Berthaud et al., unpublished data)

Pop ID	Plant ID	Species tested	Size	2n = 72	90	108	n+n	2n+n	% n+n	2n+n	2n+0
24	143	DHBV	10	10	0	0	0	0	0	0	100
28	163, 164	MZ	20	18	0	2	0	2	0	10	90
29	183	MZ	12	10	0	2	0	2	0	16.7	83.3
37	282, 283, 772	DM	46	43	0	3	1	3	2.1	6.5	91.4
43	358, 361	DM	14	14	0	0	0	0	0	0	100
47	414	BV	12	12	0	0	3	0	25	0	75
48	421, 423	DM	39	38	0	1	0	0	0	5	95
52	497	DM	10	5	0	5	0	5	0	50	50
53	545	IT	19	17	0	2	2	2	10.5	10.5	79
54	588	DH	15	13	0	2	0	2	0	13.3	86.7
55	608	DH	18	12	2	4	0	6	0	33.3	66.7
59	641	DM	7	7	0	0	0	0	0	0	100
60	654, 655	DM	23	21	0	2	0	2	0	8.7	91.3
62	675	DH	14	12	0	2	0	2	0	14.3	85.7
63	689	DH	12	11	0	1	0	1	0	8.3	91.7
67	734	DH	11	11	0	0	0	0	0	0	100
71	853	BV	14	13	0	1	0	1	0	7.1	92.8
72	879	DM	19	18	0	1	0	1	0	5.3	94.7
74	898	DM	21	20	0	1	5	1	23.8	4.8	71.4
83	960	DM	8	8	0	0	0	0	0	0	100
87	990	DM	5	5	0	0	0	0	0	0	100
96	1076	JL	35	31	0	4	1	4	2.85	11.4	85.7
98	1093	DHIT	23	23	0	0	0	0	0	0	100
100	11, 201, 121	IT	40	38	0	2	0	2	0	5	95
Total			**446**				**12**	**36**	**2.7**	**8.1**	**89.2**

Abbreviations used: TBV=*T. bravum* Gray, TDH=*T.dactyloides* var. *hispidum* (Hitchc.) De Wet et Harlan, TDM=*T.dactyloides* var. *mexicanum* De Wet et Harlan, TIT=*T. intermedium* De Wet et Harlan, TJL=*T. jalapense* De Wet et Brink, TLC=*T. lanceolatum* Ruprecht ex Fournier, TLT=*T. latifolium* Hitchc., TMZ=*T. maizar* Hernandez et Randolph.

Pennisetum flaccidum and *P. mezianum*, progeny of the $2n + n$ and $n + n$ types are produced (Table 2.6).

In summary, when progenies are produced from apomictic plants, we can observe plants of the maternal type, plants with a ploidy level different from the maternal type (genome addition), and/or plants with the same ploidy level that have undergone a cycle of recombination. With the apomictic mode of reproduction, we have a system favoring changes toward higher or lower ploidy levels. Changes toward higher ploidy levels are the result of fertilization within unreduced embryo sacs. Changes toward lower ploidy levels come from parthenogenetic development of a reduced egg cell, which is the result of meiosis and recombination. When apomixis is active, sexuality is not eliminated but rather distributed over several generations. This topic is discussed in greater detail below.

Table 2.5 Variation in chromosome number for progeny from wild populations of *Tripsacum dactyloides mexicanum*. (Seeds were collected in the wild)

Population	Genotype	Progeny tested	Chromosome number 72	90	108	2n+n	2n+n (%)
38	DM38-01	78	73		5	5	6.4
39	DM39-04	172	111	4	57	61	35.5
39	DM39-15	16	9		7	7	43.8
39	DM39-16	7	4	2	1	3	42.9
39	DM39-20	17	11		6	6	35.3
39	DM39-21	10	9		1	1	10.0
39	DM39-22	12	10		2	2	16.7
39	DM39-23	12	7		5	5	41.7
40	DM40-01	56	55		1	1	1.8
40	DM40-02	208	198		10	10	4.8
40	DM40-03	17	15		2	2	11.8
Totals/averages per population							
38		78	73		5	5	6.4
39		246	161	6	79	85	34.6
40		281	268		13	13	4.6
Totals/averages, all populations							
		605				103	17.0

Diversity in Wild Apomictic Populations

Pernes (1975) described polymorphisms observed in wild populations of *Panicum maximum* in East Africa, which is the center of diversity for this species. He identified three types of populations: (*i*) monomorphic populations; (*ii*) polymorphic, with disjointed variation and distinct genotypes; and (*iii*) polymorphic, with discrete variation.

The latter was discovered in zones where sexual diploids and apomictic tetraploids were sympatric. The IRD-CIMMYT team's observations during collections of wild *Tripsacum* led to the same typology. In the case of *Tripsacum*, however, different species can coexist in the same population. Diploid populations are more frequent than in *Panicum*, and several ploidy levels in within species have been discovered in the same populations.

Three different species were found to coexist in a multispecific wild *Tripsacum* population ("La Toma" population #39) near Tequila, Jalisco, Mexico: *T. pilosum*, a diploid sexual species, and two apomictic tetraploid species, *T.bravum* and *T. dactyloides mexicanum*. Using fingerprinting, restriction fragment length polymorphisms (RFLPs) and isozymes, M. Barré et al. (personal comm.) identified most of the diploid plants. Plants belonging to the

Table 2.6 Size of categories defined in Table 2.1 for two *Pennisetum flaccidum* x *P. mezianum* crosses. From Bashaw et al. (1992)

Progeny type	Progeny size	n+0	n+n	2n+n & n+n*	2n+n	2n+0 (%)
PI315868xPI214061	2,505	-	51	20	77	2428 (96.9%)
PI220606xPI214061	3,040	-	58	72	148	2892 (95.1%)

* This hybrid category has been recognized on morphological traits. Not all the hybrids were analyzed cytologically.

two tetraploid species were distributed in clones of variable size (Table 2.7). The genetic diversity in this population was distributed among 54 different diploid plants, six triploid clones (11 plants), and 18 tetraploid clones (83 plants). We conclude that there are almost no "widespread" genotypes in these populations. Moving from one population to another, new genotypes of the same species are found. In Mexico, populations #38 and #39 ar e about 10 km apart and both contain *T. bravum* and *T. dactyloides mexicanum*. Nevertheless, their genotypes are distinct. As a rule of thumb, the probability of finding distinct genotypes within a distance of 50 to 100 m is quite high.

In population #38, we analyzed 94 asexually reproducing triploid and tetraploid plants, distributed in 24 clones, i.e., four plants per genotype on average. Ellstrand and Roose (1987) observed 5.9 plants per clone in a literature survey of studies involving asexually reproducing plants. Wild populations of dandelion (*Taraxacum sp*, Asteraceae) and *Antennaria sp.*(Asteraceae) are comparable (Lyman and Ellstrand 1984; Ford and Richards 1985; Bayer 1990).

Table 2.7 Distribution of clones in *Tripsacum* wild population "La Toma"

Type*	Chromosome no.	Size	Type*	Chromosome no.	Size
BV1	72	33	DM12	54	1
BV2	72	3	DM13	54	2
DM1	72	4	DM14	72	1
DM2	72	2	DM15	72	1
DM3	72	2	DM16	72	1
DM4	72	27	DM17	54	1
DM5	54	5	DM18	54	1
DM6	90	1	DM19	54	1
DM7	108	1	DM20	72	1
DM8	72	1	DM21	72	1
DM9	72	1	DM22	72	1
DM10	72	1	DM23	72	1

* BV = *T. bravum*; DM = *T. dactyloides mexicanum*

In summary, studies of wild populations demonstrate that apomixis does not produce the uniformity that is often simplistically suggested. Diversity is maintained in these populations. Mechanisms generating and maintaining this diversity may involve genetic exchanges between different *Tripsacum* types and genetic recombination as previously described.

Ploidy Cycles and Organization of Agamic Complexes

In agamic complexes, two pools exist: one is sexual diploid and the other is apomictic polyploid (very often triploids and tetraploids). Plants considered to be apomictic present a certain amount of sexuality, at a rate we will call "k." Authors of reviews on apomixis (Nogler 1984; Asker and Jerling 1992) conclude that facultative apomixis is the most common. Obligate apomixis, when found, occurs when k = 0, and is under the same genetic control as facultative apomixis.

In many cases, apomixis and pseudogamy (endosperm produced after fertilization by pollen) are found together. Pseudogamy is the rule for apomictic Poaceae, Rosaceae, and Ranunculaceae. In *Taraxacum* (Asteraceae), fertilization is not needed for endosperm development (Ford and Richards 1985), while in *Parthenium*, which belongs to the same family, seeds are produced only after pollination, demonstrating that fertilization is needed for endosperm development (Powers and Rollins 1945).

Taraxacum and *Parthenium* Agamic Complexes (Asteraceae)

Taraxacum sp. is present on five continents and about 2,000 species have been described. The base chromosome number is eight, and diploid and tetraploid forms exist. Diploid forms are sexual and, depending on the species, self-incompatible or self-compatible.

Polyploid forms are autonomous apomicts, either facultative or obligate. Fruits (propagules) can be obtained without pollination, after eliminating anthers and stigmas (Mogie and Ford 1988).

In *Parthenium* (Asteraceae), diploid forms with $2n = 2x = 36$ are sexual, and polyploid forms with $2n = 54, 72, 90$, or 108, are apomictic. In this genus, pseudogamy is prevalent and therefore fruits are not produced in the absence of pollen (Powers and Rollin 1945). Ploidy buildup occurs through production of $2n + n$ progeny (Powers and Rollins 1945), and production of haploids from hexaploids has been documented (Powers 1945). In this case, a cycle exists between tetraploids, hexaploids, and triploids, with a possibility of incorporating diploid forms into the cycle through their production of $2n + n$ progeny with 54 chromosomes.

Capillipedium-Dichanthium-Bothriochloa Agamic Complex (Poaceae)

The genera of *Capillipedium, Dichanthium, and Bothriochloa* are distributed over Europe, the Mediterranean region, Asia, Australia, and the New World, and have been studied in detail by Harlan, de Wet, and coworkers. De Wet (1968) described a possible evolution in the genus *Dichanthium* based on ploidy cycles involving diploids, tetraploids, and haploids. In a broader approach, de Wet and Harlan (1970) described the interrelationships between species of the three genera of this agamic complex (Figure 2.1). The most common ploidy levels are $2x, 4x, 6x$, as well as some pentaploid forms. Diploids are sexual, and polyploids are apomictic. However, forms from the New World are sexual and polyploid. Triploid forms are not mentioned. Gene flow occurs in several directions, but in some cases is limited by incompatibility barriers. Genetic exchanges between *Capillipedium* and *Dichanthium* are effective only when species of *Bothriochloa* are involved as genetic bridges. Haploid production was detected experimentally and haploid plants were found to be either sexual or sterile. Tetraploid plants can be recovered from these dihaploids through the formation of $2n + n$ progeny, with n proceeding from pollen of tetraploid plants. Rates of $2n + n$ production of up to 15% have been observed.

Panicum maximum Agamic Complex (Poaceae)

"Guineagrass" has its origin in East Africa. It has colonized West Africa as well as the tropical areas of the New World. This agamic complex includes three species: *Panicum maximum, P. trichocladum,* and *P. infestum* (Combes 1975). *Panicum maximum* is widely distributed and sexual diploid forms have been identified (Combes and Pernes 1970), though they are very rare, having only been found in three very limited areas in Tanzania (Combes and Pernes 1970; Nakajima et al. 1979). The other forms are tetraploid and facultative apomicts. Occasionally, penta- and hexaploid forms have been detected.

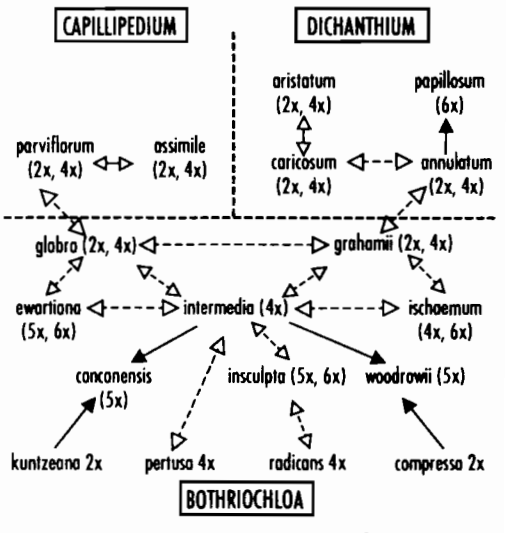

Figure 2.1 Continuous introgression and hybridization without further introgression in an apomictic complex including three genera, *Bothriochloa, Capillipedium, Dichanthium,* and 18 species. Adapted from De Wet and Harlan (1970).

Table 2.8 Distribution of clones according to ploidy level from the *P. maximum* collection established in Côte d'Ivoire (Combes 1975)

Total	2x	3x*	4x	5x**	6x**
551	19	0	506	12	13

* 3x ploidy level not found in wild populations
** 5x and 6x overrepresented in this collection (from over-collecting in these populations)

Table 2.8 shows the distribution of clones according to their ploidy level in a collection established in Côte d'Ivoire.

Triploid plants have been experimentally obtained from hexaploids ($n + 0$ progeny) as well as from diploid x tetraploid crosses. (Poly-) haploids also have been experimentally obtained from tetraploids, and the resultant plants have been either sexual or sterile (potentially apomictic as shown by embryo sac analyses). These findings led Savidan and Pernès (1982) to propose an evolutionary scheme based on ploidy cycles involving di-tetra-haploid levels as in the *Dichanthium* complex. The change from diploid to tetraploid is realized through $2n + n$ hybridization with pollen from tetraploid plants. In this system, sexuality is maintained at the diploid level. Contact between diploid and tetraploid plants allows genetic exchange between these pools (compartments) and creation of sexual tetraploid plants, allowing the release of new genetic diversity at the tetraploid level.

Paspalum Agamic Complex (Poaceae)

The center of diversity for the genus *Paspalum* is in South America. Studies conducted by Quarin (1992), Norrman et al. (1989) and collaborators at the Instituto de Botanica del Nordeste, Corrientes, Argentina (IBONE) show that many species in this genus have genetic pools at two or more ploidy levels (Table 2.9). In the pool with the lowest ploidy level, plants are sexual and self-incompatible, while in pools with higher ploidy levels, they are apomictic and self-compatible. In many cases, the two pools are at the diploid and tetraploid level (group 3 of Table 2.9). In some species, however, the sexually self-incompatible plants are tetraploid and the self-compatible apomictic plants are hexaploid or octoploid (group 6 of Table 2.9).

Some species that are sexual and self-compatible at the tetraploid or hexaploid level (groups 5 and 7 of Table 2.9) are not apomictic at higher ploidy levels. In other species, triploids are often apomictic and are found in species with sexual diploids and apomictic tetraploids.

As with previously cited agamic complexes, sexual forms are found at the lowest ploidy level and apomictic forms at the other levels. However, in this example, the relationship includes the incompatibility system. Apomictic plants are self-compatible and the corresponding sexual plants are self-incompatible. Experiments should be conducted to determine whether this also occurs in other agamic complexes.

Tripsacum Agamic Complex (Poaceae)

The *Tripsacum* genus is restricted to the New World, from 42°N to 24°S. Its center of diversity (or origin) is located in Mexico and Guatemala, and 11 of the 16 species described for the genus are found in this region. These 11 species show different ploidy levels both within and among themselves. The collection the team assembled from Mexico displayed the following distribution (unit = one ploidy level of one species in one population): diploids, 16.4%; triploids, 7.9%; tetraploids, 72%; penta- and hexaploids, 3.7%.

When compared to other agamic complexes, a high frequency of triploid plants in the *Tripsacum* complex was observed. These wild triploid plants are apomictic, produce fertile pollen, and set good seed. All of the natural polyploids we observed were apomictic (Leblanc et al. 1995; and unpublished data).

Diploids are sexual, and progeny with $2n + n$ chromosomes from apomictic plants occur at a significant frequency (Tables 2.4 and 2.5). Through this mechanism, many hexaploids were produced experimentally or detected in seeds collected from a wild population. Natural hexaploid plants in wild populations were observed at a lower frequency than in the seed progeny we analyzed.

Triploid plants can be obtained in four ways: (*i*) from $2n + n$ hybridization within diploids, (*ii*) from crosses between diploid and tetraploid plants, (*iii*) from haploidization of hexaploids ($n + 0$ progeny), or (*iv*) from asexual propagation of apomictic triploids. Evaluation of these possibilities is currently underway. In addition we have observed the presence of triploids, tetraploids, and hexaploids, and absence of diploids in some wild populations, which suggests that some triploids could have originated from haploidization of hexaploid plants. In populations containing diploids and triploids, there is a possibility of $2n + n$ hybridization, with $2n$ from the triploid female and n from a diploid male leading to the production of new tetraploid plants. We have documented such an event in seeds from one wild population. This event shows one possible route of gene exchange from the diploid to the tetraploid genetic pool. We did not discover any sexual tetraploid *Tripsacum*, but residual sexuality exists in apomicts, which permits production of $n + n$ progeny. This sexuality favors creation of new diversity at the tetraploid level by allowing crosses between apomictic plants.

Our model (Figure 2.2) suggests that in the *Tripsacum* agamic complex, sexuality fosters two stages: (*i*) a change from tetraploidy to

Table 2.9 Distribution of species of *Paspalum* according to their incompatibility system, ploidy level, and meiosis behavior (from studies at IBONE, Quarin, personal comm.)

Species	2x	3x	4x	5x	6x	8x
almum	sex, SI+	-	apo, SC*			
bertonii	sex, SI+	-	apo, SC*			
brunneum	sex, SI+	-	apo, SC*			
compressifolium	sex, SI+	-	apo, SC*		apo, SC*	
coryphaeum	sex, SI+	-	apo, SC*			
cromyorrhizon	sex, SI+	-	apo, SC*			
dedeccae	sex, SI+	-	apo, SC*			
denticulatum	sex, SI+	-	apo, SC*			
distichum	sex, SI+	-	apo, SC*		apo, SC*	
equitans	sex, SI+	-	apo, SC*			
haumanii	sex, SI+	-	apo, SC*			
hydrophilum	sex, SI+	apo, SC*	apo, SC*			
indecorum	sex, SI+	-	apo, SC*			
intermedium	sex, SI+	apo, SC*	apo, SC*			
maculosum	sex, SI+	-	apo, SC*			
modestum	sex, SI+	-	apo, SC*			
notatum	sex, SI+	apo, SC*	apo, SC*			
palustre	sex, SI+	-	apo, SC*			
procurrens	sex, SI+	-	apo, SC*			
proliferum	sex, SI+	-	apo, SC*			
quadrifarium	sex, SI+	apo, SC*	apo, SC*			
rufum	sex, SI+	-	apo, SC*			
simplex	sex, SI+	-	apo, SC*			
boscianum	-	-	sex, SC+			
dasypleurum	-	-	sex, SC+			
dilatatum	-	-	sex, SC+	apo, SC-		
regnellii	-	-	sex, SC+			
virgatum	-	-	sex, SC+			
durifolium	-	-	sex, SI+		apo, SC*	
ionathum	-	-	sex, SI+			apo, SC*
conspersum	-	-	-		sex, SC+	
inaequivalve	-	-	-		sex, SC+	
laxum	-	-	-		sex, SC+	
ramboi	-	-	-		sex, SC+	

sex = sexual mode of reproduction; apo = apomictic mode of reproduction; SC = self compatible; SI = self incompatible; + = meiosis regular; * = meiosis irregular; - = meiosis with many univalents

hexaploidy through $2n + n$ hybridization, and (*ii*) a change from hexaploidy to triploidy by meiosis and parthenogenetic development of the embryo. From triploidy to tetraploidy the pathway is as previously described ($2n + n$ hybridization) and involves diploid plants as pollinators. Complete cycles of tri-tetra-hexa-haploid plants linked to diploid plants are possible. During these cycles, recombination and fertilization events occur, helped by the parthenogenetic development of reduced embryo sacs and by fertilization of unreduced embryo sacs. Apomixis, in this case, enhances the functioning of sexuality that is distributed over several generations.

Cycles and Sexuality

In all agamic complexes, two different ploidy pools are found: a lower ploidy pool (usually diploid) with sexual forms and a higher ploidy pool (usually several ploidy levels, the most frequent being the tetraploid level) with apomictic forms. Absence of apomixis at the diploid level is thought to be due to either a lack of expression of this trait at this ploidy level or to an absence of transmission through haploid gametes (Nogler 1984; Grimanelli et al. 1998). The sexual pool is where most of the genetic recombination occurs and is therefore the pool where most of the selection on new combinations is acting.

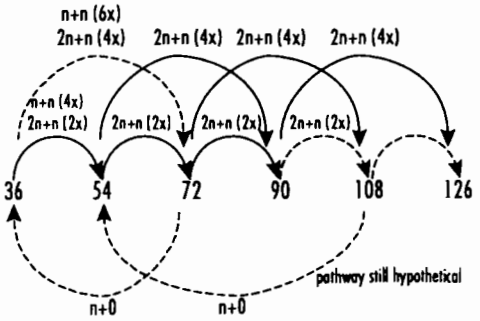

Figure 2.2 Evolution of ploidy levels in *Tripsacum* from fertilization of female gamete (n or 2n) by a male gamete (n) from 2x, 4x or 6x plants or parthenogenetic development of egg cell (n+0).

Gene flow from the diploid to the polyploid pool is realized in several ways. Diploid sexual plants, in some cases, can produce $2n$ female gametes (Harlan and de Wet 1975). If these gametes are pollinated by pollen from tetraploid plants, tetraploid progeny will be produced that will be sexual to a certain extent, providing an opportunity for a new burst of diversity to be tested at the tetraploid level. Another flow, as discussed earlier, comes from the pollination of unreduced eggs from triploid plants by normal pollen grains from diploid plants. The triploid plants can result from crosses between diploid and tetraploid plants. As can be seen, many opportunities exist for the diploid pool to contribute to the genetic diversity of the apomictic tetraploid pool. In the *Antennaria* complex, several genomes from diploid species can be accumulated in polyploid species (Bayer 1987).

In the polyploid apomictic pool, new genetic combinations may also arise through residual sexuality ($n + n$ progeny). We have also seen evidence that sexuality is distributed over several generations by creation of $2n + n$ progeny in one generation, followed by $n + 0$ progeny in the next generation. By permitting some perenniality for each stage of the sexual cycle, this wealth of genetic recombination is favored by apomixis, and it may be characteristic of the apomictic mode. More experimental data and modeling are required to isolate all of the factors involved in the genetic recombination of apomicts.

Management of Apomictic Varieties

Two types of apomictic varieties can be distinguished: forage varieties, which are already released as apomictic varieties, and apomictic varieties of crops such as maize and pearl millet, which may be released in the near future.

In the breeding of apomictic forage grasses, sexuality is involved at different steps and permits genetic recombination (Valle and Miles 1992; see Valle and Miles, Chap. 10). Released varieties are apomictic and have been distributed mainly outside their centers of diversity. In this instance, breeding activity is generating new genetic diversity.

Because projects are now underway to transfer apomixis to pearl millet, maize, wheat, and rice, we must consider the consequences of apomixis on the diversity management of landraces and that apomixis drastically reduces the recombination rate. It is important to remember that these landraces and their wild ancestors represent our current reservoir of genetic diversity. Thought should also be given to conserving the diversity of wild ancestors that grow near fields planted with apomictic varieties, which could be recipients of apomixis genes through naturally occurring gene flow.

Projects to transfer apomixis to pearl millet and maize have reached an intermediate stage: advanced generations of interspecific hybrids between apomictic forms and cultivated species have been produced that retain the apomictic trait. In the case of rice, possible sources of apomixis are yet to be identified. For wheat, F_1 and BC_1 hybrids between *Triticum* and *Elymus* have been produced (Peel et al. 1997; Savidan et al., Chap. 11). Pearl millet and maize are allogamous crops and so methods must be developed to maintain genetically adaptative processes once this new mode of reproduction is introduced. In its current design, the *Pennisetum* project considers the creation of tetraploid apomictic varieties of pearl millet (Dujardin and Hanna 1989). Upon release, the distinct ploidy levels of currently cultivated millet and the tetraploid apomictic new varieties will act as a genetic barrier between them. Dissemination of apomixis gene(s) from the tetraploid to the diploid level would involve production of triploid plants, which are usually male sterile; so dissemination through triploids should be negligible. However, in agamic complexes, apomixis seldom occurs at the diploid level. Some mechanism may suppress the expression of apomixis or impeach transmission to the diploid level. In the pearl millet program, there is no clear evidence that apomixis can be expressed at the diploid level. In contrast, a few BC_2 diploid-like hybrids in the maize-*Tripsacum* program were found to express apomixis (Leblanc et al. 1996). These plants are $2n = 28$ with $x = 10$ from maize and $x = 18$ from *Tripsacum*. Furthermore, triploid *Tripsacum* are male and female fertile. Thus, tetraploid apomictic varieties of maize will probably not restrict diffusion of apomixis gene(s) to other maize lines or its wild ancestor, teosinte. Therefore, the models of diffusion of apomixis discussed below are based on diploidy.

Apomixis fixes heterosis, thereby presenting two options for its use: (*i*) to produce apomictic F_1 hybrids through breeding programs and release them to farmers as end products; and (*ii*) to release to farmers apomictic varieties that would be used to transfer (diffuse) gene(s) to landraces, which would eventually become apomictic. In the latter case, breeding for apomixis would be a local activity. In fact, these two options are complementary and related as they pertain to the diffusion of apomixis gene(s). F_1 apomictic hybrids could be released in an area where landraces and wild relatives still exist. The transfer of the gene to these landraces and wild relatives will depend on the parameters cited above in option 2.

Transfer of Apomixis Gene(s) and Evolution of Landraces

We deduce from Sherwood (see Chap. 5), that apomixis is probably initiated by one dominant gene (see also Valle and Savidan 1996). The active *A* allele of this "apomixis gene" would be found mostly in the

heterozygous condition (*Aa*). The homozygous stage (*AA*) has been considered lethal in some cases (Nogler 1984). Nevertheless, in discussing apomixis transfer, we will consider three models: (*i*) apomixis is active as a dominant trait, either heterozygous or homozygous (*Aa* or *AA*) with the recessive homozygote (aa) being sexual; (*ii*) apomixis is active only as a heterozygote (*Aa*), with the recessive homozygote (*aa*) being sexual; and (*iii*) apomixis is only expressed as a recessive homozygote (*ss*), while *sS* and *SS* are sexual. We will also consider a residual rate of sexuality, k, in apomictic plants, with $0 < k < 1$.

Simple models of population genetics predict, in the absence of selection, the diffusion of the apomixis gene (Pernès 1971; Marshall and Brown 1981). According to the models, it is possible for the apomixis gene to transfer to landraces, such as maize or pearl millet, and to inadvertently move to wild relatives (Pernès 1971; van Dijk and van Damme 2000).

In model 1, there is one dominant allele for apomixis and three categories of genotypes at generation n: *AA* (apomictic) at a frequency of P_n, *Aa* (apomictic) at a frequency of $2Q_n$, and *aa* (sexual) at a frequency of R_n. Gametes for generation n+1 are distributed according to the following frequencies: male gametes *A* have a frequency of $P_n + Q_n$ and gametes *a* have a frequency of $Q_n + R_n$; female gametes *A* have a frequency of 0, gametes *a*, a frequency of R_n, gametes *AA*, a frequency of P_n, and gametes *Aa* a frequency of $2Q_n$.

Three genotypes will appear at generation n + 1 with the following frequencies (random mating of gametes): *AA* at a frequency of $P_{n+1} = P_n$, *Aa* at a frequency of $2Q_{n+1} = 2Q_n + R_n(P_n + Q_n)$, and *aa* at a frequency of $R_{n+1} = R_n(R_n + Q_n)$.

With $P_n + 2Q_n + R_n = 1$, we obtain $Q_n = 1/2(1 - P_n - R_n)$ and the recurrence relation:

$$R_{n+1} = 1/2R_n(1 - P_n + R_n)$$

Equilibrium is reached for $R = 1$, the population being entirely sexual, or for $R = 0$, the population being completely apomictic. This model is identical to the model proposed by Fisher (1941) for autogamy. In fact, apomictic plants self-reproduce, however they simultaneously release pollen with the dominant allele to the sexual plant forms; consequently, a portion of the progeny of sexual forms becomes apomictic.

If we take into account a rate of residual sexuality, k, the variation in frequency for *A* allele becomes $P_{n+1} + Q_{n+1} = (P_n + Q_n)(1+1/2(1-k)R_n)$ (Pernès 1971).

The change in frequency of allele *A* from generation n to generation n + 1 is a function of Rn, the frequency of the recessive allele, and a function of k. A zero value for k (obligate apomixis) maximizes the frequency of *A*, while higher values of k reduce the frequency of *A*. This variation would be zero if $k = 1$, i.e., when all plants are sexual with either the *A* or *a* allele.

In this model, we assume random mating of gametes. Transfer would be favored if an apomictic variety, homozygous for *A*, were interplanted with the variety (landrace) to be modified. In the case of maize, by detasselling and harvesting only the landrace, only heterozygous progeny would be produced. These new plants would be apomictic and genetically fixed. Their ability to evolve would rely on the rate of residual sexuality, k. A proportion k of the apomictic forms can be fertilized by pollen from other sources. Moreover, pollen from the first generation of apomictic forms can be used to pollinate the landrace. After several cycles of such backcrossing, the new variety will be identical to the landrace except that it carries the apomixis gene. Evolution in these "new" landraces will depend on the rate of residual sexuality that is retained at the end of the transfer process.

In model 2, apomixis is active in plants with the *Aa* association of alleles. The aa genotypes are sexual. If Rn is the frequency of *aa* genotypes (sexual) and Qn is the frequency of *Aa* genotypes (apomictic), frequencies in the next generation (n + 1) will be $R_{n+1} = R_n(1-1/2Q_n)$, and $Q_{n+1} = Q_n(1 + 1/2R_n)$. In this case, the apomixis allele, *A*, diffuses in the population as

$$1 + 1/2Rn > 1 \text{ and } Qn + 1 > Qn.$$

We can use this model to define conditions of equilibrium between sexual and apomictic forms if a differential fitness exists between the two forms. With a fitness of 1 + s for the *aa* and 1 for the *Aa*, the frequency changes from generation n to generation n + 1 are as follows:

$$R_{n+1} = R_n(1 - 1/2Q_n).(1 + s)/(1 + sR_n + sR_n^2)$$

$$Q_{n+1} = Q_n(1 + 1/2R_n).1/(1 + sR_n + sR_n^2).$$

In this case, equilibrium between sexual and apomictic forms will be reached for s = 1/1 + R. Initially, when apomixis starts to be established in a population, R is close to 1, and equilibrium can be reached with s values close to 0.5. The fitness advantage of the sexual forms in relation to the apomictic forms has to be at least 1.5:1 to reach the equilibrium. Once apomixis is widely established, R is lower, and equilibrium will be reached only with higher s values. In the extreme case of Q close to 1, equilibrium will be reached with s values close to 1. In this instance, sexual forms will have to produce twice as many seeds as apomictic forms to survive in the successive generations.

If model 2 applies to apomictic varieties, transfer of apomixis to landraces could be accomplished according to the process found in model 1; but the transfer will take longer (at least one more generation) because the first generation will be made from *Aa* x *aa* crosses producing *Aa* and *aa* genotypes, not from *AA* x *aa* crosses, which produce only *Aa* progeny. Conservation of diversity in the apomictic landraces will depend, as in the former model, on the rate of residual sexuality, k.

In model 3, apomixis is active only in plants that are homozygous for the recessive allele *s*. In this case, *SS* (sexual) has a frequency of Pn, *Ss* (sexual) has a frequency of 2Qn, and *ss* (apomictic) has a frequency of Rn. Using this model, it can be shown (Pernès 1971) that the frequency of *S* behaves as follows:

$$Pn + 1 + Qn + 1 = (Pn + Qn)(1 - 1/2Rn)$$

The frequency of *S* is reduced from one generation to the next, as 1 - 1/2Rn is always lower than 1.

If the genetic control of apomixis follows this model, then transfer of apomixis will require at least two generations. The pathway to transfer can be imagined as follows:

1st generation: *SS* [sexual] female x *ss* [apomictic] male = *Ss* [sexual]

2nd generation: *Ss* [sexual] female x *ss* [apomictic] male = *Ss* [sexual] + *ss* [apomictic]

3rd generation: *Ss* [sexual] + *ss* [apomictic] x *ss* [apomictic] or *Ss* [sexual] + *ss* [apomictic] = *Ss* [sexual] + *ss* [apomictic] or *SS*[sexual] + *Ss* [sexual] + *ss* [apomictic]

The apomixis gene can diffuse within the population through backcrossing between plants from the first generation and the donor variety as male parent. In order to have apomixis transferred within a reasonable timeframe, the donor must be used as the male variety of each generation. After several backcrosses, the local variety will be transformed to an apomictic variety, but it will be almost identical to the donor variety. Therefore, if apomixis is active only when recessive alleles are present, it will be difficult to transfer apomixis to landraces while at the same time maintaining the original traits of these landraces. It would require (*i*) the use of

markers to retain the *a* allele, (*ii*) the production of near isogenic lines through backcrossing with the landrace, and (*iii*) the selfing of isogenic, heterozygous (*Aa*) lines to produce *aa* apomicts.

2*n* + *n* Progeny

In *Tripsacum*, we saw an average of 10% of progeny come from 2*n* + *n* hybridization; in some samples, this rate rises to 35%. Crosses between apomictic species of *Pennisetum* also produced this type of progeny (Bashaw et al. 1992). These forms are less frequent in other species, such as *Panicum maximum*. If this trait is inherited during the transfer of apomixis, what behavior can be expected from cultivated apomictic forms?

The transfer projects now underway consider a type of apomixis linked to pseudogamy. Once apomictic varieties are produced, most probably they will be also pseudogamous. In this case, we are concerned with the ratio between embryo ploidy and endosperm ploidy, as it has been often reported that a ratio different from 2:3 (or 2:5) would introduce some developmental incompatibility at the seed level and a loss in productivity (endosperm development also depends on maternal:paternal genome ratio; see Chap. 6, 11, 12, and 13). However, for the *Tripsacum*, we observed that triploid plants produce seeds even when their pollen environment comes mostly from tetraploid plants. In this case, the ploidy ratio between embryo and endosperm is 3:8. The 2*n* + *n* progeny we detected were from normal seeds with normally developed endosperm. In *Tripsacum*, the 2:3 ratio (or 2:5) between embryo ploidy and endosperm ploidy does not appear to be necessary for seed filling. In general terms, we have two hypotheses to consider:

1. Endosperm development is deficient when the ratio of embryo ploidy to endosperm ploidy is different from 2:3 (or 2:5). In this case, ears display poorly filled kernels (with 2*n* + *n* embryo) at harvest time. There is a potential loss of production due to the presence of these 2*n* + *n* embryos, but these kernels would not be selected as seed for the next generation.
2. Endosperm development is not affected by a ratio of embryo ploidy to endosperm ploidy different from 2:3 (or 2:5). In this case, kernels with 2*n* + *n* embryos would go undetected and could be used as seed for the next generation. Apomictic plants obtained from such embryos are triploid; they may produce normal seeds but the pollen could be sterile, which could limit field production. If the pollen is still fertile, as noted with triploid *Tripsacum*, no loss in production should be detected. However, ploidy buildup will occur, and many different ploidy levels will be stored in the same variety. This ploidy buildup could raise chromosome numbers to levels far above the optimum for productivity, potentially resulting in lower production.

In nature, 2*n* + *n* progeny production is a strategy that takes advantage of genetic recombination, as these plants would give rise, after meiosis, to some haploid progeny by parthenogenetic development of reduced embryo sacs. In the case of an apomictic crop, it is a trait that should be reduced or eliminated.

Relationship between Wild Relatives and Apomictic Varieties

For the purpose of discussing the relationship between wild relatives and apomictic varieties, we will use the maize-teosinte model, however, it is our belief that it can be extrapolated to pearl millet in instances where wild relatives are still in contact with cultivated plants. Teosinte is only found in Mexico and Guatemala. Relationships between wild relatives and maize are not identical over the distribution area of teosinte. The variety

parviglumis may be found in southwest Mexico and is considered to be a very wild form, with almost no link to modern maize. In the states of Michoacan and Mexico, teosinte should be considered a weed. An incompatibility system exhibited by these weedy teosintes, which efficiently controls gene flow from maize to teosinte, has been detected and analyzed (Kermicle and Allen 1990). Moreover, as described by Wilkes (1967), teosintes generally have a flowering period that is distinct from maize. These mechanisms limit gene flow between this wild relative and maize.

If we use model 1 to explain the transfer of apomixis from apomictic plants to landraces, we can envisage the following process. The first generation hybrid between teosinte (sexual, *aa*) and apomictic maize (*AA*) would be apomictic (*Aa*), and BC_1 plants with teosinte as female would produce *Aa* (apomictic) and *aa* (sexual) progeny. At each generation, the apomictic forms are fixed but they still participate in the next generation from sexual plants through their pollen, which can transfer the apomixis allele to sexual plants. Therefore, a portion of each generation's progeny becomes apomictic. We can then deduce that the apomictic allele will diffuse into the wild population. However, the assumptions made to simplify the model may not prove accurate when applied to the relationship between cultivated plants and wild relatives.

Cultivated maize and its wild teosinte relatives are, morphologically, widely distinct. Apomictic maize x teosinte F_1 hybrids will be apomictic and will breed true. Sexual maize x teosinte F_1s are known to have a low fitness due to their intermediate morphology and adaptation, and they are easily recognized morphologically. When they grow in a field, they are not harvested. However, if the hybrid is apomictic, its pollen will transmit the *A* allele at a rate of 50%. Pollination efficiency depends on synchronization between flowering of these hybrids and the wild relatives. As a lack of synchronization between the two types of plants is anticipated, the gene flow between them should be minimal. These observations deviate considerably from the assumptions posited in the model in which apomictic plants are expected to engage in pollination in proportion to their frequency in the population. Moreover, in the long run, the apomictic intermediate forms should have a lower fitness than the sexual forms, because the latter can take advantage of more new recombinations and adapt faster to environmental changes. As noted earlier, a stable polymorphism between sexual and apomictic forms is possible when fitness values of the two forms reach a certain ratio. We have also observed that the speed of apomixis diffusion is a function of the rate of residual sexuality—a high level of residual sexuality will slow apomixis diffusion.

Promoting Genetic Diversity and the Release of Apomictic Varieties

We base our models for apomixis diffusion on the hypothesis that this mode of reproduction is under a simple genetic control. Current knowledge about the mechanisms underlying apomixis, however, is very incomplete, especially regarding the expression of an apomixis gene in a new genetic background, as would be the case with a *Tripsacum* apomixis gene transferred into a maize background. If genetic control of apomixis in landraces and new varieties involves several genes or a major gene and modifiers, the dynamics of diffusion will be more difficult to describe and transformation of current varieties to apomictic varieties would have to be carried out by professional breeders. In this instance, apomixis could be used as a genetic fixation tool and new varieties with a complex genetic structure could be created and released. Such varieties would contribute to the maintenance of diversity at the farmer's field level.

Furthermore, if apomixis is controlled by multiple genes, the probability of diffusing this trait to wild relatives is extremely low. A wild plant would need to receive several genes (probably on several different chromosomes) from the cultivated plant to become apomictic. This transfer would certainly lower its fitness to a value unacceptable for survival in the wild.

If apomixis is under a simple genetic control, diffusion of apomixis to landraces and wild relatives is possible. Apomixis reduces recombination rates and could be perceived as a danger for conservation of genetic diversity of wild relatives and landraces. In actuality, current genetic diversity is the result of a long process of domestication, which is still underway in some regions of the world, especially where wild and cultivated plants continue to exchange genes, often within a traditional agricultural system. Somewhat surprisingly, it is in regions where traditional agriculture prevails that apomixis could be the most helpful. We know that obligate apomixis is an exception and facultative apomixis is predominant (Asker 1979). If during the transfer of apomixis to crops, residual sexuality is also transmitted and expressed in the new apomictic crop, we could rely on the rate of recombination inherent in this process to generate new genetic combinations. Even at low rates, new combinations may be interesting to farmers who could select and propagate them easily. As long as apomixis is not obligate, landraces can still evolve. It may also be possible to introduce new genes from "exotic" and modern sexual varieties. Crosses will occur only in the proportion k (rate of residual sexuality). But if these new products can be detected by markers or by their hybrid vigor, following selection, they could serve as an important source of seed for the next generation. The possibility and rate of evolution of these apomictic varieties will eventually depend upon the rate of residual sexuality; therefore, it will be important to consider this parameter when transferring apomixis from wild apomixis donor plants to first apomictic varieties. This rate of residual sexuality may depend on genetic factors. Controlling these factors, in order to adapt the value of this parameter in new apomictic varieties, could be extremely useful as we seek to conserve the genetic diversity of landraces and allow for their continual evolution.

Areas of traditional agriculture are repositories for most of the genetic diversity of crops. The conservation of this diversity is threatened, however, by changes in technical practices that can suppress current gene flow and by the introduction of new modern varieties with limited genetic diversity (e.g., F_1 hybrids). Producing new varieties from local germplasm may be advantageous to farmers, and it could be more easily accomplished if apomixis is incorporated into the breeding scheme (see Toenniessen, Chap.1). In this scenario, landraces with high genetic diversity would be maintained in these farming systems, thus limiting the diffusion of varieties with low genetic diversity. This diversity would serve as a reservoir for future evolution.

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