Chapter 5

The significance of apomixis in the evolution of the angiosperms: a reappraisal

Peter J. van Dijk & Kitty Vijverberg

Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, Boterhoeksestraat 48, 6666 GA Heteren, The Netherlands. E-mail: p.vandijk@nioo.knaw.nl (author for correspondence)

Apomixis, the ability to produce asexual seeds, constitutes, along with outcrossing and selfing, one of the three major breeding systems in the angiosperms. However, apomixis is by far the least common of these three, despite theoretical advantages of apomixis over outcrossing and selfing. Darlington and Stebbins argued more than 50 years ago that no completely apomictic (sub)genera exist within the angiosperms. Clones lack genetic variation and therefore the ability to adapt to changing environments. In addition it has been suggested that clones degenerate because of the accumulation of deleterious mutations. The commonly held view is therefore that apomicts are evolutionary dead ends, doomed to early extinction. Recent genetic studies provide insight in the genetic architecture of apomixis. Apomixis appears to be a genetically complex trait and may therefore be difficult to evolve. Recent progress in angiosperm phylogeny allows for the optimisation mapping of apomixis onto phylogenetic trees. Assuming that aposporous and diplosporous apomixis are non-homologous, the results indicate that gametophytic apomixis arose many times in the history of the angiosperms. Gametophyic apomixis is especially common in the Poaceae, the Asteraceae and the Rosaceae and its phylogenetic distribution is illustrated here within the former two. In both families apomixis is clustered at the (sub)tribal level, i.e. above the genus level. It is argued that some clades may be preadapted in such a way that apomixis evolves more easily, leading to parallel/convergent evolution of the trait. In addition, it now becomes clear that genes that encode for apomixis can be transmitted through pollen in hermaphrodites. In this way apomixis-genes can escape from early extinction and survive for longer periods of evolutionary time. The clustering of apomixis at the (sub)tribal level could then be due to a common ancestry or introgression of such apomixis-genes.

KEYWORDS: apomixis; agamospermy; angiosperm evolution; phylogeny.

INTRODUCTION

More than 50 years ago, Darlington (1939) and Stebbins (1950) declared apomicts as evolutionary dead-ends, doomed to early extinction and insignificant in the evolution of the angiosperms. In this chapter the significance of apomixis in the evolution of the angiosperms is reconsidered in the light of recent progress in the genetics of apomixis as well as in angiosperm phylogeny. The term apomixis will be used here for reproduction through asexual seeds, thus as a synonym to agamospermy (Nogler 1984). In this sense apomixis is an alternative to sexual reproduction, which can either be outcrossing or selfing. In the original definition of apomixis, Winkler (1908) included vegetative asexual reproduction, but only as a substitute for sexual reproduction.

Sexual reproduction involves the formation of reduced (*n*) gametes through meiosis and the production of a *2n* zygote by fertilisation. Sexual reproduction probably evolved shortly after the origin of the eukaryotes from asexual prokaryotic ancestors, some 850 mya (Cavalier-Smith, 2001). The first angiosperms date back to the Early Jurassic (190 mya) to Early Cretaceous (140 mya) (Sanderson & Doyle, 2001). Sexual reproduction thus long preceded the origin of the angiosperms. Sexual reproduction in the angiosperms involves double-fertilisation (Nawaschin, 1898), i.e., the first sperm cell fertilises the egg cell to produce the embryo and the second sperm cell fertilises the central cell to produce the endosperm, the tissue that nourishes the embryo. The flowers of the angiosperms are clear adaptations to sexual reproduction. Floral divergence causing reproductive isolation was responsible for most of the angiosperm diversity we see today. There can be no doubt that sexual reproduction was the ancestral state in the angiosperms. Apomixis is then the secondary evolution of asexual reproduction from sexual reproduction.

Outcrossing or biparental reproduction is a very inefficient way of reproducing, compared to forms of uniparental reproduction such as selfing and apomixis (Maynard Smith, 1978; Charlesworth, 1980; Bell, 1982). Theoretically, a dominant gene for apomixis will spread rapidly in a population of outcrossing hermaphrodites (Marshall & Brown, 1981). The underlying reason for this spread is that sexuals produce some apomictically reproducing offspring in crosses with apomictic pollen donors, whereas apomicts produce no sexually reproducing offspring in such crosses. Moreover, autonomous apomicts benefit from reproductive assurance whereas outcrossing sexuals depend on uncertain transfer of pollen for their seed set. Selfing has similar advantages over outcrossing, although compared to apomixis, the evolution of selfing may be handicapped by initial inbreeding depression (Lloyd, 1988).

Despite these theoretical advantages apomixis is much less common than outcrossing or selfing. Mogie (1992) roughly estimated the incidence of gametophytic apomixis (see below) as approximately 0.1% of angiosperm species. No completely apomictic (sub)genera exist in the angiosperms (Darlington, 1939; Stebbins, 1950). Apomicts always have closely related sexual sister taxa, with

which they can cross, when acting as pollen donor. This suggests that extant apomicts are of relatively recent origin and that apomictic lineages have short evolutionary life spans. Typically, apomictic lineages are confined to the terminal branches of phylogenetic trees.

The reasons for the early extinction of apomictic lineages are seen in the lack of genetic variation and consequently the lack of adaptability to changing environments. This lack of adaptive potential will be especially detrimental to apomictic lineages with respect to evolutionary arms races with rapidly evolving parasites and pathogens with short generation times ('Red Queen hypothesis'; Levin, 1975; Hamilton, 1980; Bell, 1982). Apomictic lineages are also prone to the accumulation of deleterious mutations, due to chance events in small populations (Muller, 1964) and to inefficient purging in large populations (Kondrashov, 1982).

Most disadvantages of apomixis are long-term, whereas the advantages of apomixis, such as transmission advantage and reproductive assurance are instantaneous. Intuitively this will lead to a rapid take-over of sexuality by apomixis, but Nunney (1992) has shown, based on computer simulations, that even if apomicts have a strong short-term advantage, increased extinction rates of apomicts will result in the 'twiggy' phylogenetic distribution outlined above. Moreover, clade selection results in the proliferation of sexual lineages, in which apomixis is difficult to evolve (Nunney, 1989). This corroborates with Lloyd's notion that the rarity of apomixis is probably due to the fact that apomixis is difficult to achieve in developmental terms and is often associated with infertility (Lloyd, 1988).

Types of apomixis. — There are different developmental pathways that can result in apomictic seeds. The main types will be described here briefly, for more detailed descriptions see Nogler (1984), Asker & Jerling (1992), Koltunow (1993) and Crane (2001).

In sporophytic apomixis somatic embryos are formed directly from a sporophytic cell in the nucellus (nucellar embryony) or the ovular integuments, without an intervening gametophytic generation. This type of apomixis is also called adventitious embryony, because normally also a sexual embryo is formed in the seed. Sporophytic apomixis is relatively common in (sub)tropical fruit trees such as citrus and mango (Richards, 1986). Plants with adventitious embryony are generally diploid, whereas plants with gametophytic apomixis are nearly always polyploid.

In this chapter we focus on gametophytic apomixis, which is the best-studied form of apomixis. Here an unreduced gametophyte or embryo sac is produced with an unreduced egg cell that develops parthenogenetically into an embryo. There are two forms of gametophytic apomixis: apospory and diplospory. Both types circumvent reduction of the chromosome number by normal meiosis (apomeiosis). In the case of apospory the unreduced gametophyte is formed from an unreduced nucellar initial which competes with the reduced gametophyte. In diplosporous apomixis, the normal reductional meiosis is replaced by a mitosis-like division (mitotic diplospory) or by a restitutional meiosis (meiotic diplospory). Examples of species exhibiting apospory are *Hieracium pilosella*, *Poa pratensis* and

Pennisetum squamulatum. Some examples of species exhibiting mitotic diplospory are *Antennaria alpinum* and *Tripsacum dactyloides*, and of meiotic diplospory: *Taraxacum officinale*, *Erigeron annuus* and *Boechera holboelli*. The developmental pathway of the unreduced gametophyte varies between species and can be used for further classification (Nogler, 1984; Crane, 2001).

In autonomous apomicts, the central cell of the embryo sac develops without fertilisation into the endosperm; in pseudogamous apomicts, fertilisation is necessary for endosperm development. Most apomicts are pseudogamous, but autonomous apomixis is common among apomicts in, for instance, the Asteraceae.

CONSTRAINTS IN THE DE NOVO EVOLUTION OF APOMIXIS

Recent studies on the genetics of apomixis have shed light on the genetic architecture of gametophytic apomixis. Crosses between sexuals and apomictic pollen donors indicate that apomixis (or parts thereof) is inherited as a dominant monogenic trait (see Grossniklaus & al., 2001a, for a review). However, even in species with monogenic inheritance it is doubtful whether apomixis is controlled by a single gene. In a number of species apomixis-recombinants have been reported that lack either parthenogenesis or apomeiosis: *Taraxacum officinale* (Van Dijk & al., 1999 & 2003), *Erigeron annuus* (Noyes & Rieseberg, 2000) and *Poa pratensis* (Albertini & al., 2001). These findings suggest that apomixis in other species may be controlled by a complex of closely linked genes.

A theoretical scenario for the de novo evolution of a two-gene apomixis system is outlined in Fig. 1. Assume that two dominant mutations occur in a population of outcrossing hermaphrodites. The first mutation m_1 changes a plant from meiotic into apomeiotic ($a \rightarrow A$) and the second mutation m_2 from fertilisation dependent embryo development to parthenogenetic embryo development ($p \rightarrow P$). Because a strict parthenogenetic plant cannot function as a seed parent, these mutations can only be combined in the cross between an apomeiotic seed parent and a parthenogenetic pollen parent. This automatically results in a triploid apomictic hybrid, suggesting that the relationship between gametophytic apomixis and polyploidy could be a direct one. This new apomictic plant can function as pollen donor in crosses with sexuals, thereby generating new, secondary apomictic clones. High clonal and microspecies diversities, commonly found in populations of apomicts, can be explained this way (Van Dijk, 2003).

However, the problem with this evolutionary scenario is that the mutations for apomeiosis and parthenogenesis are individually deleterious and will be selected against (Mogie, 1992). An apomeiotic mutant (which makes unreduced egg cells requiring fertilisation), when crossed with the common non-parthenogenetic genotype, produces offspring with a ploidy level that is elevated each generation: *2x* produces *3x*, *3x* produces *4x* and so on. Such a cycle of increasing ploidy levels is deleterious, because the ploidy levels that are tolerated by plants are limited (such

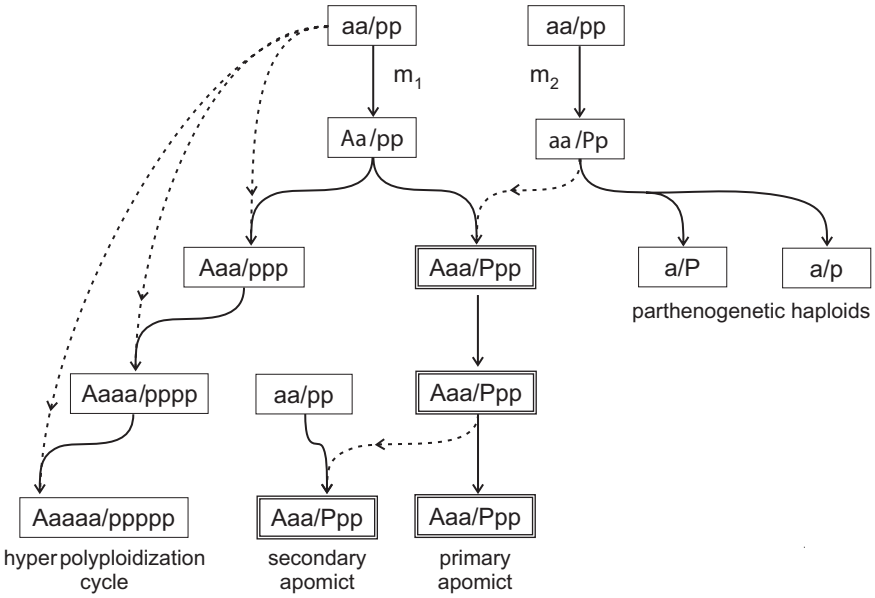

Fig. 1. A scheme showing a possible scenario for the evolution of apomixis based on an apomeiosis gene (A) and a parthenogenesis gene (P). Two dominant mutations occur in the same population: m_1; the mutation of meiosis (a) to apomeiosis (A). m_2: the mutation from non-parthenogenetic (p) to parthenogenetic (P). Individually these mutations are deleterious: repeated back-crossing of the apomeiotic plants to sexual pollen donors results in a cycle of increasing and lethal ploidy levels. Parthenogenetic diploid plants produce weak haploid offspring, in which all recessive deleterious mutations are expressed. Intercrossing of the two mutations generates a primary apomict. Backcrossing of this primary apomict with sexual seed parents generates a series of secondary apomicts. The solid lines indicate egg cells, the dotted lines pollen grains.

plants will "polyploidize themselves out of existence", Stebbins, 1950; page 389). Conversely, a diploid parthenogenetic mutant without apomeiosis will produce only weak and sterile haploid offspring, in which the recessive mutation load becomes fully expressed. Natural selection acts against these individual mutations and therefore the chances of both being present at the same time in a population are very remote. The chances of combining the two mutations via intercrossing are increased by perenniality and outcrossing. This may explain why sexual relatives of extant apomicts are nearly always long-lived and outcrossing (Gustafsson, 1947).

When apomixis has been established by two unlinked apomixis-genes, there will be selection for reduced recombination in pollen meiosis between the genes encoding for apomeiosis and parthenogenesis during the secondary formation of clones. This can be achieved via chromosomal rearrangements (e.g., inversions, translocations). In contrast to sexual plants, chromosomal rearrangements have no

negative effect on apomictic seed fertility, because apomicts circumvent female meiosis. Because selection for increased linkage between apomixis genes is only effective at a clonal time scale, i.e. when new clones are formed, this will be a very slow process. This and the age of the apomictic system may explain why apomixis genes are tightly linked in some species, but not in others. In this view a single apomixis locus is thus a complex of different co-adapted genes, comparable to the supergene of, for example, heterostyly in *Primula* (Ernst, 1936; Grant, 1975).

Another major constraint in the evolution of apomixis may be the development of the endosperm, a tissue that nourishes the embryo. In diploid sexual angiosperm species the endosperm is formed after fertilisation of the diploid central cell nucleus of the embryo sac (arisen through the fusion of the two haploid polar nuclei) by one of the haploid generative pollen nuclei. The ratio of maternal to paternal genomes in the endosperm is therefore 2m:1p. In many angiosperm species, the endosperm is only viable when the genome ratio is 2m:1p. This crucial parental genome ratio is apparent in the collapse of the endosperm, followed by starvation and abortion of the embryo in sexual interploidy crosses such as *2x* **?** *4x* and *4x* **?** *2x*. Endosperm collapse is suggested to be the result of parental imprinting of genes essential for endosperm development (Haig & Westoby, 1991; Vinkenoog & al., 2000; Grossniklaus & al., 2001b). Parental imprinting of endosperm genes probably evolved as a consequence of a conflict between maternal and paternal interests in sexual species (Haig & Westoby, 1989). When the female gamete in a pseudogamous apomict is unreduced and the male gamete not, the parental genome ratio of the endosperm becomes 4m:1p, which is lethal in most angiosperm species. In autonomous apomicts, in which the endosperm develops without fertilisation, the ratio becomes 6m:0p (for triploids). Apomictically produced seeds in a new apomict will thus abort, unless the endosperm imprinting problems are circumvented (see below). Thus, the genetic complexity of apomixis and the parental imprinting of endosperm genes are likely to constrain the evolution of apomixis in the angiosperms.

THE PHYLOGENETIC DISTRIBUTION OF GAMETOPHYTIC APOMIXIS IN THE ANGIOSPERMS

In order to assess the phylogenetic distribution of apomixis, it is important to determine which types of apomixis are homologous and which are not. By definition, non-homologous types of apomixis must have evolved independently. Sporophytic and gametophytic apomixis are two entirely different developmental processes that cannot be homologous. Apospory and diplospory also appear to be fundamentally different, the first affecting megagametophyte development, the second megasporophyte development, but according to Nogler (1984) it remains to be seen if these types have a different genetic basis. Recently it has been suggested that apomixis is caused by ectopic expression of genes normally involved in sexual reproduction (Carman, 1997; Grimanelli & al., 2001; Spillane & al., 2001).

Different ectopic expression of the same genes might result in different forms of apomixis. For example, Bicknell & al. (2000), based on interspecific crossing, showed that two different forms of apospory in *Hieracium* were probably allelic. Nevertheless, we consider it likely that apospory and diplospory are non-homologous.

Over the years several surveys of the occurrence of gametophytic apomixis within angiosperm genera have been published, the most recent one by Carman (1997) who lists 91 genera with apospory and 51 genera with diplospory. In nineteen genera both types of gametophytic apomixis are found (e.g., *Hieracium*, *Poa* and *Parthenium*). Assuming that apomixis cannot spread between genera and that apospory and diplospory are non-homologous, this implies that gametophytic apomixis has evolved at least 142 times independently in the history of the angiosperms. Below, however, we will argue that this first assumption is unlikely to be correct.

In Fig. 2 we have mapped the distribution of gametophytic apomixis onto the phylogeny of the angiosperms according to Soltis & al. (1999). Both apospory and diplospory have a scattered distribution, apospory being more common than diplospory (34% and 23% of the 35 orders, respectively). Both apospory and diplospory are found in the eudicot subgroups rosids I and II and asterids I and II (see Fig. 2).

There are clear differences in the occurrence of apomixis at the lower taxonomic levels. At the family level, almost 70 % of all the genera with gametophytic apomixis are found in only three families: the Asteraceae (27 genera with apospory; 15 with diplospory), the Poaceae (31 genera with apospory; 9 with diplospory) and the Rosaceae (12 genera with apospory; 5 with diplospory). Within other families, apomixis is restricted to isolated species-complexes. For example, in the Brassicaceae apomixis is restricted to the (*Boechera* (= *Arabis*) *holboelli/drummondii* species-complex (Naumova & al. 2001) and in the Ranunculaceae to the *Ranunculus auricomus* species-complex (Nogler 1984).

The phylogenetic distribution of apomixis is further assessed at lower taxonomic levels within Poaceae (Figs. 3 and 4) and Asteraceae (Figs. 5 and 6). Within the Poaceae, apomixis is more or less evenly distributed over the subfamilies (Fig. 3). Apomixis has so far not been reported in the large subfamily Bambusoideae. Fig. 4 shows the distribution of apomixis in the Panicoideae, the subfamily with the highest incidence of apomixis. There is a striking clustering of gametophytic apomixis in the tribe Andropogoneae (7 genera: 6 aposporous, 1 diplosporous) and in the *Panicum/Urochloa/Setaria* clade (Guissani & al. 2001) (genera *Urochloa*, *Pennisetum*, *Cenchrus*, *Brachiaria*, *Panicum maximum*, *Eriochloa*, all with apospory). Apomixis occurs incidentally in the Panicoideae in *Tripsacum*, *Paspalum* and *Echinochloa*.

In the Asteraceae we see a similar clustering of gametophytic apomixis at the lower taxonomic levels. Fig. 5 indicates a more or less even distribution of apospory and diplospory over the Lactuoideae and the Asteriodeae subfamilies. However, within the tribe Lactuceae there is a clear clustering of both forms of

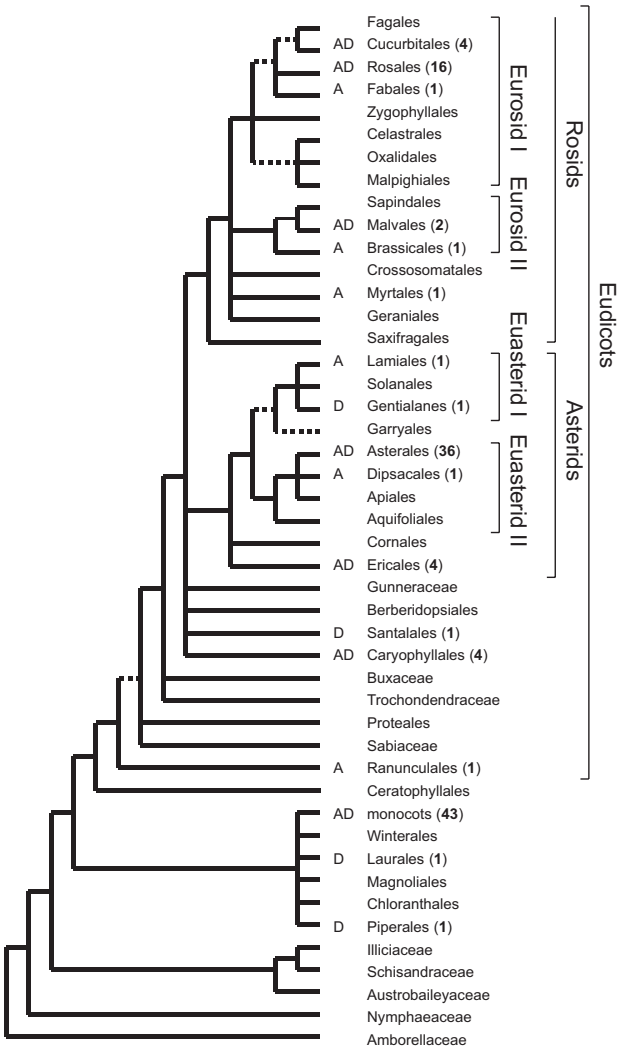

Fig. 2. The phylogenetic distribution of aposporous (A) and diplosporous apomixis (D) over the orders/families of the angiosperms. The phylogeny is from Soltis & al. (1999), based on parsimony analysis of *rbcL*, *atpB* and *18S* rDNA sequences. The jackknife consensus tree is shown; all groups have at least 75% support, except the dashed branches, which have between 50 and 75% support. The occurrence of apomixis is based on the list in Carman (1997). The number of genera in which apomixis is reported, is indicated between brackets.

gametophytic apomixis within subtribes Crepidineae and Hieraciinae and of apospory in the Hypochaeridineae (Fig. 6). Within the genus *Hieracium* apospory is restricted to one subgenus, *Pilosella* and mitotic diplospory to the other sub-

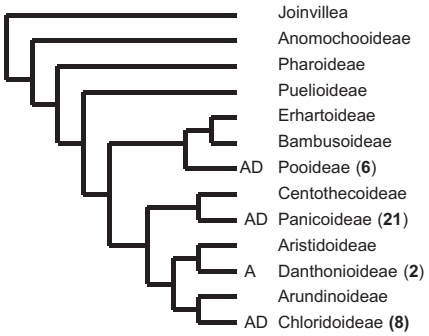

Fig. 3. The phylogenetic distribution of aposporous (A) and diplosporous apomixis (D) over the sub families of the Poaceae. The is cladogram based on parsimony analysis of six sequence data sets (*ndhF*, *rbcL*, *rpoC2*, *PhyB*, *ITS-II* and GBSSI or *waxy*), chloroplast restriction site data and morphological data (Grass Phylogeny Working Group; Barker & al., 2001). More than half of the internal nodes had a bootstrap support of more than 90%. The apomixis information is based on Carman (1997). The number of genera in which apomixis is reported is indicated between brackets.

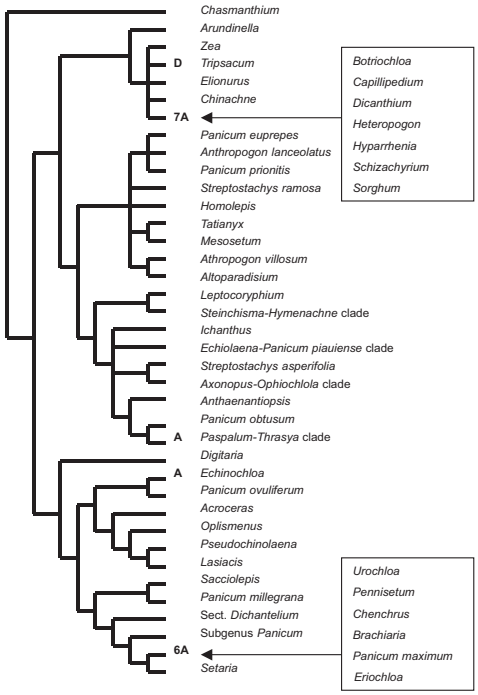

Fig. 4. The phylogenetic distribution of genera with aposporous (A) and diplosporous apomixis (D) within the sub family of the Panicoideae (Poaceae). The cladogram is a strict consensus tree based parsimony analysis of the chloroplast gene *ndhF* (Giussani & al., 2001) the apomixis information (genera with apomixis) on Carman (1997). The number of genera in which apomixis is reported, is indicated between brackets.

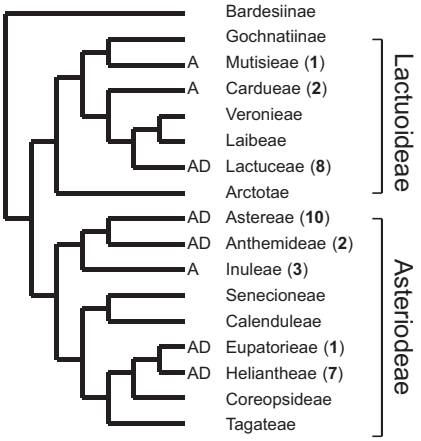

Fig. 5. The phylogenetic distribution of genera with aposporous (A) and diplosporous apomixis (D) over the tribes of the Asteraceae. The cladogram is based on parsimony analysis of 328 chloroplast restriction sites (Jansen & al., 1992); the apomixis information on Carman (1997). The number of genera in which apomixis is reported, is indicated between brackets.

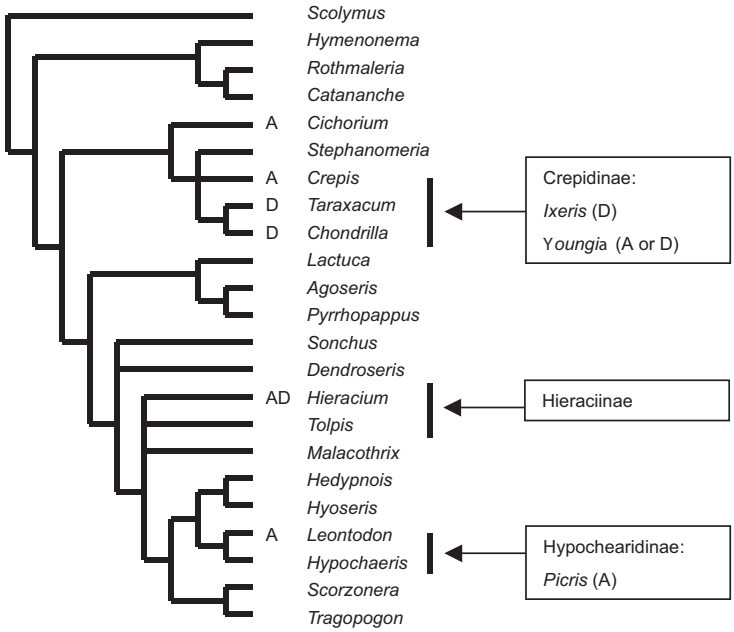

Fig. 6. The phylogenetic distribution of genera with aposporous (A) and diplosporous apomixis (D) within the tribe of the Lactuceae. Subtribes with apomixis are indicated. The cladogram is based on the parsimony analysis of morphological characters (Bremer, 1994); the apomixis information on Carman (1997).

genus, *Euhieracium*. Meiotic diplospory is clustered in the Crepidinae (*Taraxacum*, *Chondrilla*, *Ixeris*). Meiotic diplospory is also found outside the Crepidinae in *Erigeron* and *Townsendia* (subfamily Asteriodeae), indicating parallel or convergent evolution of this syndrome within the Asteraceae.

We can conclude that although gametophytic apomixis is developmentally and genetically complex (which will have constrained its evolution), it must nevertheless have evolved independently several times in the phylogenetic history of the angiosperms, given its scattered distribution at the ordinal level. Although there are no fully apomictic genera, as pointed out before by Darlington (1939) and Stebbins (1950), there seems to be a clustering of gametophytic apomixis at the supragenetic level, more specifically, the (sub)-tribal level. In the next section some possible explanations for this pattern are discussed.

PREADAPTATION

It was argued above that differential parental imprinting of genes in the endosperm is likely to be an important developmental biological constraint in the evolution of gametophytic apomixis. Deviations from the normal 2m:1p genome ratio result in endosperm collapse causing starvation and abortion of the apomictic embryo. The pseudogamous apomicts of the *Panicum/Urochloa/Setaria* clade (see Fig. 5; e.g., *Pennisetum*, *Cenchrus*, *Brachiaria*, and *Panicum maximum*) have overcome the problem of imprinting of the endosperm by a 4-nucleate embryosac with only a single polar nucleus (Warmke, 1954; Savidan, 1980). Sexual relatives have two polar nuclei that fuse, creating a normal 2m:1p genome ratio after fertilization. In apomicts fertilisation of a single unreduced polar nucleus with a reduced pollen nucleus produces a 2m:1p genome ratio, thus avoiding the imprinting problems. It is likely that the latent potential for 4-nucleate embryo sac development has enabled the evolution of apomixis in this group, explaining the (sub)-tribal level clustering of apomixis.

Autonomous endosperm development is common in the Asteraceae, but rare in other angiosperm families. In triploid autonomous apomicts, like *Taraxacum*, *Chondrilla*, *Ixeris* and *Erigeron*, the endosperm ratio is 6m:0p. In *Taraxacum*, crosses between sexual *2x* and sexual colchicine-induced *4x* plants produce many 3x offspring plants (in both cross-directions; Warmke, 1945; P. J. van Dijk, unpublished results). This suggests that in *Taraxacum* sexual diploids tolerate 2m:2p and 8m:1p genome ratios in the endosperm, which further implies that the endosperm of sexual *Taraxacum* is not strongly imprinted. In addition, triploids in interploidy crosses are viable in some sexual Asteraceae species, suggesting that parental endosperm imprinting is not important (e.g., *2x* **?** *4x Tripleurospermum*; Kay, 1979). A similar situation is found in the Brassicaceae where *Boechera* (*Arabis*) *holboellii*, an apomictic close relative of *Arabidopsis*, shows partial autonomous endosperm development (Noumova & al., 2001). In *Arabidopsis 2x* **?** *4x* and reciprocal crosses produce viable *3x* embryos with functional endosperm, implying that

the endosperm of *Arabidopsis* is not heavily imprinted (Vinkenoog & al., 2001). These observations suggest that autonomous apomixis may have evolved in plant groups in which for unknown reasons endosperm imprinting was not strong. For instance, selfing in an ancestor would relax the genomic conflict and imprinting. A mutation for autonomous endosperm would produce viable endosperm in these plants and would provide the strong advantage of reproductive assurance. In *Arabidopsis*, three mutations have been described that control autonomous endosperm development: MEA (MEDEA) / FIS1, FIS2; and FIE / FIS3 (reviewed in Preuss, 1999). Such mutations could have led to autonomous endosperm in plants without endosperm imprinting.

It seems likely that the evolution of apomixis in these groups was enhanced because of the absence of endosperm obstacles. These groups were preadapted, which may explain the high incidence of gametophytic apomixis in some families and in some (sub)tribes. Preadaptations may also exist for other elements of apomixis, but these are less easy to envision.

Common origin. — Phylogenetic clustering of apomixis may also be explained by common origin, either horizontally, via hybridization, or vertically, because of common ancestry. Many studies on the genetic basis of apomixis involve interspecific sexual five apomictic crosses (Grossniklaus & al., 2001a), suggesting that introgression of apomixis factors (genes) is possible. Two closely related *Boechera* (*Arabis*) species, *B. drummondii* and *B. holboelli*, share chloroplast DNA haplotypes, suggesting gene flow across the species borders and probably a common origin of apomixis (Sharbel & Mitchel-Olds, 2001). In the genus *Erigeron* apomixis is transmitted in an experimental cross between *E. strigosus* (diploid, sexual) five *E. annuus* (triploid, apomict). Genetic mapping studies identified two dominant apomixis-loci, one for diplospory and one for parthenogenesis (Noyes & Rieseberg, 2000). A phylogenetic study of *Erigeron* shows that apomixis occurs in three distinct clades, indicating three independent origins of apomixis. However, apomixis is clustered in the clade with *E. strigosus* and *E. annuus* (Noyes, 2000), suggesting that apomixis genes may have spread by intogression.

Hybridization between distantly related lineages is often easier at higher ploidy levels than at the diploid level, and since gametophytic apomixis is generally associated with polyploidy, hybridization involving facultative apomixis may be quite common. De Wet & Harlan (1970) documented intergeneric hybridisation and introgression of apospory in the grass genera *Botriochloa*, *Capillipedium* and *Dichanthium*. Aposporous species in the genera *Pennisetum* and *Cenchrus* share an apospory specific chromosomal region, as was revealed by molecular markers (Roche & al., 1999). *Taraxacum* and *Chondrilla* are closely related Crepidinae genera (Bremer, 1994), which have a cytogenetically identical system of meiotic diplospory (Bergman, 1950). Hybridisation between these genera is unknown and basic chromosome numbers ($x = 8$ and $x = 5$, respectively), make horizontal gene transfer unlikely. This could be a case of common ancestry across different genera (Van Dijk, 2003).

These insights have consequences for estimating the number of occurrences in

which apomixis arose during the evolution of the angiosperms. If apomixis in different genera were of common origin, then the number of independent origins would be expected to be lower than the total of 140 apomictic genera given above. However, apomixis may not have been detected yet in many genera, and assuming a single origin per genus is a conservative estimate. For example, in the genus *Hieracium* apospory is restricted to the subgenus *Pilosella* and diplospory to the subgenus *Euhieracium*, suggesting that apomixis evolved at least twice in this genus. Especially when a genus is predisposed to the evolution of apomixis, it may have developed apomixis multiple times independently.

Both Darlington (1939) and Stebbins (1950) considered apomixis an evolutionary blind alley. We agree that apomixis has played only a minor role in the evolution of the angiosperms. However, it is important to distinguish between the evolutionary fate of apomictic clones, apomictic species and apomixis genes. Clones may have a limited evolutionary life span, because they lack adaptive potential and because they accumulate deleterious mutations. However, apomixis appears to be controlled by genes and these apomixis genes can be transferred to new clones in crosses between sexuals and apomictic pollen donors. In new clones, apomixis genes can become associated with new genetic backgrounds, that are potentially adaptive (Mogie, 1992). In addition, they may carry a reduced mutational load, because being derived from a sexual gene pool (Van Dijk, 2003). The individual clones may become extinct for the reasons outlined above. However, the apomixis genes can escape from early extinction via hybridization. Such apomixis genes therefore parasitise on the sexual gene pool for adaptive and cleansed genetic backgrounds. In such a system apomixis genes can survive for long evolutionary periods and could even predate the splits of genera.

ACKNOWLEDGEMENTS

This chapter is publication 3254 of the NIOO-KNAW Netherlands Institute of Ecology.

LITERATURE CITED

Albertini, E. A., Porceddu, F., Ferranti, L., Reale, G., Barcaccia, B., Romano B. & Falcinelli M. 2001. Apospory and parthenogenesis may be uncoupled in *Poa pratensis*: a cytological investigation. *Sex. Pl. Reprod.* 14: 213–217.

Asker, S. E. & Jerling, L. 1992. *Apomixis in Plants*. CRC Press.

Barker, N. P., Clark, L. G., Davis, J. I., Duvall, M. R., Guala, G. F., Hsiao, C., Kellogg, E. A., Linder, H. P., Mason-Gamer, R. J., Mathews, S. Y., Simmons, M. P., Soreng, R. J. & Spangler, R. E. 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). *Ann Missouri Bot. Garden.* 88: 373–457.

Bell, G. 1982 *The Masterpiece of Nature: the Evolution and Genetics of Sexuality.* Univ. California Press, Berkeley.

Bergman, B. 1950. Meiosis in two different clones of the apomictic *Chondrilla juncea*.

Hereditas 36: 297–320.

Bicknell, R., Borst, N.K. & Koltunow, A.M. 2000. Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. *Heredity* 84: 228–237.

Bremer, K. 1994. *Asteraceae. cladistics & classification.* Timber Press, Portland.

Carman, J. G. 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol. J. Linn. Soc.* 61: 51–94.

Cavalier-Smith, T. 2001. Origins of the machinery of recombination and sex. *Heredity* 88: 125–141.

Charlesworth, B. 1980. The cost of sex in relation to mating system. *J. Theor. Biol.* 84: 655–671.

Crane, C. F. 2001. Classification of apomictic mechanisms. Pp. 24–43 in: Savidan, Y., Carman, J. G. & Dresselhaus, T. (eds.), *The Flowering of Apomixis: from Mechanisms to Genetic Engineering*. CIMMYT, IRD, European Commission DG VI (FAIR), Mexico.

Darlington, C. D. 1939. *Evolution of Genetic Systems*. Cambridge Univ. Press, Cambridge.

De Wet, J. M. J. & Harlan., J. R. 1970. Apomixis, polyploidy, and speciation in *Dichantium. Evolution* 24: 270–277.

Ernst, A. 1936. Heterostylie-forschung. Versuche zur genetischen Analyse eines Organisations und "Anpassungs" Merkmales. *Z. Indukt. Abstammungs-Vererbungsl.* 71: 156–230.

Giussani, L. M., Cota-Sanchez, J. H., Zuloaga, F. O. & Kellog, E. A. 2001. A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C4 photosynthesis. *Amer. J. Bot.* 88: 1993–2012.

Grimanelli, D., LeBlanc, O., Perotti, E., & Grossniklaus, U. 2001. Developmental genetics of gametophytic apomixis. *Trends in Genetics* 17: 597–604.

Grant, V. 1975. Genetics of Flowering Plants. Columbia Univ. Press, New York.

Grossniklaus, U., Nogler, G. A. & Van Dijk, P. J. 2001a. How to avoid sex: the genetic control of gametophytic apomixis. *Pl. Cell* 13: 15–21.

Grossniklaus, U. Spillane, C., Page, D. R. & Köhler, C. 2001b. Genomic imprinting and seed development: endosperm formation with and without sex. *Curr. Opinion Pl. Sci.* 4: 21–27.

Gustafsson, Å. 1947. Apomixis in higher plants. Part II. The causual aspect of apomixis. *Lunds Universitets Arsskrift* Bd 42: 71–178.

Haig, D. & Westoby, M. 1989. Parent specific gene expression and the triploid endosperm. *Amer. Naturalist* 134: 147–155.

Haig, D. & Westoby, M. 1991. Genomic imprinting in endosperm: is effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. *Phil. Trans. Roy. Soc. London, Ser. B* 333: 1–13.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. *Oikos* 35: 282–290.

Jansen, R. K., Michaels, H. J., Wallace, R. S. Kim, K.-J., Keeley, S. C., Watson, L. E. & Palmer, J. D. 1992. Chloroplast DNA variation in the Asteraceae: Phylogenetic and Evolutionary implications. Pp. 252–279 in: Soltis, P. S., Soltis, D. E. & Doyle, J. J. (eds.), *Molecular Systematics of Plants*. Chapman and Hall, New York.

Kay, Q. O. N. 1969. The origin and distribution of diploid and tetraploid *Tripleuropspermum inodorum* (L.) Schultz Bip. *Watsonia* 7: 130–141.

Kondrashov, A. S. 1982. Selection against harmful mutations in large sexual and asexual populations. *Genet. Res.* 40: 325–332.

Koltunow, A. M. 1993. Apomixis: embryo sacs and embryos formed without meiosis or fer-

tilization in ovules. *Pl. Cell* 5: 1425-1437.
Levin, D. A. 1975. Pest pressure and recombination systems in plants. *Amer. Naturalist* 109: 437–452.
Lloyd, B. G. 1988. Benefits and costs of biparental and uniparental reproduction in plants. Pp. 233–252 in: Michod, R.E. & Levin, B.R. (eds.), *The Evolution of Sex: an Examination of Current Ideas*. Sinauer, Sunderland.
Marshall, D. R. & Brown, A. H. D. 1981. The evolution of apomixis. *Heredity* 47: 1–15.
Maynard Smith, J. 1978. *The Evolution of Sex.* Cambridge Univ. Press, Cambridge.
Mogie, M. 1992. *The Evolution of Asexual Reproduction in Plants.* Chapman and Hall, London.
Muller, H. J. 1964. The relation between recombination to mutational advance. *Mutat. Res.* 1: 2–9.
Naumova, T. N., Van der Laak, J., Osadtchivy, J., Matzk, F., Kravchenko, A. Bergervoet, J., Ramulu, K. S. & Boutilier, K. 2001. Reproductive development in apomictic populations of *Arabis holboelli* (Brassicaceae). *Sex. Pl. Reprod.* 14: 195–200.
Nawaschin, S. 1898. Resultate einer Revision der Befruchungsvorgänge bei *Lilium martagon* und *Fritillaria tenella*. *Bull. Acad. Imp. Sci. St. Petersburg* 33: 39–47.
Nogler, G. A. 1984. Gametophytic embryogenesis Pp. 475–518 in Johri, B.M., (ed.), *Embryology of Angiosperms.* Springer, Berlin.
Noyes, R. D. 2000. Biogeographical and evolutionary insights on *Erigeron* and allies (Asteraceae) from ITS sequence data. *Pl. Syst. Evol.* 220: 93–114.
Noyes, R. D. & Rieseberg, L. H. 2000. Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics* 155: 379–390.
Nunney, L. 1989. The maintenance of sex by group selection. *Evolution* 43: 245–257.
Preuss, D. 1999. Chromatin silencing and *Arabidopsis* development: a role for Polycomb proteins. *Pl. Cell* 11: 765–767.
Richards, A. J. 1986. *Plant Breeding Systems*. Allen & Unwin, London.
Roche, D., Cong, P., Chen, Z., Hanna, W. W., Gustine, D. L., Sherwood, R. T. & Ozias-Akins, P. 1999. An apospory-specific genomic region is conserved between Buffelgrass (*Cenchrus ciliaris* L.) and *Pennisetum squamulatum* Fresen. *Pl. J.* 19: 203–208.
Sanderson, M. J. & Doyle, J. A. 2001. Sources of error and confidence intervals in estimating the age of the angiosperms from *rbcL* and *18S* rDNA data. *Amer. J. Bot.* 88: 1499–1516.
Savidan, Y. 1980. Chromosomal and embryological analyses in sexual x apomictic hybrids of *Panicum maximum* Jacq. *Theor. Appl. Genet.* 57: 153–156.
Sharbel, T. F. & Mitchel-Olds, T. 2001. Recurrent polyploid origins and chloroplast phylography in the *Arabis holboelii* complex (Brassicaceae). *Heredity* 87: 59–68.
Soltis, P. S, Soltis, D. E. & Chase, M. W. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Science* 402: 402–404.
Spillane C., Steimer, A. & Grossniklaus, U. 2001. Apomixis in agriculture: the quest for clonal seeds. *Sex. Pl. Repr.* 14: 179–187.
Stebbins, G. L. 1950. *Variation and Evolution in Plants.* Columbia Univ. Press.
Van Dijk, P. J., Tas, I. C. Q., Falque, M. & Bakx-Schotman, J. M. T. 1999. Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83: 715–721.
Van Dijk, P. J. 2003. Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. *Phil. Trans. Roy. Soc. London, Ser. B* 358: 1113–1121.
Van Dijk, P. J.,Van Baarlen, P. & De Jong, J. H. 2003. The occurrence of phenotypically

complementary apomixis-recombinants in crosses between sexual and apomictic dandelions (*Taraxacum officinale*). *Sex. Pl. Reprod.* 16: 71–76.

Vinkenoog, R., Spielman, M., Adams, S., Fisher, R. L., Dickinson, H. G. & Scott, R. J. 2000. Hypomethylation promotes autonomous endosperm development and rescues post-fertilization lethality on fie mutants. *Pl. Cell* 12: 2271–82.

Warmke, H. E. 1945. Experimental polyploidy and rubber content in *Taraxacum* Koksaghyz. *Bot. Gaz.* 106: 316–324.

Warmke, H. E. 1954. Apomixis in *Panicum maximum*. *Amer. J. Bot.* 41: 5–11.

Winkler, H. 1908. Ueber Parthenogenesis und Apogamie im Pflanzenreich. *Progr. Rei Bot.* 2: 293–454.