Chapter 22
An Apomixis-Gene's View on Dandelions

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Abstract In asexual organisms, the clone constitutes a level above the individual. Most dandelions (*Taraxacum officinale s.l.*) reproduce asexually through apomixis, asexual reproduction through seeds. A clone can be seen as a superorganism that is born, that growths, degenerates and eventually dies. Apomixis in dandelions is controlled by a few dominant genes, the so called apomixis-genes. This implies that there should be three hierarchical levels in a field of dandelions: 1. the individual plant, 2. the clone and 3. the apomixis gene. Using co-dominant genetic markers that are linked to a dominant apomixis gene, we provide evidence that this hierarchical structure indeed exists in apomictic dandelion populations. The apomixis gene view implies that whereas individual clones may go extinct due to deleterious mutation accumulation or the lack of adaptive potential, apomixis genes can prevail much longer periods of evolutionary time in a succession of clones. We provide evidence that an apomixis-gene in *Taraxacum* is not transmitted to diploid offspring, which could explain the absence of apomixis in diploid dandelions. Haploid non-transmission may be caused by a mutation load that is linked to the apomixis genes as a consequence of the deep asexual reproduction history of these genes residing in many clones in the past.

22.1 Introduction

In the last chapter of his book *The Extended Phenotype* (1989), Richard Dawkins writes about different ways to view a field of dandelions (*Taraxacum officinale s.l.*): "A certain type of ecologist may gain illumination from comparing a field full of dandelions with a single tree. But for other purposes it is important. . .to see the single dandelion ramet [plant] as analogous to the tree". Dawkins referred to an article written by Daniel Janzen in 1977 in *The American Naturalist* with the intriguing title: "What are dandelions and aphids?" (see also Chapter 25). Most dandelions

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reproduce by apomixis, i.e., asexual reproduction by seed. Janzen considered an apomictic dandelion clone as a kind of superorganism and argued that the individual dandelion plants, the clone mates, were comparable to the leaves of a tree. Clone mates and tree leaves are both genetically identical units, only differing in whether or not they are connected. Janzen argued that the whole dandelion clone and not the individual apomictic plant is the evolutionary individual, just like we consider the tree and not the individual leaf as the evolutionary individual. In this chapter, we will argue that there is even a higher level of biological hierarchy above the clone (see Chapter 9), namely that of the genes that encode for apomixis (shortly the apomixis genes). Through the pollen, the male sexual function of hermaphroditic flowering plants, these apomixis genes can enter the sexual gene pool and generate new clones. This way the apomixis genes can survive clones, which may have a limited evolutionary life span, and persist for much longer periods of evolutionary time than the individual clones. Here, we will provide new evidence that this apomixis gene level is a reality and that the apomixis genes can explain why apomixis is restricted to polyploids in *Taraxacum* and why diploid apomicts have not been found in nature. For an introduction to the biology of apomixis in plants, we refer to Chapter 3.

22.2 The Genetics of Apomixis in *Taraxacum*

Dandelions are hermaphrodictic plants, which produce both egg cells and pollen grains (Van Dijk 2003). Most dandelions in Northern Europe and North America are triploid ($3x = 24$) and produce seeds through apomixis: parthenogenetic development of an unreduced egg cell into an embryo and autonomous development of the central cell into the endosperm. Although most apomicts produce pollen, fertilization is not required for the production of seeds. Hence, from the point of view of the individual plant, pollen has no function.

In Central Europe, diploid sexuals ($2x = 16$) are found, co-occurring with triploid apomicts (higher ploidy levels are rare). Diploid sexuals depend on insect pollination and cross fertilization for seed set. Mixed populations of sexual and apomictic dandelions are rather common in Central Europe. Sexual dandelions reach their Northern distribution limit in Europe in The Netherlands; at higher latitudes only polyploid apomicts are found (Van Dijk 2003).

Under experimental conditions, it is rather easy to demonstrate apomixis. Sexuals do not set seeds in the absence of pollinators, whereas apomicts produce abundant seeds. When the top of a flower bud is decapitated, apomicts will still produce many viable seeds, despite the fact that the styles and the anthers of the florets are removed. Of course, sexuals fail to produce seeds after decapitation. In triploids, high seed set is an indicator for apomixis, because sexual triploids would even after cross pollination hardly produce any seeds due to aneuploidy caused by an unbalanced meiosis. Triploidy is therefore a good indicator for apomixis in the field. Triploidy can be determined in the field microscopically by the irregularly sized pollen grains or in the laboratory by DNA flow cytometry of field collected leaf samples. Apomictic reproduction can of course also be proven by genetically identical heterozygous offspring, but this is more time consuming.

Apomicts that produce functional pollen can act as pollen donors in crosses with diploid sexuals, enabling the study of the genetics of apomixis. Two unlinked dominant apomixis loci have been identified, controlling: 1. The avoidance of meiotic reduction (*DIPLOSPOROUS-Dip*) and 2. Parthenogenesis of the embryo (*PARTHENOGENESIS-Par*). In *Dip*-plants female meiosis I is restitutional, resulting in unreduced 2*n* egg cells. Van Dijk and Bakx-Schotman (2004) investigated the transmission genetics of the *Dip*-allele using two linked co-dominant Simple Sequence Repeat (SSR) markers (in coupling phase, both at 7 cM from the *Dip* allele). This led to the conclusion that the *Dip*-allele is dominant and that triploid apomicts can be genotypically represented as *Ddd*. The expression of the *Dip*-allele is sex-specific and does not affect pollen meiosis. Egg cells are therefore always *Ddd*, whereas pollen can be *Dd*, *dd*, *d* or *D* (discarding aneuploid pollen, which is inviable in *Taraxacum*). Genotypically triploid apomicts are *Ppp* for the *Par*-locus. There are indications that a third major locus is involved in apomixis, which may be related to the autonomous endosperm development, but this needs further investigation. A single dominant allele is sufficient in triploids to suppress the sexual reproductive pathway. Interestingly, this implies that loss of an apomixis allele, for example by non-disjunction, could lead to a reversal from apomixis to sexuality. This has indeed been observed by Sørensen and Gudjonsson (1946) and Sørensen (1958) who showed that rare 3x-1 offspring (aberrant *tenuis*) of a triploid apomictic lineage could be pollinated by other triploid apomicts, resulting in some diploid sexually reproducing offspring. *Tenuis* aberrants had lost one of the three Nucleolus Organizer Region chromosomes and the frequency of occurrence was estimated as 5×10^{-4} (3 out of 6000 clone mates). Since the *Dip*-locus was shown to be located on the NOR-chromosome (Van Dijk and Bakx-Schotman 2004), the modern interpretation is that a NOR chromosome carrying the D-allele was lost in the *tenuis* plants. According to Sørensen (1958), not all *tenuis* plants reverted to sexuality, which is consistent with the *Ddd* genotypic constitution. Most research so far has focused on the *Dip*-locus and therefore, the remainder of this chapter will deal with this locus.

22.3 Clones as Superorganisms

As mentioned before, mixed sexual and apomictic populations are not uncommon in Central Europe. Through crosses of diploid sexuals with polyploid apomicts (mainly triploid) new clones can be formed. In terms of a superorganism this is the "birth" of a new clone. Verduijn et al. (2004) found that in mixed populations with diploid sexuals and triploid apomicts in the Netherlands, ~2% of the natural progeny of diploid sexual plants were triploids. It was estimated that in a rather small peripheral sexual population of 2500 reproducing sexual plants, each year some 10,000 triploid seeds would be produced by diploids. If three apomixis genes would segregate independently, ~ 29% of these seeds could reproduce by apomixs. In reality most *neo*apomicts are partly seed sterile, probably depending on the action of additional modifier genes (Van Dijk 2007). Since established apomictic clones have full apomictic seed set, there will be strong fertility selection between neoclones. Even neoclones with high fitness run a high risk of extinction by demographic

stochasticity shortly after formation when the numbers of clone mates are still low. Nevertheless, it is clear that many new clones are generated in mixed populations. Some of these clones will be well adapted and will proliferate, or grow in terms of the clone as a superorganism.

It is widely accepted that clones are prone to early extinction because of the lack of adaptability and the accumulation of slightly deleterious mutations. According to the Red Queen Hypothesis (Bell 1982; see also Chapter 7), rapidly evolving pathogens could cause the elimination of clones. The frequency distributions of clones in apomictic *Taraxacum* populations are typically L-shaped, with a few common clones and many rare ones (Van der Hulst et al. 2000; Van Dijk 2003). Frequency-dependent rust fungus infection has been demonstrated in apomictic populations of *Condrilla juncea*, a close relative of *Taraxacum* (Chaboudez and Burdon 1995).

Theoretically, asexual lineages will accumulate slightly deleterious mutations due to stochastic loss of the least loaded class in small populations (Muller's Ratchet, Muller 1964; see also Chapter 5), genetic hitchhiking with beneficial mutations (Rice 1987) and inefficient purging in large populations (Kondrashov 1982). Frequency-dependent selection may accelerate the accumulation of slightly deleterious mutations (West et al. 1999). Active transposable elements can cause slightly deleterious mutations and theoretical studies indicate that it is likely that asexual lineages become extinct because of transposon accumulation (Dolgin and Charlesworth 2006). Docking et al. (2006) analyzed transposable elements in a number of Canadian apomictic lineages of *Taraxacum*. Sequence comparisons indicated purifying selection acting on these transposable elements since substitution rates at non-synonymous sites were much lower than at synonymous sites. Transposons, such as Ty1-*copia*, Ty2-*gypsy* and LINE-like retroelements, were probably still functional in the *Taraxacum* apomicts and could reduce the fitness of clone mates. The decline of the number of clone mates and, eventually, the extinction of the apomictic clone causes the degeneration and the death of the superorganism (Fig. 22.1).

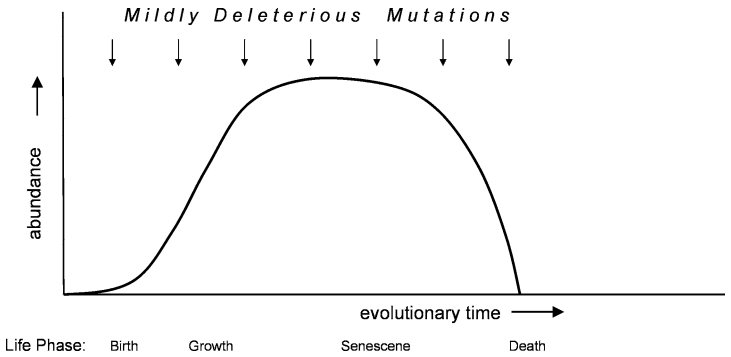

Fig. 22.1 The life phases of a clone viewed as a superorganism. Abundance refers to the number of clone mates. During its life time a clone will accumulate slightly deleterious mutations

22.4 The Superstructure of Asexual Populations

Taraxacum officinale apomicts are considered to be obligate apomicts. In other *Taraxacum* species, rare facultative apomixis has been reported, but we have never observed residual sexuality in apomictic *T. officinale*, despite searches for it. Notwithstanding the lack of sexuality, even in regions far north of the sexual distribution area dandelions exhibit a great deal of morphological variation. For a long time, morphologically distinct clones have been described as microspecies by certain taxonomists. Many different microspecies can be found growing together in grasslands. Recently, molecular studies have discovered even more genetic variation. Van der Hulst et al. (2000, 2003) investigated the clonal structure of a triploid dandelion population in a park in Viborg, Denmark (Jutland), more than 700 km north of the nearest known sexual diploid population (in the Netherlands). This study revealed an interesting hierarchical population structure and will be described here in some more detail. Allozymes, cpDNA, AFLPs and SSR markers were used to analyse a sample of 65 plants. AFLP fingerprinting generated on average about 100 fragments per plant. Thirty three different AFLP fingerprints were found, of which 14 occurred more than one time and 19 only once. The redundancy of these 14 fingerprints clearly indicated clonal reproduction by apomixis in this population. Two AFLP fingerprints were identical to AFLP fingerprints found in a Dutch apomictic population, more than 600 km to the South. These two clones had thus likely spread over a distance of 600 km or more. This finding suggests that it should be possible that clones are generated in the area of sympatry between sexual and apomictic dandelions and spread over hundreds of kilometres and over evolutionary time frames. Dandelions are effectively dispersed by wind and clones may have a long lifespan. This provides an explanation for the high clonal diversity found in the asexual regions. Alternatively the diversity of clones may have a local origin possibly caused by a rare local reversal to sex caused by loss of one or more apomixis genes, as discussed above.

Analysis of the Viborg sample for three SSR-loci resulted in 32 groups, closely resembling the AFLP groupings. Figure 22.2 shows schematically the variation at the three SSR-loci, after removal of the clone mate redundancy. Some somatic mutations within clones were detected at the *MSTA72* locus. However, more interesting was the genetic variation at *MSTA78*. All clones shared one allele, 164 bp, at this locus. The locus however, was not fixed; there was a lot of variation for the other two alleles. *MSTA78* is one of the SSR-loci that was shown to be linked to the *Dip*-locus by Van Dijk and Bakx-Schotman (2004). The other two SSR loci, *MSTA72* and *MSTA64*, are not linked to *Dip* (nor to *Par*). The fixation of the *MSTA78-164* allele can thus be explained by strong linkage disequilibrium between this allele and an allele controlling diplospory, an essential element of apomixis. Van Dijk and Bakx-Schotman (2004) originally estimated the size of the linked-allele of *MSTA78* to be 160 bp, but later, single gel analysis showed that this allele is of the same size as the fixed 164 bp allele in Viborg.

A more extensive survey furthermore indicated that the *MSTA78-164* allele was almost completely fixed in apomictic dandelion populations in the Netherlands,

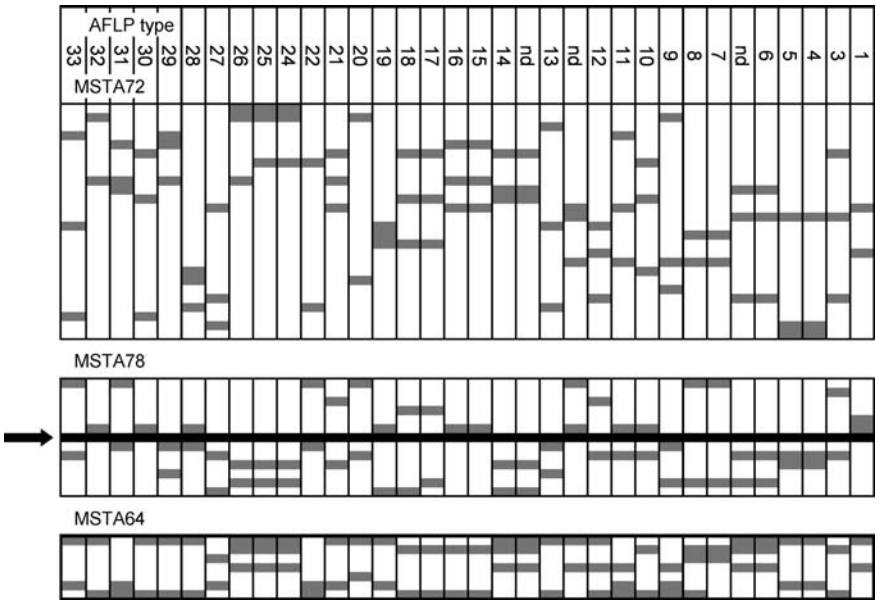

Fig. 22.2 A virtual SSR gels of the different apomictic clones in the Viborg population (Van der Hulst et al. 2003), based on real data. At the top, the different AFLP fingerprint types are indicated. All clones share the 164 bp allele at locus MSTA78 (between the arrows, black fragments). This allele is most likely in linkage disequilibrium with the *Dip* allele, which controls diplospory, an element of apomixis in dandelions

Northern Germany and Denmark. In contrast, in France, the 164 bp allele was not fixed in apomicts and was also found in diploid sexuals (Van Dijk, Van Culemborg, Vijverberg and Van der Hulst, unpublished results).

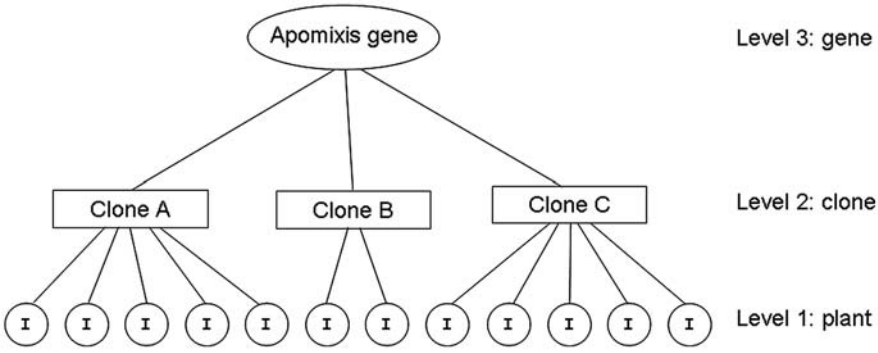

Fig. 22.3 Apomixis at three hierarchical levels. The individual plants form the lowest level (*level 1*). Plants can be clone mates sharing the same genotype, belonging to the same clone (*level 2*). Different clones may share the same apomixis gene(s) (*level 3*) because of common ancestry. *Clones A* and *C* are larger clones than *clone B*, because they have more clone mates

The structure of the Viborg population clearly shows that there are three hierarchical levels within apomictic dandelions (Fig. 22.3). From the lowest to the highest level these are: 1. the individual plant or clone mate, 2. the clone, or the evolutionary individual, and 3. the apomixis gene. In the remainder of the chapter we will have a closer look at the evolutionary implications of the highest level, the apomixis genes.

22.5 Apomixis Genes are Older than Clones

In mixed sexual-apomict populations, a so called sex-asex cycle will operate (Fig. 22.4). Through the pollen, the male sexual function, genes can cycle between the sexual gene pool and the asexual clone pool. Most of the pollen formed by triploid apomicts will be chromosomically unbalanced, but a low percentage will possess the full haploid or diploid genome. Through haploid pollen genes from the clones can flow into the sexual gene pool and through diploid pollen new clones will be formed. As we will show below, apomixis genes cannot be transmitted through haploid pollen and cannot enter the diploid gene pool. For the moment the most important aspect of the cycle is that new clones are being formed and that apomixis genes can survive within successive generations of clones. While non-apomixis genes can cycle between the sexual and the asexual complement, apomixis genes can only cycle within the asexual complement.

In the sexual gene pool, slightly deleterious mutations will be purged. Hence, the haploid genome of sexual egg cells will be cleansed from deleterious mutations. If such a haploid egg cell becomes fertilized by a diploid pollen grain carrying a dominant apomixis gene, a new apomictic clone will be formed, which will have a

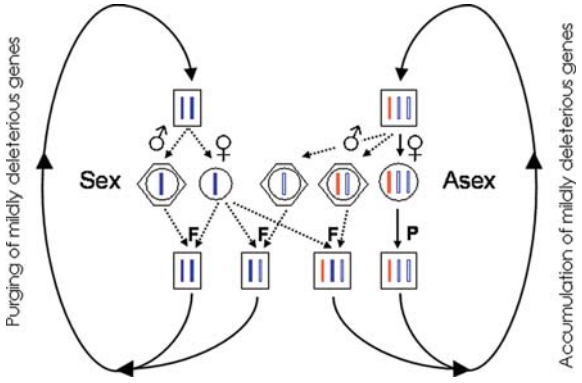

Fig. 22.4 The sex-asex cycle in dandelions. Sexual diploid dandelions and hermaphroditic apomictic triploid dandelions are linked by functional pollen produced by the apomicts. Only one chromosome set is shown. Pollen grains are shown with thick cell walls, egg cells with thin cell walls. F = fertilization; P = parthenogenesis. The arrows indicate directions of possible gene flow. The chromosomes from the sexual gene pool are in dark blue, the chromosome carrying a dominant apomixis gene is in red. The non-apomixis gene chromosomes in the triploid apomicts are indicated in light blue

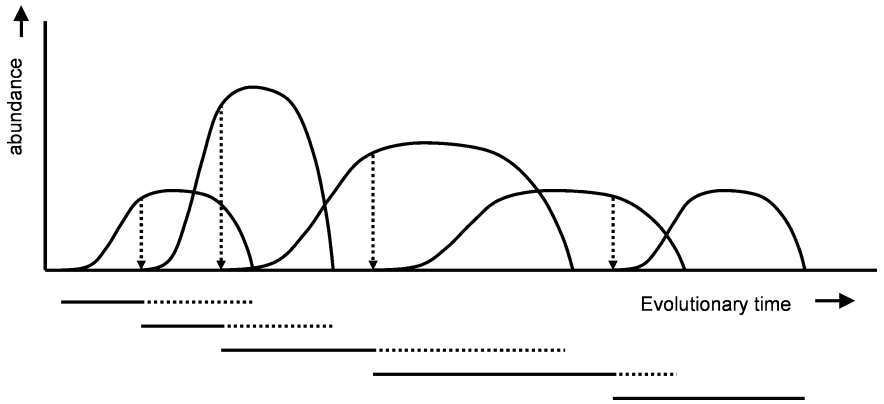

Fig. 22.5 This figure illustrates the idea that apomixis genes can predate apomictic clones. Abundance means the number of clone mates. Each time a new clone is formed (*dashed arrows*), the apomixis genes "jump" into a new clone. Apomixis genes can survive much longer in evolutionary time than individual clones and will have a far deeper asexual reproduction history. Consequently, the chromosomal regions surrounding the apomixis genes will have strong signatures of a long asexual reproduction history, especially when recombination during pollen meiosis in these regions is suppressed

reduced mutation load compared to its father clone. Apomixis genes become associated with a new genetic background that is partly cleansed. If a clone degenerates by an increasing mutation load, the apomixis gene may escape extinction by backcrossing to sexuals. The idea that apomixis genes can be much older than the genes that they reside in, is illustrated in Fig. 22.5.

Looking backwards in time, the three *Dip*-alleles in any triploid dandelion clone will differ in their asexual reproduction history. One recessive allele, *d1*, descends from the sexual mother and has only experienced asexual reproduction since the formation of the present clone. The other recessive allele, *d2*, descends from the triploid apomictic father clone and there is an equal chance (0.5) that it descends from the apomictic grandfather or the sexual grandmother. However, the chance that the *d2*-allele has resided in more than six previous clone generations is less than five percent ($(0.5)^5 = 0.031$). In contrast, the *D*-allele has cycled through the asexual part of the sex–apomixis cycle in numerous successive clones since its origin by mutation.

22.6 A Mutation Load Linked to Apomixis-Genes

During pollen meiosis, the *D* chromosomes can recombine and exchange flanking regions with the *d1* and *d2* chromosomes which were recently derived from the sexual gene pool. Through this process, large parts of the *D*-linked mutation load can be removed; however, uncoupling will be infrequent for regions close to the apomixis loci. The regions close to the apomixis loci are therefore expected to carry a higher

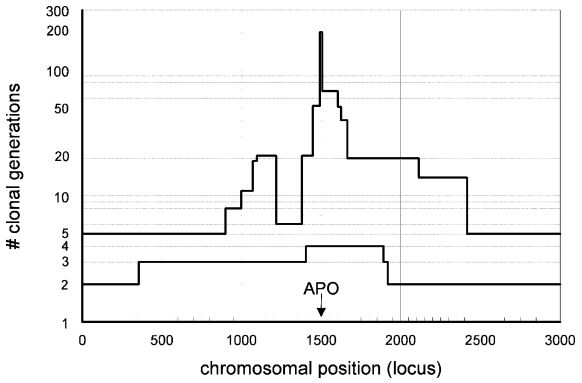

Fig. 22.6 The depth of the asexual reproduction history along a chromosome carrying a dominant single dose apomixis gene, after 200 clonal generations cycling in the sex-asex cycle. The three simulated chromosomes contain 3000 loci with one apomixis locus in the middle. One chromosome carries the dominant apomixis allele *D*, the other two the recessive sexual reproduction allele *d*. Bivalent chromosome pairing occurs at random (autotriploidy) and one randomly positioned cross-over occurs on each flank of the apomixis locus

genetic load than regions elsewhere in the genome. We have simulated this for a triploid with a single chromosome of 3000 loci carrying an apomixis locus in the middle (Van Dijk, unpublished results). Crossovers were randomly positioned on each flank of the apomixis locus. Figure 22.6 shows the results of a typical simulation after 200 clonal generations as the depth of the asexual reproduction history -the number of clonal generations that the chromosomal region resided in a clone- across the three chromosomes. The *d1*-chromosome of the sexual mother has only resided in the present clone. Parts of the *d2*-chromosome have resided in three ancestral clones. Almost one third of the *D*-chromosome has resided in more than 19 clonal generations. A small region surrounding the *D*-allele has resided in clones since the origin of the D-allele itself. The mutation load across the chromosome would have a similar pattern, thus increasing towards the apomixis locus. A chromosome walk towards the apomixis gene will be a walk into deeper asexual history and potentially offers a way to study the long term effects of asexual reproduction. This will also apply to other apomixis loci. The genome of an apomictic clone is thus a mosaic of regions differing in asexual reproduction history and asexual mutation load. Neoclones originate from a single cell, a zygote. Therefore, the new clone will be fixed for all the ancestral deleterious mutations in the fertilizing diploid pollen grain. Single cell descent is a powerful mechanism for slightly deleterious mutation fixation in the asexual lineage. This is illustrated in Fig. 22.7.

Over evolutionary time, recombination between apomixis chromosomal regions and their non-apomixis homologs will further decrease. Since the chromosomal regions surrounding apomixis genes do not undergo exchanges with their homologs in the sexual gene pool, they will diverge from these homologs. Sequence divergence will further decrease recombination (Opperman et al. 2004; Li et al. 2005), which will further increase sequence divergence, as a self-reinforcing process.

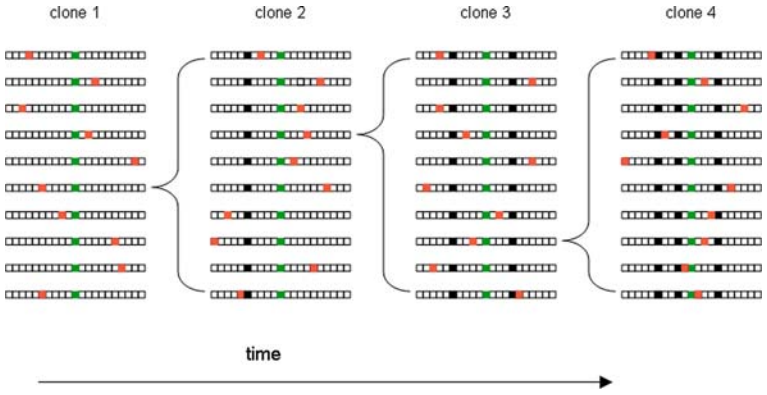

Fig. 22.7 The sampling effect in pollen grains giving rise to new apomictic clones. Each successive clone is represented by 10 clone mates. Only the chromosome carrying the dominant apomixis allele (*green square*) and 21 other loci (*white square*) is shown. For clearity, recombination is not included. Slightly deleterious mutations that newly occurred during the life of a clone are indicated in red. The mutations inherited from the previous clonal generation are indicated in black. The result is a fixation of slightly deleterious mutations in the vicinity of the apomixis locus

Moreover, apomixis chromosomal regions will accumulate chromosomal rearrangements (inversions, translocations), because there is no meiotic sterility sieve against chromosomal rearrangements in apomictic clones, as female meiosis is circumvented in seed formation. Mutants in apomictic clones with chromosomal rearrangements will not have decreased in seed fertility. Chromosomal rearrangements will suppress recombination, again leading to increased sequence divergence. Thus, the chromosomal region with a deep asexual reproduction history surrounding the apomixis gene will expand as will the mutational footprint of asexual reproduction.

22.7 Recombination and Structure of Apomixis Chromosomal Regions

Studies with genetic markers have indicated that recombination around apomixis loci is suppressed in many species, as predicted above. Strong suppression of recombination in an Apomixis Specific Chromosome Region (ASCR) was first reported in the apomictic grass *Pennisetum squamulatum* (Ozias-Akins et al. 1998). A large number of genetic markers were co-segregating with the apomixis trait. Subsequently, suppression of recombination has been described in many other apomict species (reviewed in Ozias-Akins and Van Dijk 2007). Interestingly, especially in grass species, apomixis is inherited as a single dominant factor, whereas in the Asteraceae family, apomeiosis and parthenogenesis are inherited as separate genetic factors, e.g., *Taraxacum* (Tas and Van Dijk 1999), *Hieracium* (Catanach et al. 2006) and *Erigeron* (Noyes et al. 2007). This suggests that the single apomixis

locus in some apomicts may in fact contain several tightly linked genes with different function. Given that apomeiosis and parthenogenesis are different processes occurring in different cells, control by different genes at different points in time is to be expected.

Analysis of the structure of ASCRs is also consistent with the idea of a deep asexual reproduction history. Fluorescent in situ hybridisation (FISH) has revealed hemizygosity of the ASCR in tetraploid apomictic *Pennisetum squamulatum* (Goel et al. 2003). The size of the non-recombining ASCR in *Pennisetum squamulatum* has been estimated to be about 50 Mb. Recently, the first sequences of ASCRs have been published. Shot gun sequencing of Bacterial Artificial Chromosomes of the ASCR in *Pennisetum* indicated many duplications and insertions of transposable elements and only few genes (Conner et al. 2008). Similar results were obtained in another apomictic grass species, *Paspalum simplex* (Calderini et al. 2006). Transposon insertions, small deletions and point mutations caused a loss of coding capacity in the ASCR compared to homologous rice chromosomal regions. Chromosomal FISH showed that in this apomict the ASCR in *Paspalum* is also hemizygous.

In *Taraxacum*, there is no indication for suppression of recombination around the *Dip*-locus (Vijverberg et al. 2004). In contrast, recombination around the *Par*-locus is strongly suppressed (Van Dijk, unpublished results). Although there is no evidence of suppression of recombination around the *DIP* locus, below we will provide evidence for segregation distortion of *Dip*-alleles in haploid pollen grains.

22.8 Why are Apomicts Not Diploid?

Above, we referred to the study by Van Dijk and Bakx-Schotman (2004) who investigated the inheritance op the *Dip*-locus in a cross between a sexual diploid and a diplosporous tetraploid plant. This *Dd1d2d3* tetraploid had balanced pollen meiosis and produced highly fertile diploid pollen (*Dd1*, *Dd2*, *Dd3*, *d1d2*, *d1d3* and *d2d3*). A *Dd1d2* triploid apomictic pollen donor would produce both haploid and diploid balanced pollen (*D*, *d1*, *d2* and *Dd1*, *Dd2* and *d1d2*, respectively), albeit at a low rate, because most pollen grains would be aneuploid. A recessive mutation load coupled to the *D*-allele, in a triploid apomict would result in a segregation bias against the *D*-allele in haploid pollen, but not in diploid pollen where the recessive lethals would be largely masked by the wild type alleles linked to *d1* or *d2*. To test this hypothesis of segregation distortion in haploid pollen grains, we made a diploid sexual X triploid apomict cross (TJX3-20 X A68; see Appendix). It was previously noticed that although diploid sexuals have a sporophytic self incompatibility system, high selfing rates occur in diploid sexual X triploid apomict crosses. Since no selfing occurs in diploid sexual X diploid sexual crosses, it seems that pollen from triploids has a mentor pollen effect on selfing (Tas and Van Dijk 1999). To avoid the confusing effects of selfing, a male-sterile diploid sexual plant TJX3-20 was used. Although pollen fertility of the triploid clone A68 was very low, by crossing more than 62 diploid inflorescences 192 hybrid offspring could be raised in

total. Although pollen grains of triploid A68 were highly irregularly sized - indicating many aneuploid pollen grains-, DNA flow cytometry revealed that only viable euploid offspring plants were produced: 96 diploids, 95 triploids and 1 tetraploid. This suggests that only pollen grains with balanced genomes are capable of fertilization, or that only genomically balanced zygotes are viable. Since the diploid mother plant only produces haploid eggs, the ploidy level of the fertilizing pollen grains can be deduced. In the case of diploid offspring, the pollen grain from A68 was haploid, in triploid offspring the pollen grain from A68 was diploid and in the single tetraploid plant, the pollen grain from A68 must have been triploid (unreduced). Carrying the full paternal apomictic genome of A68, the tetraploid F1 hybrid was as expected apomictic. The 92 triploid hybrids segregated for apomixis, with approximately one third being apomictic (30.4%) and two thirds non-apomictic (69.6%). This was as expected, because apomixis in dandelions is controlled by several unlinked loci, which can recombine during pollen meiosis. Most remarkable, however, none of the 97 diploid hybrids reproduced apomictically.

To investigate whether the lack of apomixis in the diploids was due to non-expression or non-transmission of the apomixis genes, the transmission of two SSR markers that were linked to the *Dip*-allele was analyzed. As mentioned before, *MSTA78* and *MSTA53* had been mapped at 7 cM distance on the same side of the *Dip*-locus in a diploid x tetraploid cross (Van Dijk and Bakx-Schotman 2004 and Van Dijk, unpublished results; Fig. 22.8A). High allelic diversity between the crossed plants allowed the unambiguous deduction of all egg cell and pollen grain genotypes. Approximately two third of the triploids carried the *MSTA78-164* allele and the *MSTA53-202* allele. All apomicts carried these two alleles, however, not all

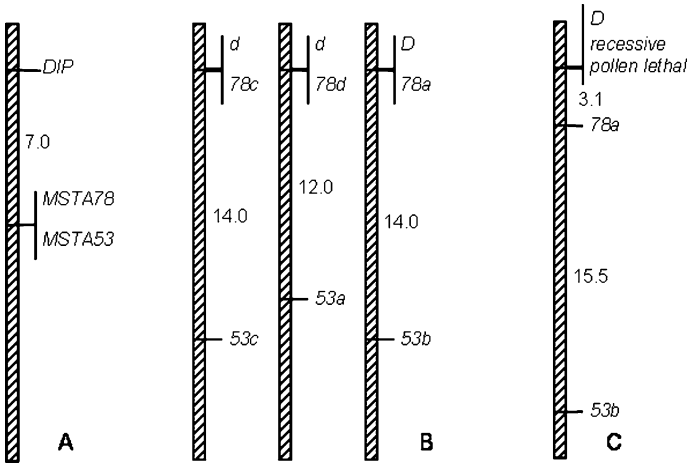

Fig. 22.8 Three genetic maps of the *Dip* chromosomal region in *Taraxacum officinale* based on: A. a *dd* X *Dddd* cross (Van Dijk and Bakx-Schotman 2004), B. Diploid pollen grains in a *dd* X *Ddd* cross and C. haploid pollen grains in the same *dd* X *Ddd* cross, assuming a recessive pollen lethal factor completely linked to the D-allele. The *MSTA78a* allele is the 164 bp allele, the *MST53b* allele is the 202 bp allele. For further explanation see the appendix

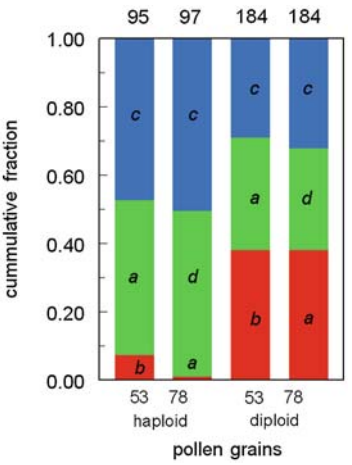

Fig. 22.9 Segregation of the *MSTA78* and the *MSTA53* SSR loci in a *dd* X *Ddd* cross, for haploid and diploid pollen grains. The number of alleles analyzed is indicated above the bars. The *MSTA78a* allele in the graph is the 164 bp allele, the *MST53b* allele is the 202 bp allele

carriers of these alleles were apomictic, which can be explained by the lack of other elements of apomixis, due to segregation.

Figure 22.9 shows the segregation of the SSR alleles in the haploid and the diploid pollen grains of the apomictic pollen donor A68. In the diploid pollen grains, the segregation ratios of the three paternal alleles were not significantly different from 1:1:1 Mendelian equality (*MSTA78*: χ^2 =1.96; d.f. = 2; $P = 0.375$; *MSTA53*: $\chi^2 = 2.36$; d.f. = 2; $P = 0.307$). In haploid pollen however, the segregation ratios were highly distorted (*MSTA78*: $\chi^2 = 45.61$; d. f. = 2; $P < 0.001$; *MSTA53*: $\chi^2 = 28.89$; d.f. = 2; $P < 0.001$). Haploid segregation distortion was caused by the two SSR-alleles that were linked to the *D*-allele. The *MSTA78-164* allele was found only in one out of 97 diploid plants, the *MSTA53-202* only in seven out of 95 diploid plants.

Figure 22.8B shows the genetic map of the *Dip*-chromosomal region, as constructed from the diploid pollen grains. The *MSTA78-164* and the *MSTA53-202* alleles were linked to the *D*-allele.

The allele-specific segregation distortions in haploid pollen grains cannot be explained by preferential chromosome pairing during pollen meiosis, because the frequencies of di-allelic genotypes in diploid pollen did not differ significantly from random assortment (*MSTA78*: $\chi^2 = 3.35$; d. f. = 2; $P = 0.187$; *MSTA53*: $\chi^2 = 4.19$; d. f. = 2; $P = 0.123$). A plausible explanation is that the *D*-allele was not transmitted via haploid pollen because of recessive pollen lethality due to a *D*-specific mutation load, as predicted above. A pseudotest-cross indicated that the single diploid F_1 plant carrying the paternal *164 bp*-allele for *MSTA78* lacked the dominant *D*-allele. A crossover probably uncoupled this marker from the *D*-allele and its linked mutational load. The seven haploid pollen grains transmitting the *MSTA53b* allele can also be explained this way. The genetic map, based on the haploid pollen grains and assuming recessive pollen lethality is shown in Fig. 22.8C. There were no segregation distortions between the other alleles at the SSR loci, suggesting that *d2* had not

accumulated a significant number of linked deleterious mutations in the previous clonal generation (s) compared to *d1*, which has only resided in clone A68.

An alternative explanation is that the *D*-specific segregation distortion is not due to a linked mutation load, but that the *D*-allele itself has, besides the female meiosis I restitution, a pleiotropic recessive pollen lethal effect. Nogler (1984) suggested that the dominant apospory (*A*) factor in apomictic *Ranunculus auricomus* acted as a recessive pollen lethal. Both the *Dip*-gene in *Taraxacum officinale* and the *A*-factor in *Ranunculus auricomus* avoid meiotic reduction, but via entirely different cytological mechanisms, namely meiotic restitution (diplospory) and meiotic by-passing (apospory), respectively (see Chapter 3). It is unlikely that dissimilar genes would have similar pleiotropic effects. In contrast, what these genes have in common is that they reside permanently in asexual lineages and we consider it therefore more likely that recessive lethality is a consequence of a linked mutation load.

Apomixis has been described in $\sim$ 400 plant taxa (Bicknell and Koltunow 2004). Nearly all investigated gametophytic apomicts (apomixis *s.s.*) are polyploids and reports of natural diploid apomicts are rare. The well-known case of diploid apomixis in *Potentilla argentea* was shown to be selfing by the use of genetic markers (Holm et al. 1997). At present, *Boechera holboellii* is the only robust case of a natural diploid apomict (Naumova et al. 2001; see also Chapter 23). Non-transmission of apomixis genes in the haploid state caused by a linked recessive mutation load may be a general explanation for the strong association between gametophytic apomixis and polyploidy. A similar hypothesis was developed by Richards (1996), however without explaining how the load would become specifically linked to the dominant apomixis allele, nor providing empirical evidence for its existence.

22.9 Conclusions

If asexual lineages go extinct because of the accumulation of deleterious mutations or a lack of adaptability, the apomixis genes may escape extinction via crossing with sexuals. Apomixis genes become incorporated into new clones, with a genetic background that is partly drawn from the sexual gene pool, that is freed from deleterious mutations and that is potentially adaptive.

There are at least two conditions for this system to persist. First, it is essential that the sexuals do not go extinct. This is a problem, because theoretically, a dominant gene for apomixis will rapidly go to fixation in a sexual outcrossing population (Marshall and Brown 1981; Van Dijk 2007). The haploid non-transmission of the *D*-gene described here protects diploid sexual dandelion populations from being taken over by apomixis. Since the haploid non-transmission of apomixis genes due to a linked genetic load takes time to build up, the diploid sexual gene pool must have initially been isolated from the pollen producing apomicts, e.g., by habitat differentiation or geographic isolation. Another possibility would be a reversal to sexuality by loss of apomixis genes after the linked genetic load was established.

A second condition is that the clone must continue to produce at least some functional pollen; otherwise, the apomixis genes will become trapped in a clone and will go extinct with it. Because pollen is not needed for seed production, pollen function will degenerate over evolutionary time. There may even be selection against pollen production in clones if resources for pollen production can be reallocated to increase fitness (e.g., more or bigger seeds). This creates a conflict of interest between apomixis genes and apomictic clones. Indeed, dandelion clones that lack pollen are not uncommon. It would be interesting to theoretically explore the parameter values under which sexuals and pollen producing apomicts can coexist over long periods of time.

The apomixis gene transmission system depends on the male function in hermaphroditic apomictic organisms. Hermaphroditic apomixis is not restricted to plants; certain animals like earth worms or flatworms also reproduce by hermaphroditic parthenogenesis. Moreover, the apomixis/parthenogenesis gene transmission system is not limited to asexual hermaphrodites but also applies to asexual organisms, in which males can be induced by environmental triggers and in which there is a genetic basis for asexual reproduction, as has been demonstrated in aphids (Delmotte et al. 2001; see also Chapter 25) and in *Daphnia* (Innes and Hebert 1988; Paland et al. 2005; Lynch et al. 2008; see also Chapter 15). The three-level superstructure of asexual populations may therefore be more common.

John Maynard Smith wrote in his book *The Evolution of Sex*: "Asexual clones of the dandelion *Taraxacum officinale* continue to produce functionless yellow petals and many produce functionless pollen. It is difficult to suggest any explanation of these facts, other than that these clones may be relatively recent in origin, and that evolutionary adaptation in asexual populations is slow, so that the maladapted features are retained" (p 41, second edition, 1978). The apomixis-gene view provides an additional explanation, namely that without a pollen transmission system for apomixis-genes, apomictic dandelions would likely have become extinct long ago. Clearly, biologists may gain illumination from an apomixis-gene's view on dandelions.

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Appendix

A pollen-sterile sexual diploid dandelion (TJX3-20) was crossed with a pollen-fertile apomictic triploid (A68) (Fig. 22.10). TJX3-20 originated from Langres (France). A68 originated from Heteren (The Netherlands). Viable seed set in the TJX3-20 X A68 cross was low (on average 2.1%), reflecting the high frequency of inviable aneuploid pollen grains produced by the unbalanced pollen meiosis of triploid A68. Sixty-two crossed capitula contained only 192 viable seeds in total. Ninety-six F_1 plants were diploid (50%), 95 triploid (49.5%) and one (0.5%) was

Fig. 22.10 Spontaneous seed set of bagged flowers (excluding cross pollen) in the diploid sexual male sterile TJX3-20 seed parent (*left*) and the triploid apomictic pollen parent A68 (*right*). Large seed heads indicate apomictic seed set, small seed heads indicate the absence of spontaneous seed development

tetraploid. These plants were the products of the fertilization of a haploid egg cell by a haploid, diploid and triploid pollen grain, respectively.

To induce flowering, eight week old F_1 plants were vernalized for 9 weeks in a cold room at 4°C. One hundred eighty two F_1 plants (94.7%) were tested for the ability to form apomictic seeds (six seedlings died and four adult plants did not flower). In order to prevent contamination by cross-pollination, the flowers were covered with small paper bags before opening. All F_1 plants were male sterile, like TJX3-20, hence seed set due to selfing can be excluded. The development of a large seed head is an indication for apomictic seed set (see Fig. 22.10). To determine the degree of apomictic seed set, for each F1 plant two batches of 50 randomly chosen seeds were germinated and the number of seedlings germinating was counted. Most of the apomicticly reproducing triploid F_1 plants had a high penetrance of apomixis (> 90% seed set), some however had a much lower penetrance.

The segregation of two microsatellite loci, *MSTA53* and *MSTA78*, which were known to be linked to the *DIP*-locus were analysed, was investigated using the methods described in Falque et al. (1998) and Van Dijk and Bakx-Schotman (2004). The *MSTA53* and *MSTA78* genotypes of TJX3-20 were respectively 202/202 and 162/166 (in base pairs). For convenience these genotypes are renamed as *b*/*b* and *a*/*b*. The *MSTA53* and *MSTA78* genotypes of A68 were respectively 198/202/222 and 164/170/174. For convenience these genotypes are renamed as a/*b*/*c* and *a*/*c*/*d*.

All 28 F_1 triploids that reproduced apomictically carried the paternal *MSTA78-164* allele ($\chi^2 = 12.65$; d. f. = 1; $P = 0.0004$), supporting the previously reported tight linkage between *MSTA-78* and the *Dip*-locus. Twenty four of the 28 F_1 triploids that reproduced apomictically carried the paternal *MSTA53-202*-allele ($\chi^2 = 2.05$; d.f. = 1; $P = 0.15$). This implies that the *MSTA78-164* allele is closer to *D* than the *MSTA53-202* allele – in the 2x X 4x cross described by Van Dijk and Bakx-Schotman (2004) no recombinants between *MSTA53* and *MSTA78* were found. The other paternal and maternal alleles of *MSTA78* and *MSTA53* were not significantly associated with apomixis in the triploid offspring, supporting the *Dip*-genotype constitution *Ddd*.

The fact that the microsatellites are codominant and that *D* occurs in a single dose, allowed genetic mapping of all three homologs in the diploid pollen grains of A68 (Wu et al 1992; Van Dijk and Schotman 2004). Because the genotypes in diploid pollen grains derived from a triploid segregate in a 2:1 and not in a 1:1 ratio, we balanced the data set by constructing a complementary haploid counterpart of each diploid pollen grain. The modified data set was analyzed with Joinmap® 3.0 (Van Ooijen and Voorrips 2001) using the BC1 module and the Kosambi mapping function. Figure 22.8B shows the genetic map of the *D*-chromosomal region, based on the diploid pollen grains.

For the linkage between the haploid pollen lethal and the *MSTA78-164* allele we assumed that the number of 164-pollen grains formed was equal to the number of *c* and *d*-pollen grains, on average 48. In only one of these *MSTA78-164*-pollen grains there was a cross-over between the *164*-allele and the recessive lethals, resulting in a Kosambi distance of 2.1 cM. Similarly a Kosambi-distance between the *MSTA53-202* allele and the recessive pollen lethal was estimated as 23.3 cM. Figure 22.8C shows the genetic map, based on haploid pollen grains, assuming recessive pollen lethality completely linked to the *Dip*-allele.

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