The significance of sexual reproduction for local adaptation in *Taraxacum*

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The significance of sexual reproduction for local adaptation in *Taraxacum*

De betekenis van seksuele voortplanting voor lokale aanpassing in *Taraxacum*

(Met een samenvatting in het Nederlands)

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Carolien Gerda Franciska de Kovel
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Contents

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General Introduction

Introduction

Sexual reproduction, such as is practiced by eukaryotes, possibly arose as a means to ensure diploidy and mask deleterious mutations (Maynard Smith & Szathmáry 1995), or as a way to facilitate DNA repair (Bernstein *et al.* 1987). Sexual reproduction, however, has turned out to have many other effects, be they main or side effects, including the creation of genetically variable offspring by a single mother. It seems to have opened up new alleys for evolution: all complicated multicellular organisms, including those that currently reproduce asexually, have evolved through repeated cycles of sexual reproduction. Obligatory asexual organisms are all relatively simple. To understand the ecological and evolutionary significance of sexual reproduction is a challenge for biology. What makes it different from asexuality? What are the short-term and long-term consequences of reproducing by way of sex?

The 'simple organisms' that have never known sexual reproduction do fine and survive for millions of generations. In the 'complex' organisms though, lineages that have reverted to asexuality seem to be all, but for a few noteworthy exceptions, of recent origin (Burt 2000). This suggests that asexual lineages of this type cannot survive long. Their way of life has become dependent on sexuality, but in what respects (Lenski 1999)?

Why asexuality is different

The level of selection and evolvability

One of the major consequences of the mode of reproduction is the level at which selection operates. In a sexual population, recombination reshuffles the genes in every zygote. A particular allele will find itself in different combinations of other alleles on its own and other loci every generation. As a consequence, natural selection can operate almost independently on different genes, though depending on the frequency of alleles on other loci in the population. Some of the variation, however, is not accessible for selection: the non-additive part of the genetic variation, which results from interaction among alleles and loci. This means that, though some combinations with higher fitness may be extant in the population, it is not always possible to fix the whole population at this genotype.

In asexual lineages, alleles are not reshuffled and new combinations can only arise by mutation. The effect of a particular allele in an asexual genome cannot be separated from the effects of alleles on other genes in the genome. Selection can therefore act solely through the differential survival and reproduction of clonal lineages. Selection acts on the additive part of the genetic variation as well as on the non-additive parts (Fischer *et al.* 2000). Well-adapted combinations are faithfully copied in the next generation, but evolutionary potential within the lineage is low, because new genotypes cannot arise from recombination.

When the asexual population is extremely large and genetically diverse, such as bacterial populations, the situation may approximate the situation in sexuals. Yet, even in bacteria the response to selection is probably less rapid, when counted in generations, and less extensive than in sexual eukaryotes (Joshi 1997).

The different ways in which selection operates on sexuals and asexuals can affect the rate and extent of their adaptation to their local environment, especially if this environment varies on a relatively small scale. This will be discussed in the section “Coping with environmental heterogeneity”.

Population growth rate: the cost of sex

Another consequence of asexuality is that it has a higher population growth rate than sexuality, when all other things are equal. Sexuals invest in both males and females, asexuals only in females. If the number of progeny that a female can produce limits total reproduction, and the sex ratio of sexual populations is 1:1, then asexuals have a two-fold advantage (Maynard Smith 1978). If a sexual couple produces two children, it just replaces its own number. If an asexual female produces two daughters, she doubles her number. In a more formal representation, the growth rate of sexual and asexual populations is:

$$N(t) = N_0 \cdot r^t \cdot n^t$$

with r the population’s sex ratio, n the number of surviving offspring per mother and N_0 the initial number of individuals in the population. For asexual populations $r = 1$.

As an additional advantage, asexuals –except for the pseudogamous forms– have reproductive assurance: they can reproduce even if they do not find a mate. For many plants, such as *Taraxacum,* this also means independence from insect pollinators.

A high and assured reproductive output may affect the optimal life-history. The optimal allocation of resources to reproduction and somatic growth could be different between sexual and asexual forms. A high fecundity could partly be an alternative to over-all adapteness.

Aim of the thesis

The purpose of this thesis is to study the consequences of sexual and asexual reproduction for adaptation. By comparing sexually reproducing organisms with relatives that have lost this capacity, I hope to obtain insight in the significance of sexual reproduction: what are the options that are opened by sexual reproduction and which options are closed? How can the mode of reproduction affect the further evolution of a species? Of course, these are high aims for a single thesis and only a small part of the issue can be addressed. This study is concerned with microevolution: the adaptation to local conditions. How do populations adapt to their local environment, which may include spatial heterogeneity, random fluctuations and directional change, and what is the role of the mode of reproduction in this.

A complicating factor is that asexuality is often associated with hybrid origins and polyploidy, in plants as well as in animals. The significance of this, I will discuss in the section 'polyploidy and mode of reproduction'.

Coping with environmental heterogeneity

The different ways in which selection can act on sexually and asexually reproducing organisms could have important implications for the evolution in those populations. It is likely to influence how well adapted the species will be to local conditions. One of the areas in which differences are expected between sexually and asexually reproducing organisms is with respect to niche width.

Environmental variation in time

If the environment fluctuates randomly over time on a so-called coarse-grained time-scale *sensu* (Levins 1968), i.e. once every generation or slower, all narrowly adapted lineages in an asexual community will be eliminated when for each in turn conditions occur that they cannot tolerate. Eventually, the genotypes with the highest geometric mean fitness over many generations have survived, and variation has decreased (Lynch 1984;Vrijenhoek 1998). In particular abiotic conditions, such as climate, may vary randomly from year to year and affect the survival of that year's juveniles. This idea assumes so-called hard selection: unfit genotypes are eliminated. The prediction is that extant asexual genotypes are tolerant to the kind of environmental fluctuations that occur from one generation to the next. These genotypes have been named General-Purpose Genotypes or GPG's (Baker 1965;Lynch 1984).

Sexuals, on the other hand, can produce genetically variable offspring. In any given environment some of these may not do well, but others will. The genotypes that were not favoured in one generation will be recreated by recombination in the next and are not lost from the population forever. The pattern of fluctuations in the environment, as well as demographic factors such as effective population size, will determine how much variation can be maintained in a sexual population and whether this variation is an alternative for a broad tolerance. Producing non-fit genotypes every generation, of course, constitutes a load for the sexual parents (Charlesworth 1993b;Lande & Shannon 1996).

Changes in the environment are not necessarily random changes on a time scale of about one generation. Climate change and many other factors may cause directional change or very slow fluctuations relative to the organism's life span. Genetic variation combined with recombination will enable sexual species to track such changes if they are slow enough. After initial standing variation has been exhausted, asexuals, which are unable to combine independent mutations from different clonal lineages, will adapt more slowly and lag behind the sexuals. A difference in trait values between sexuals and asexuals may be the result, despite identical selection pressures (Bürger 1999). Under these conditions, the asexuals will need a higher reproductive output than the sexuals; otherwise they will go extinct, because they are less well adapted to current conditions (Bürger 1999). Whether such environmental variation will affect the optimal degree of specialism of individual genotypes is not clear.

Frequency dependent selection can create its own temporal dynamics. A special case is negative frequency dependent selection that can, for instance, be exerted by pathogens that become adapted to the most frequent immunotype in the population (Hamilton 1980;Sorci *et al.* 1997). Ongoing selection because of interactions between species has been called Red Queen dynamics (Hamilton 1980). Though this model makes no predictions about the traits that sexual and asexual genotypes will possess on average, it predicts that rare or new asexual genotypes will establish more easily, like in the frozen niche variation model discussed below. Multiple clones are likely to co-exist. If asexual populations do not generate new genotypes often enough, they are likely to have a fitness disadvantage with respect to sexuals. Red Queen dynamics as a consequence of pathogen infections have been studied relatively often (Clay & Kover 1996), but only one clear case has been demonstrated so far (Dybdahl & Lively 1998).

Environmental variation in space

Environmental variation in space is expected to have entirely different consequences for the evolution of niche width than variation in time has. It has long been known that spatial heterogeneity is more likely to maintain genetic variation in a population than temporal heterogeneity is (Hedrick 1986). The degree of local adaptation in a heterogeneous environment will depend, among other things, on the dispersal distance of individuals or gametes, and the structure of the environment (Kirkpatrick 1996;Scheiner 1998). Asexuals have no dispersing gametes, so one could argue that sexuals have more dispersal and should therefore be more generally adapted than asexuals (Levin 1988).

Locally adapted sexual organisms will not automatically produce adapted offspring, because recombination will break up the genotype and produce variation.

Asexuals, though, produce offspring identical to themselves. This may be particularly important for adaptation to conditions at the border of the species' natural range. Many of the traits offering adaptation to a certain niche are quantitative traits governed by a number of genes. Offspring of a random mating between any two individuals in the population will probably show a normal distribution of those trait values around the mid-parent value. In a randomly mating population, the trait values among the zygotes in the whole population will become normally distributed around the population mean. Rare genotypes on the edge of the distribution are likely to mate with someone with a genotype closer to the mean, and their offspring will on average regress towards the mean relative to the extreme parent. Therefore, even if niches on the edge of the population's current distribution are favourable, for instance because intra-specific competition is less severe for those niches, the sexual population will only slowly extend their distribution into those marginal niches (Roughgarden 1972;Kirkpatrick & Barton 1997).

Asexuals, however, who happen to have an extreme genotype, will produce offspring identical to themselves. Those asexual offspring can quickly fill an open niche to carrying capacity without much competition from other genotypes (Case & Taper 1986;Weeks 1993). In such cases, it should pay for the asexuals to have a highly specialised genotype (Case & Taper 1986). This model supposes variation in available niches and the possibility for individuals to choose their niche, e.g. their preferred nesting location or preferred prey type. Selection in this model is assumed to be frequency- and density dependent, so-called 'soft selection'. This model predicts co-existence of multiple specialised clonal lineages together with sexual forms. The idea has been termed the Frozen Niche Variation model (Vrijenhoek 1984).

Many other ideas have been proposed about the consequences of sex for fitness (Kondrashov 1993;Bell 1982;Crow 1994), but these fall outside the scope of this thesis. This thesis focuses on relatively short-time effects of the mode of reproduction for local adaptation. Theories for long-term consequences can probably better be studied in other organisms.

Environmental tolerance and phenotypic plasticity

An organism's optimal phenotype will depend on the current conditions, and no phenotype is optimal for all possible conditions an organism can find itself in. Many species are able to adjust their phenotype to current or expected conditions through phenotypic plasticity (Schlichting 1986;Schmid 1992;Stearns 1989). The same genotype will develop different phenotypes depending on the information it gets about its environment. By varying certain aspects of the phenotype such as morphology, the genotype is able to maintain a high fitness under varying conditions (Thompson 1991). A well-known example is stem and leaf morphology of plants depending on light intensity (Huber *et al.* 1998). Some traits can vary throughout the lifetime of an organism, every time conditions change (e.g. fur colour in some temperate mammals); other traits are determined once, in early development, and are subsequently fixed (Smith-Gill 1983).

A genotype that has a broad environmental tolerance, such as general-purpose genotypes, is likely to be more plastic in certain traits than a specialist genotype. As a consequence, its fitness will drop off less dramatically in sub-optimal conditions than that of specialist genotypes (Guntrip & Sibly 1998). Knowledge of phenotypic plasticity in traits known to affect adaptedness can help us inferring a genotype's environmental tolerance.

Patterns of occurrence of sexuality and asexuality

The occurrence of sexuality and asexuality in nature may be informative of the environmental conditions under which sexuality or asexuality is favourable. Two patterns are of interest: cyclical parthenogenesis, in which case sexual and asexual reproduction within the same organism are separated in time, and geographic parthenogenesis, in which sexual and asexual reproduction within the same species or taxon is separated in space.

Cyclical parthenogenesis

Cyclical parthenogenesis is a strategy employed by a wide range of organisms, such as aphids, daphnids, algae, fungi etc. A number of parthenogenetic generations are followed by a sexual generation. Quite often, sexual reproduction is induced by a change in environmental conditions, in particular deterioration of the environment. In most of these species, the sexually produced generation has a form that is tolerant to adverse conditions, a resting stage such as a winter egg in *Daphnia*, a zygospore in *Chlamydomonas*, or a telospore in Basidiomycetes.

The pattern of cyclical parthenogenesis suggests that asexual reproduction is advantageous when conditions are favourable. Parthenogenetically produced offspring can be produced much more rapidly than sexual offspring in these species. A short generation time conveys a high fitness in a growing population (Stearns 1992). The advantage of sex is not dependent on the timing within the life cycle, but the opportunity costs may be lowest when population sizes are not increasing (Burt 2000). This pattern is not likely to teach us much about the environmental conditions that sexual or asexual organisms are better adapted to.

Geographic parthenogenesis

Geographical parthenogenesis is very common in plants. In many species the range of the asexual forms exceeds the range of the sexual forms. Often the range of the asexuals extends to higher latitudes and higher altitudes where abiotic conditions may be more severe (Bierzychudek 1985). In animals, the general pattern is the same (Glesener & Tilman 1978).

Though in cyclically parthenogenetic organisms, adverse conditions induce sexuality, the pattern of geographic parthenogenesis shows that harsh conditions are by no means impeding asexual reproduction. A number of explanations for the pattern have been offered. At lower densities, the sexuals have a lower probability of finding a mate, or being pollinated, which gives asexuals an advantage in these conditions. Another explanation is that asexuals are better colonisers than sexuals (Mogie & Ford 1988) and have been able to extend their range faster than sexuals when the northerly areas became habitable again after the ice ages. Areas occupied by asexuals are difficult to invade afterwards for sexuals, because of the surplus of unsuitable partners (Mogie & Ford 1988). When population densities are lower than elsewhere, immigrants are relatively numerous and this puts sexuals at a disadvantage because mating with immigrants breaks up adapted genomes (Peck *et al.* 1998), as argued above. Sex, on the other hand, is proposed to convey benefits in conditions where biotic interactions, e.g. with pathogens, are numerous, that is, at the lower latitudes (Glesener & Tilman 1978). The reason for this would be that they could uphold the continuous arms' race with interacting species better than asexuals. Though this pattern gives some hints about the opportunities and limitations of the two modes of reproduction, its causes are lacking proof.

Polyploidy and mode of reproduction

Asexual reproduction is often associated with polyploidy, both in plants (Gustafsson 1946;Bierzychudek 1985) and in animals (Vrijenhoek 1999). In plants, it seems all asexuals are polyploid, though many polyploids are sexual (Bierzychudek 1985); in animals all polyploids are probably asexual, though diploid asexuals occur as well (Vrijenhoek 1999;Suomalainen *et al.* 1987). Polyploid as well as asexual forms often have their origins in hybridisation events involving related sexual species. It has been suggested that because of this hybrid origin, asexuals and polyploids exhibit some degree of hybrid vigour or heterosis (Cullum 1997). Empirical evidence does, however, not support this notion as a general rule (Cullum 1997;Wetherington *et al.* 1987). For the same reason of having multiple alleles per locus, it has been argued that polyploids have a broader ecological tolerance, or general-purpose genotypes (Levin 1983). Polyploidy certainly affects physiology (Grime & Mowforth 1982;Tal 1980), but general effects of polyploidy are hard to find (Gottschalk 1976;Levin 1983). In plants, where many sexual polyploids exist, differences in distribution patterns of diploids and polyploids have commonly been observed. Polyploids are, for instance, often more widely distributed than diploid species, especially into formerly glaciated areas (Bierzychudek 1985). Since polyploidy and asexuality are coupled, it is difficult to distinguish between the effects of each on life history and other traits.

If we want to understand the consequences of sexual reproduction from the comparison of diploid sexual and polyploid asexual organisms, we must understand the consequences of polyploidy as well and take them into account.

The *Taraxacum* system

Based on Sterk 1987b, unless otherwise cited.

Morphology

Taraxacum, or dandelion, is a well-known herbaceous plant. It is a perennial species. The taproot, in which carbohydrates are stored, can grow up to a meter in length and constitutes the major part of the plant's biomass. The lobed leaves grow in a rosette, close to the ground. The capitula appear on hollow, unbranching stems. Each capitulum contains 15-400 yellow florets. The capitulum develops into a spherical seedhead. The elliptic seeds (*achenes*) weigh typically around 0.5 mg and carry a parachute or *pappus*. All plant parts contain bitter, white latex.

Life cycle

Seeds mature around May in the Netherlands. Maximum dispersal distance has been assessed to be about 2.3 m in open vegetation at high wind speed, but the majority of seeds probably land closer to the mother. The few seeds that are caught in turbulence can possibly travel much larger distances (Sheldon & Burrows 1973). Most seeds germinate in the same summer as they have been produced, and few seeds survive for more than a year in the seedbank. Under favourable conditions plants flower in the subsequent spring, usually March-April. Most genotypes in the Netherlands require a cold period to induce flowering. The flowers attract insect pollinators, in particular bumblebees and bees.

Polyploidy

Dandelions form a so-called agamic complex. The base chromosome number of dandelions is n=8. Diploid plants (2n=16) all reproduce sexually. The majority of individuals, however, is triploid and reproduces apomictically. Higher ploidy numbers up to decaploid appear in lower frequencies. Unlike some other species, polyploid dandelions, even ploidy levels as well as odd levels, all reproduce apomictically.

Geographical distribution

Dandelions are indigenous in Eurasia. A few varieties are indigenous in the Americas, but most plants in North America are descendants of plants that were introduced by European settlers. In the southern hemisphere, dandelions are rare.

The distribution of sexual diploids and apomictic polyploids is a clear example of geographic parthenogenesis. Whereas apomicts grow in the whole range of *Taraxacum* distribution, sexuals are confined to the lower latitudes. In Europe, sexual distribution ranges to the north of Belgium with a few occurrences in the Netherlands, and into the Czech republic and Bohemia (Den Nijs & Menken 1994), apomicts occur as far north as Iceland.

Sexual and apomictic reproduction and their interactions.

Sexual dandelions are usually self-incompatible (Jenniskens 1984) and function normally. Apomictic plants produce seeds by apomixis of the *Antennaria* type. This means that the female gametes show restitutional meiosis (*diplospory*). The male meiosis in apomicts, however, is usually not restitutional, but irregular. Pollen grains can probably contain any number and any combination of chromosomes. Each flower produces thousands of pollen grains, and viable combinations are likely to occur.

Apomictic plants produce both pollen and nectar (Jenniskens *et al.* 1984) and pollinators visit both reproductive types. In mixed populations of sexuals and apomicts, pollen exchange can occur. It has been shown that pollen produced by triploids can father offspring in diploid plants. Hybrid offspring can be diploid, triploid or tetraploid. Diploid offspring, if fertile, reproduces sexually. Triploid offspring, when fertile, can be apomictic. So, two-way gene flow between the apomictic triploid community and the sexual diploid population is possible (Tas & Van Dijk 1999b;Tas & Van Dijk 1999a;Morita *et al.* 1990). The frequency of such hybridisation is unknown. A survey in one mixed population showed that sexual mothers had less than 2% triploid offspring (P. van Dijk, pers. comm.). Allozyme studies show that generally apomictic communities share more alleles with local sexual populations than with distant apomictic communities (Menken *et al.* 1995).

Genetic diversity of apomicts

The genetic variation among triploid, apomictic dandelions is high. Local populations of triploid dandelions usually contain many genotypes. Many of these clonal genotypes are restricted to a local population, whereas a few genotypes are very widespread (Lyman & Ellstrand 1984;Ford & Richards 1985;Menken *et al.* 1989;Battjes *et al.* 1992) (Richards 1996). High genetic variation is found in areas where sexuals are absent, as well as where they are present. Most of the data suggest multiple hybridisation as the source of this variation, with a much smaller role for within clonal mutation (King 1993;Van der Hulst *et al.* 2000). *Taraxacum* apomicts do not seem to have a heterozygote excess compared to Hardy-Weinberg expectations (Menken *et al.* 1995).

Phenotypic plasticity

Dandelions have been shown to be plastic in many traits, just as most plant species (Bradshaw 1965;Schmid 1992). Phenotypic plasticity has been found, for instance, in the number of capitula per plant (Van Loenhoud & Van der Heijden 1980), the number of ovules per capitulum (Van Loenhoud & Van der Heijden 1980;Sukatschew 1928), the shape and serratedness of leaves (Sánchez 1967), and in growth rate and root:shoot ratio (Hommels 1991;Cox & Ford 1987). Most of these studies report genetic variation in degree of plasticity.

Outline of thesis

The main question of this thesis is how the mode of reproduction affects local adaptation. I studied whether sexually and asexually reproducing organisms have evolved different strategies for survival and reproduction. Special interest was paid to the way the two modes of reproduction cope with environmental heterogeneity. A number of theories have been discussed in the literature about the characteristics individual genotypes and populations evolve when they reproduce in either way. A few of these have been discussed above. Despite general interest in the subject, empirical data are rather scarce (West *et al.* 1999a). The main body of this thesis concerns studies on a model species dandelion, *Taraxacum,* comparing sexual and asexual genotypes. As in all areas of biology, understanding of specific systems in sufficient detail is necessary to understand the general pattern.

As mentioned before, a number of ideas have been developed about the way in which sexual and asexual species adapt to their environmental conditions, and the differences between sexuals and asexuals that will result from it. We will study those differences and will try to reason backwards to understand the significance of sexual and asexual reproduction. The original ideas were in many instances verbal. Subsequent modelling by various authors has elucidated some of the issues involved, but no models specifically allowed evolution of the degree of specialism. Therefore, we developed a simulation model with which we could study the evolution of specialism or generalism under various conditions. The results of this study can be found in chapter 2.

The model species of this study is dandelion, *Taraxacum*. Selection requires genetic variation, and affects both the mean and the distribution of traits in the population. Different responses to selection pressures or different selection pressures on sexuals and asexuals, as described in section "Why asexuality is different" can result in differences between sexuals and asexuals. Chapter 3 describes some morphological and life-history traits and their variation of sexual and asexual types of dandelions, when grown under common-garden conditions.

A very important environmental variable is light. Amount and quality of light can vary at different scales in time and space, though variation in space is probably most important. Variation in time is not likely to be random. Chapter 4 describes how genotypes of sexual and asexual dandelions respond to different light levels. Can both types grow equally well at different levels or do they specialise on high light or shaded conditions?

Since asexual dandelions are triploid and sexual dandelions are diploid, differences between sexuals and apomicts can either be associated with the type of reproduction or with the ploidy level. Chapters 5 and 6 describe studies that were set up to disentangle the effects of ploidy and reproductive mode and to get insight in the dynamics of establishment of new asexual lineages. When new asexual lineages are generated, they may differ from the average sexuals because of their higher ploidy level. Alternatively, triploids may be similar to diploids in certain traits. Selection may subsequently cause differences between the average trait values of the sexual and the asexual types. In chapter 5 differences in morphological and life-history traits between sexual plants, asexual plants from the field and new asexual plants from the lab are studied. In chapter 6, asexual genotypes from the field were compared to new asexual genotypes from the lab under two light levels. This way, it can be studied whether asexual genotypes were selected for a particular response to shading, be it more plastic (generalist) or more static (specialist).

The genotype composition of an asexual community can only change as a result of mutation or by the generation of new types from backcrosses with sexuals. Potential changes in genotype composition of the asexual community in response to selection pressure are therefore expected to be slower than in sexual populations. Biotic interactions may exert continuous selection pressure, for instance on the competitive ability of genotypes. In chapter 7, an experiment is described that tested the reaction of sexual and asexual seedlings to competition with other dandelions. It tests whether sexuals are better at upholding the arm's race than asexuals. This experiment can also give insight in the way sexual and asexual dandelions are able to continue co-existence.

Asexual dandelion communities always contain numerous different genotypes. If these genotypes are functionally different, a combination of genotypes may be able to occupy a broader niche than single genotypes. This can also have consequences for the co-existence of sexuals and asexuals. In chapter 8, an experiment is described that tested the significance of genetic variation in a heterogeneous environment.

Chapter 2

Evolution of specialisation in sexual and asexual organisms

Evolution of specialisation in sexual and asexual organisms

Carolien G.F. de Kovel & Daniel J. van der Post

Abstract

In a variable environment, producing genetically variable offspring, such as sexual organisms do, may have different consequences than producing genetically identical offspring, such as asexual organisms do. Theoretical work has coined the concept of general-purpose genotype: asexual clones that survive for many generations will be tolerant to a variety of environmental conditions. Another concept is the Frozen Niche Variation model: in a spatially heterogeneous environment, asexual specialists can easily invade a sexual population if the individual organisms can choose their favourite niche. So, these ideas have different expectations about the degree of specialism asexual organisms should have, though how this relates to the degree of specialism in sexual organisms is not entirely clear. In the natural world, the environmental variation may comprise both temporal and spatial components. Also, many organisms do not find their own niche, as is assumed by the Frozen Niche Variation model, but disperse a certain distance from their parents. This paper describes the results of a simulation model. It studies the evolution of specialism and generalism in sexually and asexually reproducing organisms under different patterns of environmental variation in both space and time. The results show that general-purpose genotypes will evolve in a fluctuating environment in sexuals as well as asexuals if population sizes are small, so genetic variation gets depleted in the sexuals. In a spatially structured environment with limited dispersal, many specialist genotypes can co-exist, and under restricted conditions the optimal asexual genotype may be more specialist than the optimal sexual genotype. Some consequences of environmental variation in both space and time are shown, as well as some effects of interaction between sexuals and asexuals.

Keywords: asexuality, environmental heterogeneity, frozen niche variation, general-purpose genotype, sex, simulation model

Introduction

Sexual reproduction is the rule for higher organisms, though many forms of asexual reproduction occur in a wide range of species (Suomalainen *et al.* 1987). Over the last decades, a number of ideas have crystallised about the role of recombination and sexual reproduction in enhancing adaptation in heterogeneous environments.

Asexuals produce offspring that is genetically identical to themselves. This ensures a faithful copying of a possibly well-adapted genotype, but it also reduces the evolutionary potential of asexual families relative to sexuals. Based on these characteristics of asexuality two different theories about asexual adaptation to environmental heterogeneity have been proposed in the literature: the theory of the general-purpose genotype (GPG) (Lynch 1984) and the frozen niche variation theory (FNV) (Vrijenhoek 1984).

General-purpose genotype

The idea of the general-purpose genotypes assumes that asexual lineages that survive for many generations must have been able to tolerate all of the conditions that they have encountered over those generations. The genotypes with the highest geometric average fitness over all these generations have survived. The prediction therefore is that asexual genotypes are tolerant to the kind of environmental fluctuations that occur from one generation to the next.

Sexuals, on the other hand, can produce genetically variable offspring. In any given environment, some of these may not do well, but others will. The genotypes that were not favoured in one generation will be recreated by recombination in the next and are not lost from the population forever. Each individual genotype does not need to be tolerant to a wide range of conditions. The pattern of fluctuations in the environment, as well as demographic factors, will determine how much variation can be maintained in a sexual population and whether this variation is an alternative for a broad tolerance. Producing non-fit genotypes, of course, constitutes a load for the population (Lande & Shannon 1996).

Frozen niche

The other idea, the frozen niche variation hypothesis (FNV) was designed by Vrijenhoek (Vrijenhoek 1984). Several authors have elaborated on the idea (Weeks 1993). The model argues that the faithful reproduction of a specialised genotype, such as asexuals do, is a good strategy in a spatially heterogeneous environment, or at least in an environment in which several niches are available simultaneously. Many of the traits offering adaptation to a certain niche are quantitative traits governed by a number of genes. In a randomly mating population, the offspring of any given pair of parents will probably show a normal distribution of those traits values around the mid-parent value. As a result, in the whole population, before selection the trait values among the zygotes will be normally distributed around the population mean. Rare genotypes on the edge of the distribution are likely to mate with someone with a genotype closer to the mean, and their offspring will on average regress towards the mean relative to the extreme parent. Therefore, even if niches on the edge of the population's current distribution are favourable, for instance because competition is less severe for those niches, the sexual population will only slowly extend their distribution into those marginal niches (Roughgarden 1972;Kirkpatrick & Barton 1997).

An asexual mutant, however, who has an extreme genotype, will produce offspring identical to herself. Those offspring can quickly fill an open niche to carrying capacity without much competition from other genotypes (Case & Taper 1986) (Weeks 1993). In such cases, it should pay for the asexuals to have a highly specialised genotype (Koella 1988). Sexuals break up their genotypes every generation by recombination. It is possible that a more generalist strategy is optimal for sexual organisms. The Frozen Niche model supposes variation in available niches and the possibility for individuals to choose their niche, e.g. preferred nesting location or preferred prey type. In addition, those niches are exhaustible.

In many species, and in particular in plants, the population is spatially structured with mating being local and offspring growing close to their parents. In addition, the possibility for choice for a particular niche is rather limited. It is not clear whether the frozen niche model will work under those conditions.

The GPG model predicts that with respect to temporal fluctuations of the environment, asexuals will evolve broader tolerance than sexual genotypes. The FNV model expects asexual genotypes to be specialised with respect to spatial environmental variation. Some environmental conditions will fluctuate both in time and in space, e.g. water availability or temperature. According to these models, this can lead to conflicting selection pressures.

Using Monte Carlo simulations, we studied the degree of specialisation that sexual and asexual organism will evolve under different situations of environmental heterogeneity. We implemented a spatially structured habitat. Simulations with different levels of migration were used to study local adaptation or specialisation in sexuals and asexuals. Random fluctuations in environmental conditions over time at a scale of 1 and 10 generations were employed to study whether asexuals are more likely to evolve general-purpose genotypes than sexuals. A mixture of both migration and environmental fluctuations was used to study realistic conditions.

Model

The environment

The environment consisted of a one-dimensional array of 41 habitats, numbered 0 through 40. These habitats represented a cline with an environmental variable varying from 0 to 40.

The organisms

Organisms could be either sexual or asexual. Sexually reproducing organisms were self-incompatible hermaphrodites. Phenotypes of the organisms consisted of two traits: mode and breadth. The mode denoted the environmental condition at which the maximal fitness was obtained; the breadth signified the tolerance for environmental conditions deviating from this optimal value (see below). For example, one could think of the mode as the temperature at which a crucial enzyme performs optimally; breadth would then be the stability and functioning of the enzyme at deviating temperatures. Organisms were diploid. Each of the traits was coded for by five loci. At each of the loci, five alleles were present in the population. The values of the alleles ranged from 0 through 4. Allele effects on the trait within and among loci were additive and resulted in trait values ranging from 0 to 40 (2 alleles * 5 loci * value 4 = 40). With these values, many genotypes will code for the same phenotypes. More different combinations will code for phenotypes with mode and mean of 20 than for extreme phenotypes. Since breadth values of 0 were not biologically relevant, the breadths of individuals homozygous for allele 0 at all loci governing breadth values were assigned a breadth of one. No linkage was assumed between any of the loci: in gamete formation, all loci would segregate independently. No mutation towards other allele values was implemented.

Life-history

Parents were chosen randomly and with replacement within the habitat. If the organism was asexual, one offspring identical to the parent was produced. If the individual was sexual, a second organism was chosen at random until a sexual individual was found. One offspring was produced by recombining the parental

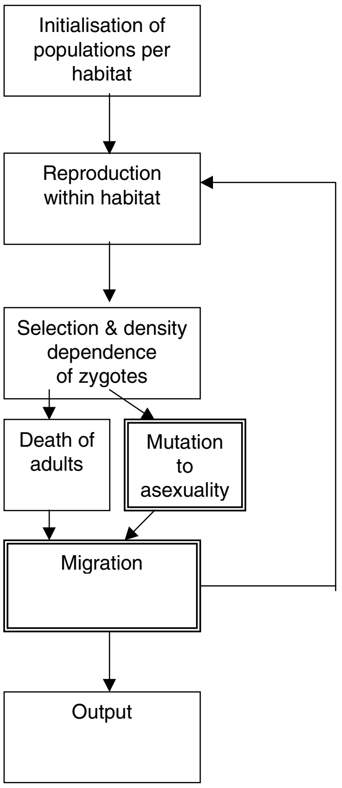

Fig 1. Flowchart of simulation model. Double-lined boxes represent steps that are not present in all simulations.

genomes randomly. Fitness of the zygote was determined according to its phenotype and the environmental condition within the habitat, as described below. Fitness determined the chance that the zygote actually survived this stage and was added to the new generation. Reproduction and selection continued until population size in each habitat equalled its carrying capacity. However, each individual could only produce a limited number of offspring as a mother, and so, if offspring viability was low, the habitat would not be filled to its carrying capacity. This procedure did not allow the asexuals the usual two-fold advantage, but created equal fecundity for both reproductive modes. The zygotes matured in their habitats of birth and could as adults migrate to neighbouring habitats. Migration comprised 0, 25, or 50% of the total number of inhabitants of a habitat. Half of the migrants travelled towards a neighbouring habitat, one quarter travelled two habitats away etc. Migrants passing the border beyond 0 or 41 bounced back. Census was on surviving zygotes within their habitats of birth (Fig 1).

Mutation towards asexuality

In a number of simulations, mutation of sexuals to asexuals was implemented. Sexual offspring was produced as described above and tested for survival. Surviving zygotes could mutate towards asexuality with a probability of 10^{-4}. No back mutation was considered, as this is supposed to be much more difficult in nature.

Selection

Selection proceeded according to an individual's phenotype and the value of the environmental variable in the habitat it was born in. The following quadratic function was used to define a zygote's fitness w_i:

$$\begin{aligned}&|E-M| < 0.5 * B \rightarrow w_i = w_{\max} - \frac{4w_{\max}}{B^2} * (E-M)^2\\&else \rightarrow w_i = 0\end{aligned}$$

with:

$$w_{\max} = \frac{1}{\sqrt{B}}$$

With M the individual's mode, B its breadth, E the value of the environmental variable in the habitat of birth. When the difference between the individual's mode M and the state of the environment E is $0.5B$ or more, then fitness is 0. This function shows a trade-off between generalism and maximal fitness w_{max}: Jack-of-all-trades is master-of-none. A tolerant genotype is therefore not automatically the best option of all.

Initial conditions

The genotypes of the individuals were assembled at random from a uniform distribution of all alleles at each locus. Individuals having extreme phenotypes for mode and breadth were rare at initiation by this procedure.

Simulations

In control simulations neither migration nor temporal variation was implemented. Each habitat was therefore isolated from others. Two rates of migration were tested, as mentioned above: 25% and 50%. Temporal environmental fluctuations involved random fluctuations about the initial environmental value (which equalled the habitat number). The environmental value fluctuated by maximally 4 units on either side of the initial value and therefore had amplitude of 9: the environment within each habitat had nine possible states. Subsequent values were not correlated. The environmental state either changed every generation, so-called short-term variation, or every ten generations, the long-term variation. A combination of temporal and spatial variation was made in a set of simulations in which the environment within each habitat changed state every ten generations, whereas either 25% or 50% of the population migrated each generation. All simulations mentioned above were run with either populations solely consisting of sexuals or of asexuals. Simulations ran for 2000 generations. Each simulation was repeated five times.

The same set of simulations was run starting with sexual populations of 200 individuals per habitat for 2000 generations, after which sexual zygotes were allowed to mutate into asexuals with a probability of 10^{-4}. Then simulations were continued for another 10,000 generations.

Data analysis

Data were collected for generation 1-20 and from then on once every 20 generations until the end of the simulations. Collected data comprised the average mode and breadth per habitat (for sexuals and asexuals separately), its variation, and its range, as well as the number of individuals per reproductive strategy.

Most variables were not normally distributed, and therefore non-parametric tests were used. To compare values between habitats within the same set of simulations (independent-samples) Kruskall-Wallis H test was used. When variables of sexuals and asexuals were compared within a set of simulations, over all habitats, values within habitats were considered related samples. Such variables were compared with Wilcoxon signed rank test. Similarly, when variables were compared between two sets of simulations, e.g. with and without migration, the Wilcoxon signed rank test was used. To assess the effect of habitat number on a given variable, Spearman's rank correlation was used. As the habitat array was symmetrical about habitats 19 and 20, correlations were calculated separately for habitats 0-19 and 20-40. In a number of cases, computer memory was insufficient to process complete data sets. In these cases habitats 0, 10, 18, 20, 30 and 40 were used or analysis.

Results

Control

In the control simulations, each patch was initiated with 100 individuals and no migration occurred. While the mode of the purely sexual population fixed at the local optimum, breadths fixed on average at 2.7 (±1.9). In the asexual population, evolution was severely constrained by the lack of genotypes with low breadth or extreme modes. Optimal modes were only obtained in the central habitats, whereas in the extreme habitats modes at fixation were 10 units away from the optimum. Breadths in the central habitats were 11.4, in extreme habitats 25. In all subsequent simulations, the evolution of asexuals appeared severely constrained by the genotype distribution at initiation, and the results of those simulations will not be presented.

Because sexual populations went to fixation within 2000 generations, mutation to asexuality did not change modes or breadths. Since mutation was one-way, asexuals eventually took over the populations.

Migration

Migration increased the availability of genetic variation. Consequently, when the migration probability was 25%, all sexual populations became completely specialised on local conditions: their modes coincided with the local optimum and breadths decreased to 1.

Increasing migration to 50% led to a strategy of bet-hedging or general-purpose genotypes; the breadths of the sexuals increased to 7.2 (±0.2) with slightly lower values in the extreme habitats (Fig 2.). In the extreme habitats, optimum modes were not attained: mode values were 1 unit closer to the central value. In addition, genetic variation was maintained for modes and breadths until the end of the

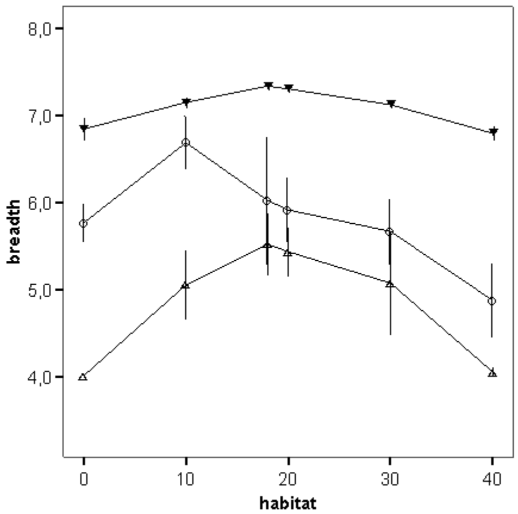

Fig 2. Average breadth values for selected habitats in simulations with only sexuals (black triangles), and simulations with asexuals (open circles) spinning off from sexuals (open triangles) when migration is 50%.

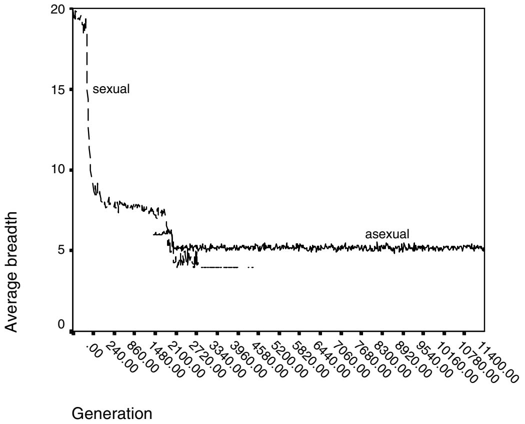

Fig 3. Typical pattern of evolution of breadths for sexuals(dashed) and asexuals (solid) in a simulation with 50% migration. Mutation to asexuality starts at generation 2000. Habitat 20.

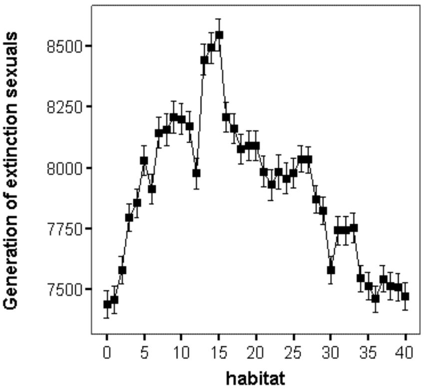

Fig 4. Generation in which sexuals go locally extinct in each habitat in simulations with mutation to asexuality. Migration is 50%. Error bars show among simulation standard error.

simulation. All possible values for the modes were still found in the population.

Asexuals spinning off from the sexual population when migration probability was 25% resembled their sexual parents: they had the optimal mode and breadths of one. In simulations with 50% migration, the interaction between the sexuals and the asexuals that had spun off from those sexuals resulted in an interesting pattern. Originally, the sexuals had a stable breadth of around 7.2. The first asexuals that managed to become established had breadth values slightly lower than this. The interaction between the sexuals and the invading asexuals drove breadths of both types steadily down. After 2000 generations of mutation to asexuality, the sexuals settled at a breadth of 4.0±0.0, even lower than the asexuals, who settled at 5.3 ± 1.0 ($p<0.05$) (Fig 2 & 3.). Though coexistence lasted, on average, longer than with 25% migration, most sexual populations eventually went extinct, starting from the extreme habitats (Fig 4.) . Range and variation in breadths were higher in the sexuals than in the asexuals. At the end of the simulation, on average 20 different mode values remained in the asexual population, while sexuals were all extinct.

Random environmental fluctuations

When the environment fluctuated with a period of one generation, fixation of breadth and mode took longer than in the static simulations ($p<0.05$). The breadth became fixed in sexuals on average at 13.7 ±1.6 and modes fixed close to the average optimum value of the habitat. All populations survived. As most sexual populations had gone to fixation by generation 2000, not much interesting happened when mutation to asexuals was introduced.

Long-term environmental fluctuations

When the environment remained stable for 10 generations in-between shifts, fixation of modes in sexuals occurred more rapidly than with environmental fluctuations every generation. Breadths became fixed around value 13.3 ±1.9, which should be enough to ensure survival over the whole range of possible environmental values. Extinction of a habitat occurred more often than with fluctuations every generation ($p<0.01$). Asexual populations spinning off from the sexuals had the same breadths as the sexuals, though at the end of the simulations, breadths of asexuals may have been slightly higher than those of sexuals (sexuals breadths = 12.0, asexuals = 13.5, $p=0.052$). Like the sexuals, the asexuals went extinct more often in these simulations than with one-generation fluctuations.

Migration combined with long-term environmental fluctuations

Whereas long-term fluctuations alone led to breadth values in the sexuals of around 12.5, fluctuations combined with 25% migration lowered the breadths to 9.6±0.3 ($p<0.01$), a value that gives a survival probability of less than 10% in the most extreme states of fluctuations within a habitat. Variation in breadths and modes was maintained for a long time. At the end of the simulation, 20 different mode values were found in the whole meta-population.

The breadths of invading asexuals in the mutation simulations were lower than those of the sexuals: 9.0±0.7 and 9.6±0.5 respectively after 100 generations of mutation (ANOVA $p=0.022$). Breadths of both types decreased over the generations to about 8.0 for asexuals and 8.5 for sexuals respectively, and this small difference between reproductive modes was maintained during the remainder of the generations ($p<0.05$). Despite larger breadths, sexuals had a higher average fitness at the moments of census ($p<0.05$). The sexuals also had higher levels of variation in modes and breadths than the asexuals ($p<0.05$); breadth values within the sexual population spanned a much larger range than within the asexual populations.

With the proportion of migrants increased to 50%, the breadths of the sexuals without asexuals were around 13, like without migration. With asexuals invading, sexuals rapidly went extinct. Surviving asexuals had breadths of 11.1±1.1. The asexuals maintained limited variation: at the end of the simulation, on average 7 mode values were found in the whole metapopulation.

To get insight in the evolution of modes and breadths during the 10-generation periods of stasis, census was held every generation between generation 5000 and 6000 in simulations with environmental change every 10 generations and 25% migration. Sexuals were on average closer to the optimum at census than asexuals ($p<0.001$). Breadths fluctuated, but no clear pattern was visible within the periods of stasis.

Discussion

The aim of our simulations was to study whether sexuality and asexuality would lead to different genotypic and population level adaptations to environmental heterogeneity.

Spatial heterogeneity: the frozen niche model

In the simulations with no migration, no fluctuations, the populations were expected to evolve towards the local optimum and become maximally specialised. This did not happen in most habitats, owing to lack of genetic variation.

With a limited amount of migration, 25%, it turned out that maximal specialisation was still the favoured phenotype. Migration solely served to eliminate lack of genetic variation.

A higher migration probability of 50%, though, resulted in a bet-hedging, or generalist, strategy for the sexuals. Asexuals that invaded under these conditions had more specialised genotypes than the resident sexuals. Asexuals, like sexuals, had a high chance of their offspring being a selected in an environment different from where the parent was selected. Sexuals, in addition, had a chance of mating with someone different from themselves, even if they remained in their own habitat. Because in the asexuals, genotypes were not broken up by recombination, their evolved breadth was lower than for the sexuals.

The interaction between sexuals and asexuals subsequently drove sexuals to specialisation as well. Whereas without asexuals, the sexuals settled for a breadth around 7.2. After invasion of asexuals, their breadths evolved towards 4.0. After invasion of the asexuals, within each habitat, asexuals with an optimal mode and a breadth lower than the sexuals' have higher chances of surviving selection than sexuals with optimal mode and larger breadth. The interaction between sexuals and asexuals selects sexuals with a low breadth, even though only a small percentage of the offspring of those specialised sexuals will survive. The dynamics of the model eventually lead to sexuals that are more specialised then asexuals. They probably compensate their loss of mal-adapted offspring by having a high fitness locally.

Though migration leads to relatively generalist genotypes, the dynamics of these simulations are those of the frozen niche model, that is: invading asexuals have smaller breadths than resident sexuals. The ensuing pattern of sexuals being driven to specialisation may be dependent on our specific formulation of the fitness and migration functions, as well as on the life-history we implemented. The negative effect of the breaking up of adapted genotypes by recombination is clear from other models as well. Earlier models of sexual populations have shown mal-adaptedness of individuals living at the ends of clines as a result of crossing with more common genotypes from the central regions (García-Ramos & Kirkpatrick 1997;Kirkpatrick & Barton 1997). In a spatially structured model, similar to ours, Peck et al. (Peck *et al.* 1998) found that high migration disrupted local adaptation of sexuals more than of asexuals. In their model, this led to extinction of sexuals in habitats with migration surpluses. In our model, sexuals could respond by adapting the breadth of their tolerance, though at a cost. It seems likely from our data and the literature, that sexuals will respond to migration with the evolution of more generalist genotypes than asexuals when a trade-off between optimal performance and breadth exists. So, given sufficient migration, the Frozen niche model can be applied to a spatially structured population. We can imagine, though, that a higher fecundity would be an alternative solution for the sexuals to counter the effects of mating with strangers (Bürger 1999), whereas evolution might also find solutions in altering the dispersal or the out-crossing tendency in certain organisms.

In particular cases, the interactions between sexuals and asexuals in mixed systems could lead to specialisation of the sexuals, giving the impression that asexuals have evolved general-purpose genotypes.

Temporal variation: general-purpose genotype

Asexual lineages that survive for many generations must have been able to survive all conditions that have occurred during those generations. The argument is that sexuals can cope with these varying conditions by producing a variety of specialised genotypes: variation as an alternative for generalism. Earlier studies have shown that genetic variation creates a large load in a randomly fluctuating environment (Lande & Shannon 1996;Charlesworth 1993b). Most models agree that random environmental fluctuation will not maintain genetic variation (Hedrick 1986).

In our model, the same result was visible. Fluctuations did not maintain genetic variation within the habitat. For sexuals as well as for asexuals, random fluctuations resulted in the evolution of general-purpose genotypes around the average optimal phenotype. Other types of temporal variation, though, may have different effects.

Combined temporal and spatial environmental variation

Simulations with random fluctuations in time, but without migration resulted in breadths of ±13.7. In simulations in which 25% migration was added to temporal fluctuations every ten generations, the breadths of the sexual population decreased to 9.6. Probably, the genetic variation that comes with migration enables sexuals to track the moving optimum. Therefore, they can be more specialised. So, if genetic variation can be maintained by other processes, it possibly is an alternative for generalism.

Again, the pattern of invading asexuals having lower breadths was found, though less extreme than in simulations with migration alone. This is not surprising. Temporal fluctuations result in equal breadths of sexuals and asexuals, and spatial variation results in asexuals with lower breaths than sexuals.

With 50% migration, breadths of the sexuals were around 13 like without migration. Again, invading asexuals had smaller breadths and drove the whole system towards specialisation, so breaking up of genotypes by recombination still had an effect on adaptation. In these simulations, though, sexuals did not evolve smaller breadths than co-existing asexuals.

Interpretation of empirical results

It is difficult to determine the scale and pattern of environmental variation that is experienced by natural populations. In our simulations we studied a limited set of the possibilities. Some general results, though, emerge. Random environmental fluctuations result in evolved general-purpose genotypes in both sexuals and asexuals. Spatial structure with gene flow leads to locally adapted specialists, though specialisation in less when gene flow is higher (Scheiner 1998). In a spatially structured environment, asexuals will evolve more specialised genotypes than sexuals. In clinal environments, asexual genotypes can more easily establish when they have a relatively rare genotype, such as a genotype adapted to the extreme ends of the species' niche space.

Another result is that generalism is not necessarily an alternative for genetic variation. Generalists with largely overlapping niches could co-exist in situations with 50% migration, where each generalist still had a 'home-base' where his mode was optimal. When the environment fluctuated in time, only the optimal generalist survived. When limited migration provided genetic variation to environments that fluctuated with a ten-generation period, genetic variation seemed an alternative to generalism, as breadths declined in the sexual population.

We should, however, keep in mind that evolution may find alternative solutions to cope with environmental heterogeneity, like discussed above.

The few empirical studies on the subject do not have equivocal results. Studies with plants of *Antennaria* species were designed to test the idea of the general-purpose genotype. In a study with *Antennaria parlinii*, the sexual genotypes (Michaels & Bazzaz 1989) seemed more specialist than the asexuals in their vegetative growth, but more generalist in their reproductive growth, and it is not evident from this how fitness itself would vary across environments. In a study with *Antennaria parvifolia* (Bierzychudek 1989), apomictic genotypes could survive in a wider range of conditions than sexual genotypes. Asexual *Erigeron annuus* did not differ from sexual *Erigeron philadelphicus* in response to competition by grass (Kenny 1996).

A number of studies with animals were designed to test assumptions and predictions of the frozen niche model. In a spatially structured environment, phenotypically different clones of the snail species *Potamopyrgus antipodarum* co-existed. Despite gene flow, the clones seemed locally adapted (Jokela *et al.* 1997b). High genotypic and phenotypic variation are commonly found in asexual populations (Vrijenhoek 1998), whether they co-exist with sexuals or not. Different specialisation (different modes in the terminology of our model) of the different clones is demonstrated by the fact that mixtures of clones usually perform better than monocultures (Semlitsch *et al.* 1997) or by clearly different optima of the clones (Vrijenhoek & Pfeiler 1997).

Results of a study, of course, depend on the type of environmental variation offered in the experiment, and how this varies in the organism's natural environment. The general-purpose genotype idea and the frozen niche model are not mutually exclusive, but spatial or temporal fluctuation may be more dominant in one or other system.

It turns out that the model of the general-purpose genotype, when based on random fluctuations in the environment, does not predict differences between sexual and asexual genotypes. It is therefore difficult to compare with empirical data.

The assumptions of the frozen niche model have been demonstrated to hold in a number of studies: differently specialised asexual clones can co-exist together as well as with sexual conspecifics. Additional predictions about invasion by asexuals starting from the extreme niches, and about asexuals having more specialised genotypes than sexuals have not been demonstrated. It seems from the data that for many organisms spatial structure in the environment is important.

Other patterns of environmental variation, such as directionally changing optima (Waxman & Peck 1999), or cyclical change (Charlesworth 1993a) and different time scales (Bürger 1999) may cause differences in degree of generalism of sexuals and asexuals. Other types of competition or selection may also have unexpected effects. Interpretation of empirically found differences between sexual and asexual conspecifics must therefore be done with care.

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Chapter 3

Genetic and phenotypic variation in sexual and apomictic *Taraxacum*

Genetic and phenotypic variation in sexual and apomictic *Taraxacum*

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Abstract

Means, phenotypic and genetic variation of quantitative traits were measured for several morphological and life-history characters of sexual and apomictic *Taraxacum* from the same locality. Sexual and apomictic *Taraxacum* differed in leaf length, timing of flowering, seed weight, and biomass distribution at harvest. Most traits had substantial phenotypic and genetic variation in both sexuals and apomicts. Broad-sense heritability values were in the same range for sexuals and apomicts, and ranged from 0.0 for number of capitula to over 0.5 for biomass distribution parameters. Traits that differed between sexuals and apomicts were not associated with lower heritability values, so the differences do probably not result from strong, recent selection. Both sexual and apomictic *Taraxacum* communities will be able to show a short-term response to changing selection pressures.

Keywords: apomixis, heritability, genetic variation, phenology, polyploidy, sexual reproduction

Introduction

In *Taraxacum*, dandelion, sexual and asexual forms exist. Sexual genotypes are diploid, and asexual, or apomictic, genotypes are usually triploid. The species shows geographic parthenogenesis. In southern and Central Europe, mixed sexual-asexual communities exist, but in northern Europe dandelion communities contain asexuals only (Den Nijs & Menken 1994). Asexual communities of *Taraxacum* usually contain considerable genetic variation as measured with allozyme markers or microsatellite markers (Battjes *et al.* 1992;Ford & Richards 1985;Richards 1996;Solbrig 1970). Different apomictic genotypes can be ecologically different in many respects (Solbrig & Simpson 1974;Vavrek *et al.* 1997;Vavrek 1998). In an other apomictic species, *Erigeron annuus*, substantial genetic variation for life-history traits has been demonstrated as well.

When genetic variation is present, selection can have an effect on the composition of the population. This effect is not necessarily the same in sexual and asexual populations, even when the selective pressures are the same. The level of selection is sexual populations is the gene. In asexual populations, the level of selection is the genotype (Bell 1997). Under balancing selection, sexual and asexual populations will attain the same mean, given sufficient genetic variation, but when initially the amount of genetic variation was the same, the variation that is maintained in the asexual populations will be less than in the sexual population. When selection on a quantitative trait is directional, asexuals may lag behind the sexuals in responding. Therefore, even under the same selection, asexuals and sexuals can have different means. Additionally, asexuals will maintain less variation (Waxman & Peck 1999). When selection is not for a single optimum, but to fill many microfiches, the more effective selection in asexuals may allow rapid and precise matching of the community to particular microsites, when standing variation is sufficient (Roughgarden 1972). This idea is the basis of the so-called Frozen Niche hypothesis (Vrijenhoek 1984).

Sexual and asexual conspecifics may be under selection for different traits as well, even in the same locality. For instance, the general-purpose genotype hypothesis states that asexuals are selected for more environmental tolerance than sexual genotypes (Lynch 1984). Competition between sexuals and asexuals may select for niche divergence. The asexual independence of pollinators could affect floral traits. Whether asexuals can respond to such selection pressures, depends on the heritable variation in the population.

As part of a survey into the dynamics of sexual and apomictic *Taraxacum*, we grew genotypes of sexual and apomictic *Taraxacum* from the same locality under common-garden conditions, and measured a number of morphological and life-history traits. We studied whether the mean trait values as well as the variation in trait values were the same in both reproductive types.

Material and Methods

Study species

Taraxacum (Asteraceae), dandelion, is an herbaceous perennial, very common along roadsides and other open vegetation. Plants flower in early spring, about April, and produce seeds 2-4 weeks later. The genus *Taraxacum* contains a complex of diploid sexual and polyploid apomictic forms. The most common polyploid cytotype is triploid. Sexual plants are usually self-incompatible (Jenniskens 1984). Apomictic plants do not require pollination for seed development.

Plant material

Sexual plants were obtained from the common garden of the Nederlandse Instituut voor Oecologisch Onderzoek (NIOO-CTO) at Heteren. In 1995, these plants had been collected as seeds on the banks of the river Rhine near Wageningen (the Netherlands), in one of the few Dutch mixed sexual/apomictic populations (Roetman *et al.* 1988). In February 1997, apomicts were dug up in the same field from which the sexuals originated. Both the sexual and apomictic plants represented a random collection from those present in the field. Microsatellites were used to identify different genotypes within the collection of apomictic plants (Falque *et al.* 1998). Screening with five polymorphic markers revealed that at least 9 different types were present among the 13 apomictic mothers. Plants were cloned (3-5 March 1997) by taking 2.5-3.5 cm pieces of taproot (depending on root diameter) from the mother plant and potting these in soil in the greenhouse. (The mother plants were allowed to flower in the greenhouse after cuttings were taken. Apomixis was ascertained by studying pollen morphology and the ability of the plants to set seed in isolation.) Clonal copies from one mother plant will further be referred to as copies of one genotype. These clonal copies of the mother were repotted in plastic cylinders of 30 cm high and 15.0 cm in diameter on 16-17 April 1997 in a 1:1 mixture of sieved black soil and sand. From each of 13 apomictic and 17 sexual mother plants, three clonal copies were used. On 22 April, the pots were placed in random order in the garden, inter-pot distance 0.6 m. They were embedded in the garden soil up to 4 cm from the upper rim. Osmocote® Plus slow-release fertiliser grains (NPK = 16:8:12 + micro nutrients) were administered in adequate supply.

Measurements

Leaf height was measured on 20 June and 21 August as the highest point reached by an unsupported leaf; length of the longest leaf was measured on 18 June and at final harvest. Capitula were counted regularly and seeds were collected by covering capitula with mesh caps after they had finished flowering.

All plants were harvested in the fourth week of March 1998 (week 48 of the experiment). Leaf area was measured on a subset of mature leaves to determine the specific leaf area (SLA). Fresh weight was likewise measured on a sub-sample of mature leaves to determine fresh weight to dry weight ratio. The number of leaves over one cm long was counted. Plants were divided into taproot (> 2mm diameter), fine roots, flower buds, and leaves, and subsequently dried at 70°C for at least 48 hours.

From each plant, three capitula (if available) were selected at random. Per capitulum, ten mature-looking seeds were weighed individually. Since much seed loss had occurred owing to herbivory by birds, no attempt could be made to estimate total seed production per plant.

Data analysis

Dry weights were converted to the natural logarithm before analysis to improve normal distribution of the data. Reproductive mode effects on all traits were identified with ANOVA where reproductive mode was treated as a fixed effect, and genotype was nested within reproductive mode. For the analyses concerning the final harvest, position was treated as a covariate, because the plants were harvested in succession over a number of days. Seed weights were averaged per capitulum and subsequently averaged per plant. Differences in flowering date were analysed with a Kolmogorov-Smirnov non-parametric test.

Heritability values for each trait were estimated from ANOVAs (Falconer 1981) calculated without the covariate.

For each trait, the coefficient of variation was calculated for the sexuals and the apomicts to see whether one or the other group had more phenotypic variation within the trait. To analyse whether trait value distributions differed between sexuals and apomicts, values were standardized within the reproductive modes by subtracting the mean value. Non-parametric Kolmogorov-Smirnov z test was used for comparison of the standardized distributions.

Results

Mean trait values

During the 48 weeks of the experiment, some plants temporarily disappeared aboveground, but later regrew from the roots. From 5 July until 8 September 1997 the plants flowered. Out of 45 surviving sexual plants 34 flowered, and out of 38 surviving apomictic plants 33 flowered. The average number of capitula produced was 6.2 ± 5.4 (7.7 for flowering plants only), not different between the reproductive modes (p=0.948). Sexuals flowered on average nine days earlier than apomicts (p<0.001). Seeds of apomicts were significantly heavier than those of sexuals: 0.72 ± 0.12 and 0.54 ± 0.10 mg respectively (p<0.01, Fig. 1). Height of the leaves was higher in apomicts than in sexuals on 20 June (p=0.014) by 1.2 cm on average, but not on 21 August (p=0.250). Lengths of the leaves did not differ between the reproductive strategies on 18 June (p=0.132), but at harvest in March sexuals had the longer leaves (p=0.022) (Fig. 2).

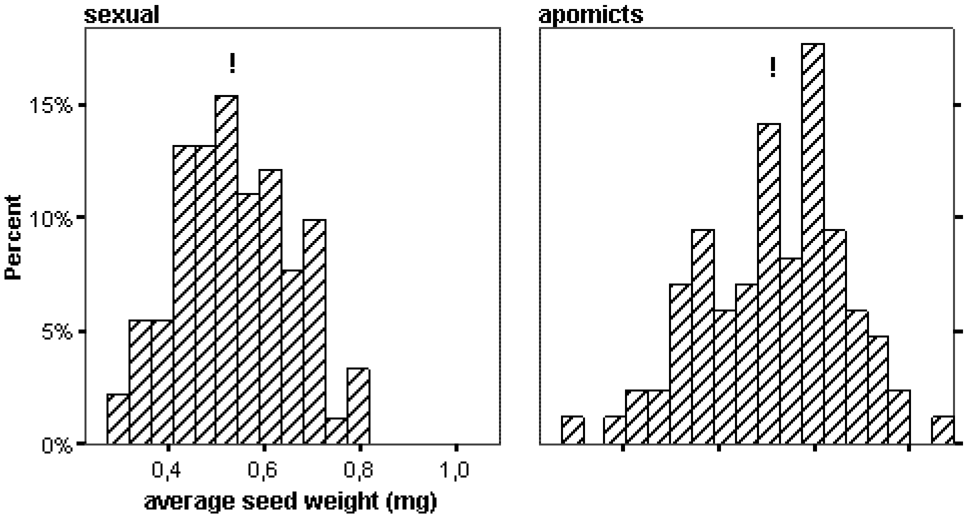

Fig. 1. Distribution of seed weight in sexual and apomictic *Taraxacum*. Arrow denotes the average.

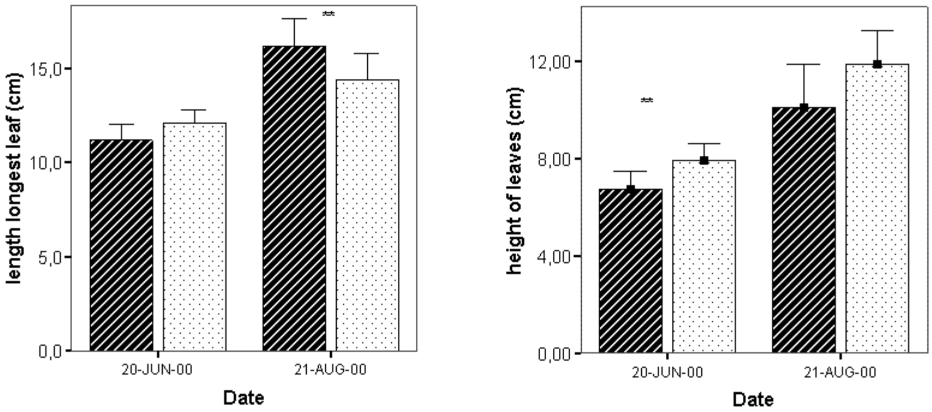

Fig. 2.a) Average length of longest leaf (cm) of sexuals (hatched) and apomictic (dotted) *Taraxacum* in June and March. b) Average height of highest leaf (cm) in June and August. Error bars show standard error of the mean.

At harvest, in early spring, 1 apomict and 7 sexuals were dead. The surviving plants had a dry weight of on average 41g ± 18.6, of which ± 65% was present in the taproot. Total dry weight did not differ between the reproductive modes (p=0.567), but biomass distribution was different between the two types (Fig. 3). Apomicts had less biomass in the flower buds (p<0.05) and more biomass in the fine roots (p<0.05). Leaf weight did not differ between apomicts and sexuals (p=0.067), but the ratio leaf weight:total weight (LWR) was significantly lower in apomicts than in sexuals (p<0.05). No significant difference was found between the number of leaves of sexuals and apomicts (p=0.075). Differences in taproot weight were not found either (p=0.433).

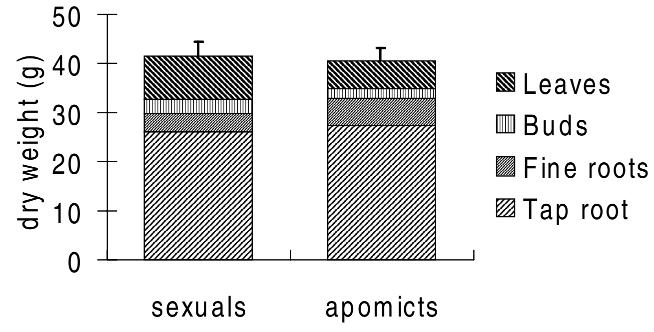

Fig. 3. Dry weight at harvest in March of sexual and apomictic Taraxacum and its distribution over leaves, flower buds, fine roots and taproot. Error bars show standard error of mean total dry weight.

The fresh weight : dry weight ratio of the leaves was significantly lower in the triploid apomicts than in the diploid sexuals, though only by 6.6% (p=0.021). Specific leaf area, though, was not different between the two types (p=0.172).

Variation and heritability

The amount of variation in each trait varied from c.v. of 10% for the fresh weight: dry weight ratio to more than 60% for the proportion of biomass present in the buds and for the number of leaves at harvest, to up to 90% for the number of capitula in the sexuals. Coefficients of variation were not consistently larger in sexuals or asexuals (Table 1). The non-parametric tests on standardised values did not identify differences in the distribution either ($p>0.05$ in all comparisons).

Table 1. Heritabilities and coefficients of variance of some morphological and life-history traits in a random sample of sexual and apomictic *Taraxacum*

Trait	**h^2 sexuals**	**h^2 apomicts**
	c.v.	**c.v.**
Dry weight (log transformed)	0.00 n.s.	0.31^{*}
	50%	40%
Number of leaves	0.08 n.s.	0.41^{*}
	74%	43%
Fresh weight/dry weight	0.11 n.s.	0.26 n.s.
	10%	15%
Number of capitula	0.18 n.s.	0.21 n.s.
	90%	83%
Specific Leaf Area	0.30 n.s.	0.16 n.s.
	16%	13%
Seed weight	0.38^{***}	0.35^{***}
	22%	21%
Leaf weight ratio	0.47^{**}	0.40^{*}
	43%	37%
Length longest leaf	0.48^{**}	0.45^{**}
	29%	28%
Tap root weight ratio	0.50^{**}	0.40^{*}
	18%	13%
Bud weight ratio	0.57^{***}	0.73^{***}
	61%	66%

The length of the longest leaf had significant h^2 values in both sexuals and apomicts. Heritable variation was also found for the distribution of biomass over different plant parts both in the sexuals and in the apomicts: the fractions in leaves, in buds and in taproots were heritable in both groups. The total number of leaves, though, had a significant h^2 in the apomicts, but not in the sexuals. The same was found for total dry weight. Specific leaf area (SLA) and fresh weight: dry weight ratio had no significant h^2 values. Despite much variation, the number of capitula per plant also had no significant h^2 in either sexuals or apomicts (Table 1).

Discussion

Differences in mean trait values between sexuals and apomicts

Sexual and apomictic dandelions differed on average in some morphological and life-history traits. Heights and lengths of leaves were different on some dates, but not on other dates. Probably the pattern of variation in length and height of leaves over the season was different between the different reproductive modes (De Kovel, submitted). Leaf length and height affect the ability of a plant to grow under shaded conditions (Van Hinsberg 1997). Apomictic *Taraxacum* have been shown to adjust their leaf morphology more strongly to shade than sexuals, and to grow better in shade as a consequence (De Kovel & De Jong 1999). Possibly apomictic dandelions can occupy microsites with a different light environment than sexuals. Sexuals flowered earlier than apomicts. Biomass distribution over plant parts at harvest was different in the two types. The apomicts had less biomass in leaves and buds and more in fine roots. Maybe the apomicts invested more in below- than in aboveground structures. It is equally likely that the shift in timing that was visible during flowering was also present in vegetative growth, and that the differences in biomass distribution are a consequence of this shift (Mølgaard 1977). Sexuals might benefit from a difference between the two types in flowering time, if this means that they receive less infertile pollen from triploids (Sterk 1987a;Van Dijk *et al.* 1991). In general, a difference in timing could reduce competition between sexuals and apomicts. The seeds of apomicts were on average heavier by 33% than those of sexuals. Heavier seeds in *Taraxacum* usually have a higher chance to germinate (Tweney & Mogie 1999), so probably, the germination ability of apomictic seeds was higher than that of sexuals.

In summary, there are a few differences between sexuals and apomicts that could result in niche divergence and a reduction in competition, though the overlap would still be rather high. In addition, apomicts have seeds that probably germinate better than those of sexuals, which increases their fitness, unless a trade-off exists with other fitness-related traits.

Variation in trait values

Variation and heritability can tell us about recent selection and the ability of a population to respond to future selection pressures. Fitness-related traits are expected to be under strong directional selection, whereas morphological traits are more likely to be under stabilising selection that may often be much weaker (Houle 1992). Strong selection will reduce heritability.

Of the traits we measured, capitulum number is likely to be closely related to fitness. Heritability of capitulum number indeed was no different from zero, but phenotypic variation in this trait was still high, the highest of all measured traits. This pattern is well known from literature (Houle 1992;Falconer 1981), though no adequate explanation is available.

In the sexuals, dry weight at harvest and number of leaves also had no significant heritabilities, despite the presence of phenotypic variation, while apomicts did have significant h^2 values for these traits. This suggests either that these traits have been under selection in the sexuals or that they are very sensitive to environmental influences. In the sexuals, one individual had a very high leaf number, which affected the estimates. The other morphological traits had significant heritabilities of about the same magnitude in the two reproductive types. The amount of phenotypic variation was similar in both groups as well. Selection on those traits has probably been weak in both groups. We infer that the differences between sexuals and apomicts in leaf length and height and in biomass distribution are not the result of strong, ongoing selection. Even for seed weight, heritable variation was present in both sexuals and apomicts. This suggests that seed weight is not directly correlated with fitness, but that some trade-off exists between seed weight and other fitness related traits. Heritable variation for seed weight was also found in apomictic *Erigeron annuus* (Stratton 1991). The differences in average seed weight and in biomass distribution or phenology between sexuals and apomicts may not be the result of selection, but a physiological consequence of triploidy (Segraves & Thompson 1999). The maintenance of genetic variation in sexual populations is still a matter of debate.

Either much of the variation is selectively close to neutral and in a balance of selection and mutation, and probably drift as well. The other possibility is that spatial heterogeneity with different micro-niches maintains the variation (Hedrick 1986). In apomictic dandelions, genetic variation is created when new lineages originate from backcrosses between sexuals and established apomicts. Loss of genotypes by drift in finite populations is much more likely in asexual than in sexual populations. Selection, of course, diminishes variation as well. Selection for single optima, either balancing or directional is expected to decrease variation in asexual communities more than in sexual populations (Bürger 1999), but no obvious differences in variation were found between the sexuals and apomicts in this study.

The distribution of trait values in asexuals suggests that apomictic genotypes are created randomly and establish without much selection, or that selection favours genotypes that are different from those already established. This last possibility is the pattern that was suggested by Vrijenhoek (Vrijenhoek 1984) in his Frozen Niche Model.

Previous studies have shown that communities of apomictic *Taraxacum* contain considerable genetic variation, not only when they co-occur with sexuals, but also in all-apomictic communities (Battjes *et al.* 1992;Ford & Richards 1985;King 1993;Lyman & Ellstrand 1984;Richards 1996). This study shows that this genetic variation codes for phenotypic variation in quantitative traits, very similar to that found in sexual populations. The apomictic species *Erigeron annuus* also contains substantial genetic variation for life-history traits. Like in *Taraxacum*, rare sexual reproduction in *Erigeron* is probably a source of new genotypes.

We tentatively suggest that genetic variation in apomictic plants is maintained by spatial environmental heterogeneity, possibly in the manner suggested by Vrijenhoek. Under changing conditions, apomictic communities can to some extent respond to selection pressures.

Acknowledgements

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Chapter 4

Responses of sexual and apomictic genotypes of *Taraxacum officinale* to variation in light

Plant Biology 1: 541-546, 1999

Responses of sexual and apomictic genotypes of *Taraxacum officinale* to variation in light

Carolien G.F. de Kovel & Gerdien de Jong

Abstract

The mode of reproduction, sexual or asexual, will influence the way populations respond to selective pressures. This can cause genetic and ecological divergence between sexual and asexual forms of the same species. Here we examine differences in morphology and phenology between sexual and apomictic types of dandelion, *Taraxacum officinale*. Sexual and apomictic dandelions were collected from a mixed population on banks of the river Rhine, the Netherlands. Clonal copies of both sexual and apomictic genotypes were planted in an experimental garden under two light levels. Sexuals flowered four days later on average than apomicts, but the number of capitula was the same. Apomicts had longer leaves and were heavier than sexuals, especially under shaded conditions. In apomicts plasticity for leaf length and height was larger than in sexuals, but for most other measured traits no differences in plasticity were observed. Trait values of apomicts were within the same range as those of sexuals.

Keywords: apomixis, environmental variation, phenotypic plasticity, *Taraxacum officinale*

Introduction

Complex organisms reproduce predominantly by way of sex. One of the properties of sexual reproduction is that recombination can take place: both through reassorting of chromosomes and through crossing-over. The higher availability of genetic variation in one mother's offspring that is the result, as well as the relatively independent movement of genes owing to crossing-over, will have effects on the way populations of sexually reproducing organisms can respond to selective pressures. As a consequence of these diverging responses to selection pressures, genotypes produced by sex are expected to differ in their relationships with their surroundings from conspecific genotypes that are produced by an asexual way of reproduction (Vrijenhoek and Pfeiler, 1997; Bierzychudek, 1989; Jokela et al. 1997; Michaels and Bazzaz, 1986; Michaels and Bazzaz, 1989). I.e. the sexual and asexual genotypes will be ecologically different: they may have different optima for ecological conditions and/or different plasticities. Most ideas concerning evolution of sexual and asexual organisms focus on the degree of specialisation of the genotypes (Vrijenhoek, 1984; Vrijenhoek, 1998; Lynch, 1984) and niche packing (Roughgarden, 1972). The expectations from these ideas depend on the way the environment is thought to vary in space and time. It is, however, often difficult to determine how the organism perceives the environmental heterogeneity.

In this study, we used the perennial plant species dandelion (*Taraxacum officinale* Wiggers) to examine the response of sexuals and asexuals to environmental variation. Of this species both sexual, diploid types and apomictic, triploid types exist. We studied the response to an environmental variable that in the species' natural habitat will show significant variation in space as well as in time: the availability of light. Light availability can vary considerably, owing to the presence of other plants. Plant responses to reduced light availability comprise the production of longer leaves (Van Hinsberg, 1996) or longer internodes (Huber et al. 1998), adjustment of the leaf angle (Van Hinsberg, 1996), increase of specific leaf area (SLA) (Sultan and Bazzaz, 1993), and adjustment of the root:shoot ratio (Sultan and Bazzaz, 1993). Dandelions are very sensitive to light: as typical open field plants, they usually are unable to survive in shaded habitats.

In this study, we investigated whether apomictic and sexual dandelions differed in morphological and phenological characters and in their response to shading.

Material and Methods

Study species

Taraxacum officinale Wiggers (Asteraceae), dandelion, is a herbaceous perennial. It forms a leaf rosette close to the ground and a taproot. It is very common along roadsides and in fields and other open vegetation. The genus *Taraxacum* contains a complex of diploid sexual and polyploid apomictic forms. The most common polyploid cytotype is triploid. Sexual plants are self-incompatible (Jenniskens, 1984). Apomictic plants do not require pollination for seed development.

Plant material

Sexual plants were obtained from the common garden of the Nederlandse Instituut voor Oecologisch Onderzoek (NIOO-CTO) at Heteren. Two years earlier, these plants had been collected as seeds on the banks of the river Rhine near Wageningen (the Netherlands), in one of the few Dutch mixed sexual/apomictic populations (Roetman et al. 1988). Apomicts were dug up in the same field from which the sexuals originated in February 1997. (The mother plants were allowed to flower. Apomixis was ascertained by studying pollen morphology and the ability of the plants to set seed in isolation.) All plants were kept in the greenhouse (18 °C) for some days to get them started. Then plants were cloned (3-5 March 1997) by taking 2.5-3.5 cm pieces of taproot (depending on root diameter) from the motherplant and potting these in soil in the greenhouse. These clonal copies of the mother were repotted in plastic cylinders of 30 cm high and 15.0 cm in diameter on 16-17 April 1997 in a 1:1 mixture of sieved black soil and sand. In the bottom of the cylinder five holes ensured drainage. The holes were covered with gauze to prevent root growth out of the plastic pots. The pots were embedded in the garden soil up to 4 cm from the upper rim on 22 April. They were placed in random order in a plot in the garden of 1.25 x 24 m in two rows, inter-pot distance 0.6 m. Extra water was added when necessary.

Treatments

From each of 30 mother plants, (which I will call 'genotypes') 17 sexual and 13 apomict, one daughter clone was subjected to a shade treatment, while the other grew in the full sunlight. The shade treatment consisted of cylinders of green foil, 30 cm in diameter and 60 cm high, that were placed around the pots. The tops of these shade cages were open. This created a gradient in both photosynthetically active radiation (PAR) and the ratio of red:far red light (Fig 1.), while it may also have affe

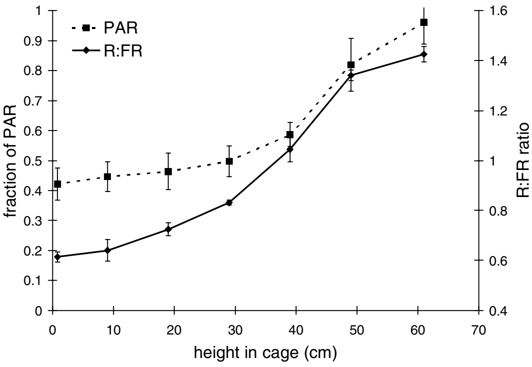

Fig 1. Gradient in photosynthetically active radiation (PAR) inside the shade cages relative to radiation outside the cages and gradient of the ratio red:far red light (R:FR) in the shade cages.

cted other environmental variables, such as temperature and humidity. This treatment mimicked conditions in high vegetation. Osmocote® Plus slow-release fertiliser grains (NPK = 16:8:12 + micro nutrients) were administered to both treatments on 14 May to a dose of 10 g N m^{-2}.

Measurements

Leaf height was measured on 13 June and 21 August as the highest point reached by an unsupported leaf; length of the longest leaf was measured on 18 June and at final harvest. Capitula were counted regularly and seeds were collected by covering capitula with mesh caps after they had finished flowering. All plants were harvested in the first week of October 1997 (week 23 of the experiment). Fresh weight was measured on a sub-sample of mature leaves to determine fresh weight to dry weight ratio. Leaf area was likewise measured on a subset of mature leaves to determine the specific leaf area (SLA). The number of leaves over 1 cm long was counted. Plants were divided into taproot (> 2mm diameter), fine roots, and leaves, and subsequently dried at 70°C for at least 48 hours.

From each plant, three capitula (if available) were selected at random. Per capitulum, ten mature-looking seeds were weighed individually. Seed set in the sexuals depended heavily on the day of flowering. In addition, seed loss had occurred due to herbivory. Therefore, no attempt could be made to estimate total seed production per plant.

Data analysis

Dry weights were converted to the natural logarithm before analysis to improve normal distribution of the data. Treatment and reproductive mode effects on all traits were identified with ANOVA where treatment and reproductive mode were treated as fixed effects. For all analyses concerning the final harvests, position was

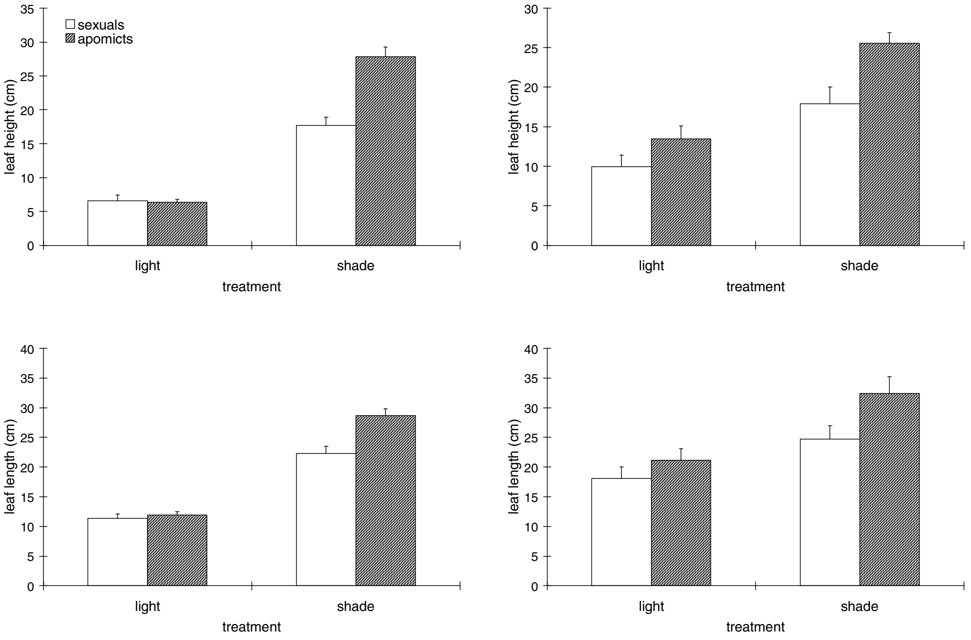

Fig. 2 *a-d.* Average height of highest reaching leaf at 13 June (a) and 21 August (b) of sexual and apomictic genotypes of *Taraxacum* in shaded and unshaded conditions. Average length of longest leaf at 18 June (c) and final harvest (d) of sexual and apomictic genotypes in shaded and unshaded conditions. Error bars represent one standard error.

treated as a covariant, because the plants were harvested in succession over a number of days. Seed weights were averaged per capitulum and subsequently averaged per plant. These data were analysed with ANOVA with reproductive mode and treatment as fixed factors. Differences in flowering date were analysed with a Kolmogorov-Smirnov non-parametric test.

Plasticity in a certain trait was expressed as differences between the trait value of a genotype in the shade treatment and in the light treatment divided by the value in the shade treatment. Effects of reproductive mode on plasticity in traits were tested with ANOVA.

Results

In the course of the experiments, some plants temporarily disappeared aboveground but regrew later from the roots. Not all plants flowered: of the sexuals 33% produced no capitula, of the apomicts 12% did not. At harvest time 5 plants were dead: 4 sexuals and 1 apomict.

Treatment effects

Shading influenced most of the measured characters. In the shade, the leaves were longer and more erect (Fig. 2), the specific leaf area was larger, the fresh weight/dry weight ratio of the leaves was higher, while the number of leaves was lower as well as the dry mass of fine roots. The distribution of biomass over different plant parts was not significantly different under both treatments. Total dry weight had a tendency to be lower under shade conditions ($p=0.089$, Fig. 3). Shading strongly reduced the number of capitula per plant: Capitulum number decreased from 5.8 per plant in the light treatments to 2.0 under shade conditions.

Effects of reproductive mode

On average, sexuals flowered four days later than apomicts (Fig. 4). The number of capitula per plant did not differ between sexuals and apomicts ($p=0.982$). Apomicts and sexuals differed from each other with respect to the length and height of the longest leaf. On 18 June the sexuals had leaves 6.6 cm high in the light and 17.7cm in the shade; the apomicts had leaves of 6.4 cm in the light and 27.8 cm in the shade (difference $p<0.05$; Fig. 2a). This constituted a significant treatment * reproductive mode interaction, indicating that the apomicts responded more strongly to shading. A similar pattern was found for the length of the longest leaf (main effect $p=0.021$, interaction $p<0.01$, Fig. 2c). At the second measuring date, heights were 9.9 and 17.9 cm for the sexuals and 13.5 and 25.6 cm for the apomicts, so apomicts had higher leaves in both treatments ($p<0.01$), while a similar pattern was again found for the leaf lengths at harvest ($p<0.01$), but no significant interactions appeared at these dates (Fig 2b and d). Heights of apomicts exceeded the range of heights of sexuals. The total weight of apomicts was higher than that of sexuals (by 31%, averaged over both treatments) at harvest time (ANCOVA, $p<0.05$). This was due to differences in taproot weight, which made up about 70% of the total weight, and to differences in fine root weight (Fig. 3). No differences between reproductive modes were found for SLA, leaf fresh weight/dry weight ratio, or the number of leaves. Seeds of apomicts were significantly lighter in the shade than in full light (ANOVA, $p<0.05$); no such differences were found for the sexuals (interaction $p<0.05$).

Plasticity

Differences in amount of genotype plasticity between the reproductive modes were found for leaf height ($p<0.05$) and length ($p<0.05$) at the first measurement., but not for the other measured traits ($p>0.05$ for all). Apomicts had responded to shading by increasing their leaf height by 337% and their leaf length by 142% on average at the first measuring date, while the sexuals increased leaf height by 169% and length

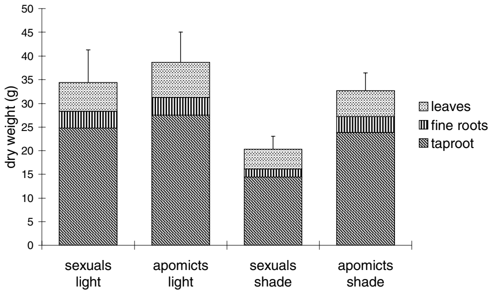

Fig. 3.Dry weight (g) at harvest of sexual and apomictic *Taraxacum* grown in full sunlight (light) or inside foil cylinders (shade) and its distribution over leaves, taproot and fine roots. Error bars represent one standard error for total dry weight.

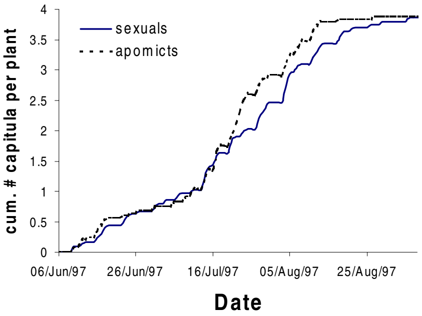

Fig. 4. Cumulative number of newly opened flowers per plant of sexual and apomictic *Taraxacum* in both treatments combined.

by 95%. From only four sexual and six apomictic genotypes seeds were available from both treatments. The ANOVA did not detect significant differences in seed weight plasticity between sexuals and apomicts (p=0.14).

Discussion

In this study, we examined the morphology and phenology of sexual and apomictic *Taraxacum officinale* genotypes under two light levels. Sexual and apomictic genotypes differed from each other in a number of traits, but trait values of apomicts were not outside the range found for sexuals, except for the compound trait height. The sexuals flowered on average four days later than apomicts, but there was large overlap as the total flowering time in the experiment was three months. Sexual dandelions usually flower later than the apomicts under natural conditions in the Netherlands (pers. obs.). At harvest (autumn), apomicts had more dry weight than sexuals. In the light treatment the apomicts had on average 13% more dry weight than the sexuals, in the shaded treatment 56%. Possibly, triploids are intrinsically larger than diploids. The apomicts had longer leaves with a steeper angle, particularly under shaded conditions. It seems likely that this higher plasticity in leaf morphology has enabled the apomicts to harvest more light under shaded conditions.

Reproductive mode and ploidy level

In dandelions, as in most other species that have sexual and asexual types, there is a difference in ploidy level between the reproductive modes (Suomalainen et al. 1987; Gustafsson, 1946). Though the differences between apomicts and sexuals can be explained as the result of diverging selection on initially identical phenotypes (see below), it is not possible in this experiment to discriminate between the effects of reproductive mode and the effects of ploidy level. Differences in flowering time between ploidy levels are not unusual (Van Dijk et al. 1991; Gottschalk, 1976). In their reviews on polyploidy both Gottschalk (1976) and Levin (1983) mention that autotetraploids of crop species frequently are larger and heavier than their diploid counterparts. However, a number of studies comparing weights among types of different ploidy level in natural species such as *Aster lanceolatus* (Chmielewski, 1995) or *Polygonum aviculare* (Meerts, 1995) found no differences. In some studies with the grasses *Arrhenatherum elatius* (Petit et al. 1996; Petit and Thompson, 1997) and *Holcus mollis* (Lamade et al. 1994) it was found that polyploids had a higher biomass than diploid types within the same taxon. Few generalizable effects of polyploidization in nature are known. In many cases, however, polyploidization has had unique effects on a number of traits (Gottschalk, 1976).

Niche width and niche location

Most ideas concerning the coexistence of sexuals and asexuals assume that on the outset genotypes of both reproductive modes are ecologically identical. Divergence is the result of subsequent selection. This is the case for the frozen niche variation hypothesis (FNV) (Vrijenhoek, 1979), the general-purpose genotype hypothesis (GPG) (Lynch, 1984), as well as for a number of other ideas. The frozen niche variation hypothesis assumes that asexual genotypes spin off from sexual populations. In doing so, they capture and freeze a phenotype present in that population. Selection among the asexual genotypes will favour those genotypes that have minimal niche overlap with established asexual clones and sexual genotypes (Case and Taper, 1986)). Sexuals can coexist with a limited number of asexual lineages, provided the cumulative niche of the clonal lineages does not completely coincide with that of the sexual population. In simulation models studying invasion of sexual populations by asexuals, clonal genotypes with niches in the margin of the sexual distribution were more likely to establish than clones with a central niche (Weeks, 1993).

The general-purpose genotype (GPG) hypothesis (Lynch, 1984) states that in temporally variable environments a clonal lineage that survives for a sufficient number of generations must have been able to tolerate all environmental conditions that it has encountered. If asexual lineages at incipience freeze the ecological variation in the sexual population, selection will favour those genotypes that have broad niches with respect to this environmental variation (Lynch, 1984). The FNV and GPG hypotheses do not exclude each other, since they refer to different aspects of environmental heterogeneity. Whereas the FNV hypothesis is concerned with the simultaneous distribution of limiting resources, the GPG hypothesis refers to coarse-grained temporal heterogeneity of the environment (Vrijenhoek, 1998). Few ecological factors affecting plant growth will, however, be relevant to one of these ideas, but not to the other.

The fact that trait values for apomicts did not exceed those for sexuals is in accordance with the FNV hypothesis. Within the framework of the FNV hypothesis, differences in mean trait values for sexuals and apomicts can be explained by assuming that only apomictic genotypes at the periphery of the sexuals' niche space have established (Weeks, 1993).

According to the GPG model apomictic lineages should be more tolerant to environmental variation than sexuals. In our experiments, we found that apomicts responded more strongly than sexuals to shading: their leaves were longer and more erect. When higher plasticity in leaves is an adaptation to become more tolerant to light variation (Schmitt et al. 1995), this should lead to smaller differences in biomass between both treatments, as biomass is probably highly correlated with fitness in dandelions (Welham and Setter, 1998; Van Loenhoud and Van der Heijden, 1980). This was not clear from our experiments, though the biomass differences between light levels seemed more pronounced in sexuals than in apomicts (Fig.3). Seed weight also showed a reproductive mode x treatment interaction, with the apomicts apparently more responsive to shading. As data on seeds were, however, incomplete, it is not possible to draw conclusions from them. In the other measured traits no differences in plasticity between sexual and apomictic genotypes were found. The data on leaf morphology, biomass and maybe also seed weight could be interpreted as a larger tolerance of the apomicts to variation in light availability, resulting in a higher fitness of the apomicts under shaded conditions. However, as light is likely to create mainly fine-grained environmental heterogeneity, in both space and time, it seems that the GPG model is not appropriate. A broader tolerance for environmental variation is not expected under the FNV hypothesis, unless these traits are somehow correlated with survival in marginal niches. It is possible that shaded habitats are marginal to dandelion populations as shading severely hampers establishment and survival of dandelions (Mølgaard, 1977).

Sexual and asexual species

In only a few studies asexual types have been compared with sexual types within the same taxon. This has been done, for instance, in *Antennaria* species, where sexual and apomictic polyploids coexist. Apomicts of *A. parvifolia* had higher growth and survival than sexuals. Apomicts were more tolerant than sexuals, especially to hot, dry conditions (Bierzychudek, 1989). Apomictic *A. parlinii* were found to produce less total biomass, but more reproductive tissue than sexuals. They were also more plastic than sexuals in the production of the reproductive mass in response to light and nutrient levels (Michaels and Bazzaz, 1989). Asexual *Daphnia pulex* were heavier than their sexual conspecifics, but did not differ from them in plasticity with respect to temperature (Scheiner and Yampolsky, 1998). It seems that sexual and apomictic types of a species do not differ from each other in their response to environmental heterogeneity in a consistent way. The results will depend on way the studied environment varies in the species' natural habitat and the correlation of the measured trait with fitness.

Summary

Summarising, sexual and apomictic *T. officinale* genotypes were found to differ from each other on average in a number of morphological and phenological traits, though trait values in both reproductive modes were within the same range. The observed differences may have resulted from differential responses to selection pressures, but also from polyploidization events. The results are not readily interpretable within the framework of the GPG or FNV hypotheses, even if we assume that polyploidization has had no effect on the morphology of the dandelions. The apomicts' larger plasticity in leaf morphology can be interpreted as a larger tolerance of apomicts for variation in light intensity or light quality.

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Chapter 5

Niche differences between sexual and apomictic *Taraxacum* as a consequence of both ploidy effects and selection

Niche differences between sexual and apomictic *Taraxacum* as a consequence of both ploidy effects and selection

Carolien G.F. de Kovel

Abstract

Multicellular organisms are usually sexual, but in some species asexual genotypes can spin off from the sexual population. In most cases, these asexual genotypes are polyploid. By comparing such newly spun off asexual genotypes with established asexual genotypes and with the sexual ancestors, the effects of selection and polyploidy on differences between sexual and asexual conspecifics can be disentangled. In *Taraxacum officinale* the triploid apomicts had larger cells than the diploid sexuals because of the ploidy difference. Polyploidy had a negative effect on leaf number. Apomicts were selected for higher proportion of viable seeds. Despite positive heritabilities for seed weight and number of ovules per capitulum, no directional selection on these traits was noticeable. Selection increased plasticity in leaf length response to shading in the apomicts. The consequences of ploidy and selection effects for the stability of the mixed sexual-asexual system are discussed.

Keywords: apomixis, niche, polyploidy, selection, sexual reproduction, *Taraxacum*

Introduction

Multicellular organisms usually reproduce by way of sex. Yet, in a number of species asexual forms coexist with the sexual forms. These asexual forms have arisen secondarily from sexual forms, quite often through hybridisation (Bierzychudek 1985;Butlin *et al.* 1999). As a result of this hybridisation, the asexual forms are polyploid in many instances (Bierzychudek 1985;Suomalainen *et al.* 1987). Studying the significance of sex by comparing sexual and asexual forms of the same species is often complicated by this difference in ploidy level.

In some species, new asexual genotypes arise regularly. In the case of *Taraxacum,* dandelion, new asexual genotypes can arise from crosses between existing apomictic plants and sexual plants, which occur in mixed sexual-apomictic populations. In *Taraxacum,* the apomictic genotypes are triploid, whereas the sexual genotypes are diploid. The origin of triploid apomictic dandelions is unknown, though it seems clear that hybridisation between related species or races played a role (Richards 1973). Contemporary apomictic genotypes of *Taraxacum* are probably formed from backcrosses between existing apomictic genotypes and sexual genotypes (Menken *et al.* 1995;Morita *et al.* 1990;Morita *et al.* 1990). Though apomictic *Taraxacum* produces seed parthenogenetically, it produces pollen through meiotic division. Most of this pollen is sterile, but some grains are able to fertilise a haploid egg-cell of a sexual plant. If this pollen is diploid, a new triploid will be formed that is usually apomictic (Tas & Van Dijk 1999a;Tas & Van Dijk 1999b;Den Nijs & Menken 1994). Sexuals and apomicts occur in mixed populations (Den Nijs & Sterk 1984b;Den Nijs & Sterk 1984a), and from allozyme data it is likely that new apomicts arise from the local population (Menken *et al.* 1995). This system allows us to study the dynamics of sexual and asexual forms more closely.

Because of the differences in ploidy level between sexuals and apomicts, direct comparison may reveal differences that are either a direct consequence of polyploidisation, or that are the result of different selection regimes on sexuals and apomicts. By studying new apomictic genotypes that have not encountered much selection together with apomictic and sexual genotypes, we may be able to disentangle the effects of polyploidy and selection.

New triploid genotypes of *Taraxacum* were generated by placing sexual plants in a field containing only apomicts, so all offspring was fathered by apomicts. Seeds were taken to the lab and screened on ploidy level with a flow-cytometer (Ulrich & Ulrich 1991). Triploid offspring plants were selected to provide seeds for this experiment. These new or hybrid genotypes have encountered little selection. If these genotypes closely resemble the established apomicts in some traits, but differ from the diploid sexuals, then these traits can be said to be directly affected by ploidy level. If, however, the hybrid genotypes are in-between the sexuals and the established apomicts, it seems likely that the sexual mother and the apomictic father have contributed different alleles to the offspring. It can then be argued that selection has favoured different alleles, hence different phenotypes, in sexuals and apomicts.

Systems containing both sexuals and apomicts can be stable, despite the more efficient reproduction of apomicts, if niche differentiation between the to forms exists. If polyploids have different characteristics from diploids because of their polyploidy, this can stabilise the system without any further effects of reproductive mode. Therefore, our first question is whether polyploidy causes differences that can lead to niche differentiation between sexuals and apomicts. Our second question is whether apomicts are under selection when they establish, and on which characters selection acts. Selection can be the consequence of competition with conspecifics and may lead to niche shifts. Other forces exerting selection may be specific for asexual reproduction, e.g. favouring general-purpose genotypes (Lynch 1984;De Kovel & De Jong 2000), or in some other way be connected to asexuality.

We compared morphological and life-history traits in sexuals, apomicts and their triploid hybrids to study the effect of polyploidy and selection on those traits. In a previous study comparing hybrids and established apomicts it was shown that apomicts are probably selected for a diverging phenology and longer leaves, in particular under shaded conditions. In the present study, growth and development were studied more closely, as well as reproduction-related traits. Also, in the present study, diploid sexuals were included, ascertaining differences between diploid sexuals and triploid apomicts.

Material and methods

Seeds from 'new' and 'established' apomicts were collected from a generation of plants grown in the greenhouse. For the origin of the new apomicts, see De Kovel & De Jong, 2000. In spring 1998, seeds of sexual plants were collected from the field that had also provided the 'mothers' for the new triploids. Seeds germinated in petri-dishes and were planted in 12x12 cm pots, filled with a 3:1 black soil:sand mixture that were placed in the greenhouse on 19 July 1999. In total, seeds from 5 diploid, 7 established triploid, and 8 new triploid mothers were used. Each mother was represented by three plants. In case of the apomicts, these were likely to be the same genotypes; in the case of the sexual plants, these were probably half-sibs. Every week, number of leaves and the length of the longest leaf were scored. In addition, height of the highest reaching leaf was recorded on 13 September and 4 October. Size of stomatal cells was measured on ten cells of a mature leaf. On 25 October, all plants were transferred to an open greenhouse, so as to experience normal seasonal changes.

When plants started to flower, the date of flowering of each capitulum was recorded. As few insects are present in the greenhouse, sexual plants were hand-pollinated with pollen from other sexual plants in the experiment. Seeds were harvested and divided into developed and undeveloped seeds. Ovules per capitulum were counted, and three sets of ten developed seeds were weighed. Thirty developed seeds per capitulum were placed on wet filter paper in a petri dish and placed in an incubator. After 14 days at 20°C in light, the number of seeds that had germinated was counted.

Plants were harvested after flowering on 10 July. Leaf area per plant was measured on the fresh leaves. Leaves and roots were dried for 48h at 70°C and weighed. Leaf area of the whole plant was determined. Specific leaf area (SLA) was calculated as total leaf area per plant / total dry weight of leaves.

Data analysis

Differences between classes in cell size and in leaf height were analysed with an ANOVA with class as a fixed factor and mother as a random factor nested within class. The same test was used for dry weights and specific leaf area (SLA) after a log-transformation to improve normal distribution of these data. SLA was calculated as the total leaf area of the plant divided by total dry weight of the leaves.

The same kind of ANOVA but with individual plant as a random factor nested within mother was used for data with a number of observations per plant. These were the number of ovules per capitulum, and weight of seeds, as well as the proportions of developed and germinated seeds after arcsine transformation. For post-hoc tests the method of Student-Newman-Keuls (SNK) was used.

To analyse leaf length and leaf number a similar ANOVA was used with date of observation as a random factor.

Pearson correlations were calculated for the relation between seed weight and germination probability, seed weight and capitulum sequence number, seed weight and the number of ovules per capitulum, number of capitula and number of ovules per capitulum, SLA and cell size, and for total dry weight and number of capitula per plant. The correlation between leaf length and leaf number was corrected for date.

Differences between classes in the number of capitula per plant and in the appearance date of capitula were analysed with Kruskall-Wallace (K-W) non-parametric test.

Heritability Estimates

The heritability of some traits was estimated from the added variance component for genotypes in an ANOVA design, following the method of Falconer (Falconer 1981). The variance components were estimated from the mean squares estimates in a Type I nested ANOVA design (SPSS software) with mothers as a random factor. Significant variance components ($p<0.05$) for mothers were interpreted as heritability values differing from zero.

Heritability of leaf length and leaf number for each class, each date was inferred from significant effects of mother in an ANOVA with mother as a random factor.

Results

General

One plant died during the experiment; it was a hybrid.

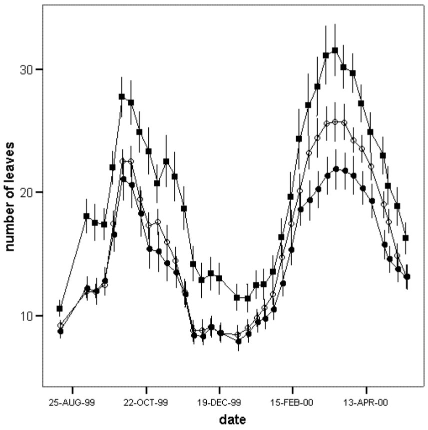

Fig. 1. Average leaf numbers through time of sexual, apomictic and hybrid plants. Error bars show one standard error. Open circles: established apomicts; closed circles: hybrids; squares: sexuals.

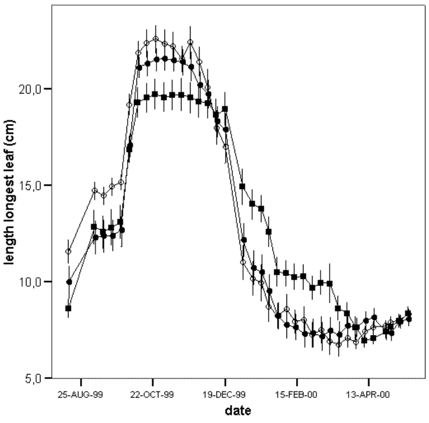

Fig. 2. Average length of the longest leaf through time of sexual, apomictic and hybrid plants. Error bars show one standard error. Open circles: established apomicts; closed circles: hybrids; squares: sexuals.

Growth and Morphology

Cell sizes differed significantly between the different classes ($p<0.001$). The stomatal cells of the sexuals were shorter than those of the apomicts and hybrids by 10.6% on average, but apomicts and hybrids did not differ significantly.

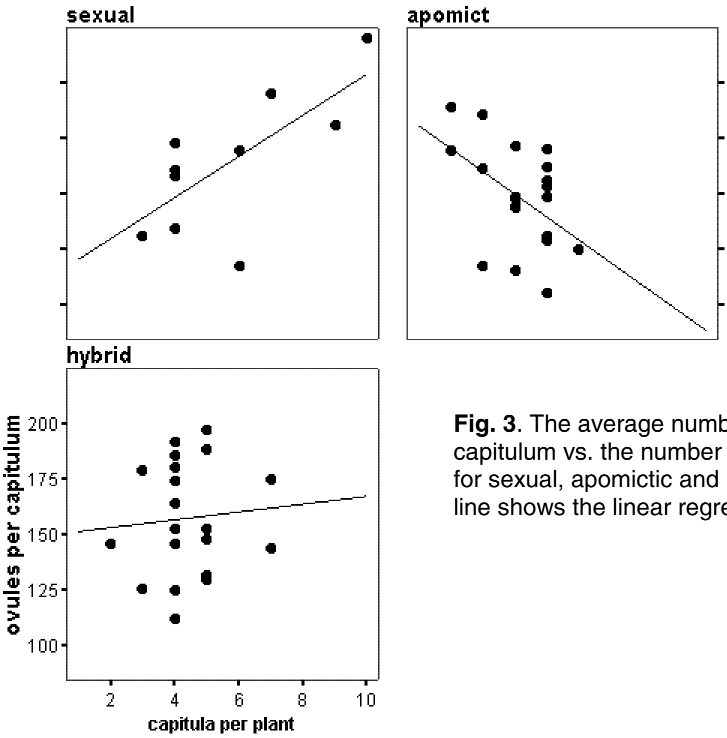

Fig. 3. The average number of ovules per capitulum vs. the number of capitula per plant for sexual, apomictic and hybrid plants. The line shows the linear regression fit.

The number of leaves per plant was significantly different between the different classes ($p<0.001$). Sexuals had most leaves whereas hybrids had on average fewest leaves, though this difference between hybrids and apomicts was mainly apparent in autumn and spring, when leaf numbers were high (Fig. 1). Leaf length differences varied with the time of year (class * time interaction $p<0.001$): sexuals had shorter leaves in the autumn when leaves were long, but longer leaves in spring when leaves were short. In autumn, hybrid leaf length was in-between sexuals and apomicts; in spring it was close to the low value of the apomicts (Fig. 2). Not one class had longer leaves than the others did on average ($p=0.644$). Timing of leaf growth, the phenology, was not conspicuously different between the different classes.

Leaf height of apomicts was significantly higher than leaf height of sexuals and hybrids on 13 September ($p<0.001$) by about 2 cm, but no significant differences were found in leaf height on 4 October ($p=0.395$). Significant differences between mothers were found for leaf height on both dates ($p<0.01$).

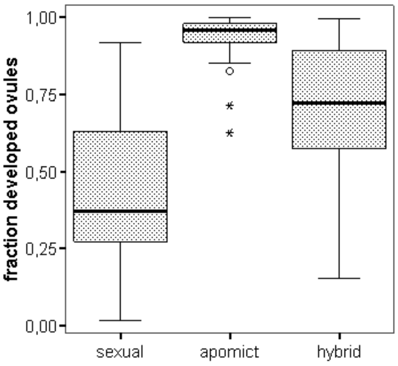

Fig. 4 The fraction of developed seeds per capitulum in sexuals, apomicts and hybrids. The boxes contain 50% of the data, the fat line shows the median, and whiskers extend from lowest to highest value excluding outliers.

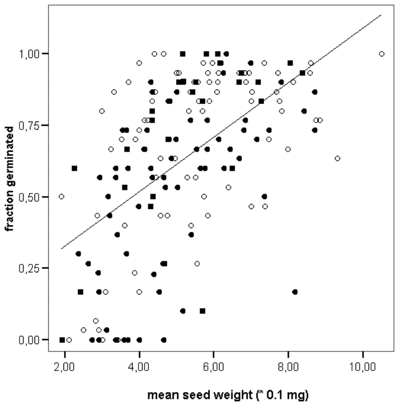

Fig. 5 The probability of germinating *vs.* the average seed weight per capitulum. Line shows the linear regression fit. Open circles: established apomicts; closed circles: hybrids; squares: sexuals.

Flowering and Seed Production

Of all surviving plants, only one did not flower; this was a hybrid.

The flowering plants produced 4.4 (±1.6) capitula per plant, and the numbers did not differ significantly between different classes of plants (p=0.599). The date of appearance of the first capitulum per plant was not significantly different between the classes (p=0.075), but timing of all capitula differed significantly: hybrid flowers appeared earliest and sexual flowers last (p<0.001). This was the same as the trend found for first capitula. Sexuals produced on average 174±37 ovules per capitulum, hybrids 158±32, and apomicts 147±33 (p=0.425). We estimated the total number of ovules as the number of capitula times the average number of ovules per capitulum per plant. This total number of ovules per plant was significantly higher in sexuals than in apomicts and hybrids, 980 (±576) in sexuals, and 608 (±149) and 693 (± 223) in apomicts and hybrids respectively (p=0.034). This difference in ovule number per plant, despite insignificant differences in capitulum number and ovule number per capitulum, was the result of a striking pattern of variation. In sexuals we found a positive correlation between the number of capitula per plant and the number of ovules per capitulum (r^2=0.42, p=0.023), whereas in apomicts we found a negative correlation (r^2=0.20, p=0.026). In the hybrids, no significant correlation was found (r^2=0.01, p=0.730). So, in sexuals, plants with many capitula also had many ovules per capitulum, whereas in apomicts plants with many capitula had few ovules per capitulum (Fig. 3).

Though the established apomicts invariably had a high proportion of developed seeds (0.94±0.06), the proportion in the hybrids varied much more and the average was lower than in the apomicts (0.71±0.22) ($p<0.001$) (Fig. 4). Sexuals, too, had often a low proportion of developed seeds (0.41±0.23), and in a number of cases the seedhead had not developed at all. However, this was probably due to pollen limitation in the greenhouse, since hand-pollination is not completely efficient.

The fraction of the mature-looking seeds that germinated was 68% (± 31) and 69% (±28) in the sexuals and established apomicts respectively. In the hybrid apomicts, the germination was 54% (±30). These differences were not significant ($p=0.223$). Variation between petri dishes, though, was rather high, because some dishes became infected by fungus.

Seed weight of mature-looking seeds decreased with capitulum sequence number ($p<0.001$). Though the number of capitula was the same in all three types, the variation in capitulum number varied among the types and this complicated the analysis of seed weight. Over all capitula, without taking capitulum number into account, seed weight did not differ significantly among the different classes ($p=0.527$). Heavier seeds had a higher probability of germinating ($p<0.001$) (Fig. 5). This relationship did not differ between the classes ($p=0.537$).

Seed weight decreased significantly with increasing number of ovules per capitulum, but r^2 was low ($r^2=0.04$, $p=0.011$ $n=159$).

Harvest

Total dry weight was 4.68 g (±1.57) per plant on average and did not differ significantly between classes ($p=0.169$), though weight of taproots separately was significantly higher in the sexuals than in the two triploid classes ($p=0.016$). Leaf area was on average 110 cm^2 (± 60) and not significantly different between classes ($p=0.887$). Specific leaf area (272 ± 43 cm^2 g^{-1}) was the same for all classes as well. The total dry weight did not correlate with the number of capitula in sexuals or apomicts ($p=0.842$ and 0.270 resp.), but did so in the hybrids ($p<0.001$, $r^2=0.58$) (Fig. 6).

Specific leaf area was not dependent on the average cell size ($p=0.678$).

Trait Heritability

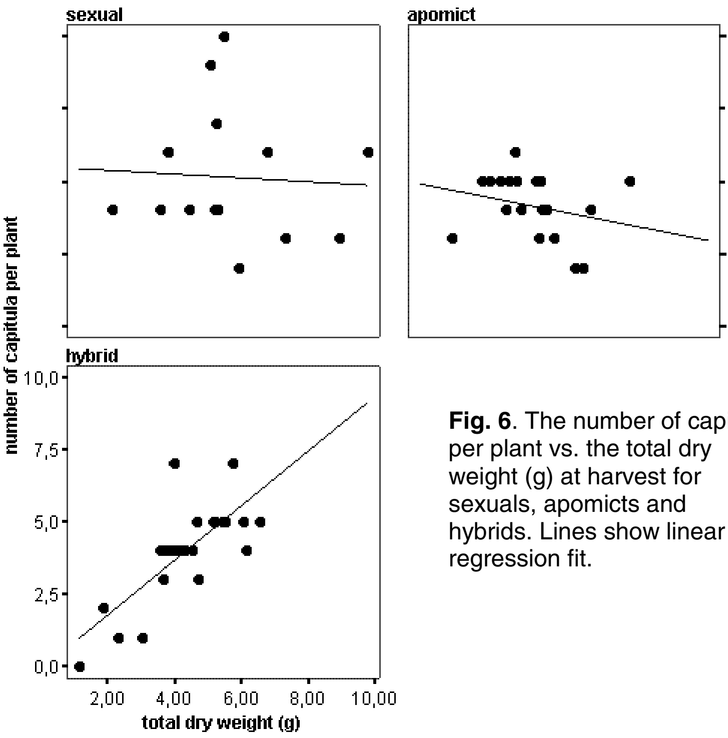

Fig. 6. The number of capitula per plant vs. the total dry weight (g) at harvest for sexuals, apomicts and hybrids. Lines show linear regression fit.

For selection to work, traits must be heritable. With the set-up that we used, an estimate of heritability of some of the traits could be made. For apomicts, heritability estimates are broad-sense, for sexuals narrow-sense. Hybrids had heritability values larger than zero for the number of seeds per capitulum, the fraction of seeds that developed, the fraction of seeds that germinated and the weight of seeds in the first capitulum. For the apomicts only the number of seeds per capitulum had a heritability significantly different from zero. For all these traits, heritability values of the hybrids were higher than those of the apomicts (Table 1).

Leaf length and leaf number had been measured 37 times on the same plants. A significant effect ($p<0.05$) of mother on leaf length was found 3 times in the sexuals, 10 times in the apomicts and 13 times in the hybrids. These positive values were found mainly from January until May, when leaves are relatively short. A significant effect of mother on leaf number was found 12 times in the sexuals and 19 times in the apomicts, mainly from November until March when leaf numbers were relatively small. For the hybrids we found a significant effect of mother on all dates but four.

Table 1. Heritability (h^2) estimates of seed-related traits. Heritability of traits in sexuals is calculated as though offspring were full sibs, though the seedheads probably contained a mixture of full and half-sibs. An asterix denotes significant differences from zero.

Trait	Sexuals	Apomicts	Hybrids
ovules per capitulum	0.14	0.22^*	0.44^*
fraction ovules developed	-	0.20	0.67^*
germination fraction	0.57	0.18	0.29^*
seed weight first capitulum	-	0.06	0.51^*

Discussion

The comparison between sexual and apomictic *Taraxacum* and their hybrids resulted in different patterns for different traits. The different patterns and their relevance will be discussed below.

No Differences

Traits that did not differ significantly between all classes of *Taraxacum* were capitula number and seed weight, when viewed over all capitula. Capitula number was not shown to differ between sexuals and apomicts in previous experiments either (De Kovel & De Jong 1999). In a field survey in Central Europe, however, sexual *Taraxacum* were found to have smaller and more numerous capitula than apomicts in the same field (Den Nijs *et al.* 1990). In the current study, sexuals on average produced more ovules per plant than the apomicts. This was connected to a particular pattern of variation in capitula number and ovule number (Fig. 3), and we can therefore not easily interpret this as an adaptation of apomicts to their reproductive assurance, as Den Nijs *et al.* do for their data.

Seed weight did not differ between the classes in this experiment, nor in a previous experiment that compared sexuals and apomicts (De Kovel & De Jong 1999).

Hybrids Traits Identical to Apomicts, Ploidy Effect

Cell size did not differ between hybrid and established apomicts, but was smaller in sexuals. It seems clear that triploidy causes larger cell sizes than diploidy. This is commonly found (Tal 1980;Levin 1983) and may affect further physiology of the plants (Warner & Edwards 1993).

Sexuals had a ± 40% heavier taproot than both apomicts and hybrids, a pattern not found in a previous comparison between sexuals and apomicts (De Kovel & De Jong 1999).

For much of the season sexuals had considerably more leaves than the two triploid classes. In spring and autumn, hybrids had even fewer leaves than the established apomicts. It is likely that triploidy caused lower leaf numbers.

Hybrid Traits not in-between Sexuals and Apomicts, Selection on Non-additive Traits

One complication is that newly formed apomictic triploids can have development errors. Such errors have been found in other sexual-asexual hybrids (Wetherington *et al.* 1987), as well as in sexual-sexual inter-generic hybrids. It is well possible that there are strong epistatic effects rather than additive effects for some traits. In that case only some combinations of sexual and apomictic genomes produce fit phenotypes, and hybrids are not in-between their parents.

One trait in which problems obviously occur is in seed production. Seed production of newly formed apomicts was poor in many cases. Though capitula were formed and ovules were formed in those capitula, parthenogenetic development of seeds was problematic. It has been shown in hybrid studies that fertility is often more vulnerable than vigour (Forsdyke 2000) (Coyne & Orr 1989). It is possible that fertility-related traits show strong epistasis (Merila & Sheldon 1999).

Even more complicated is the fact that hybrids had on average fewer leaves than either sexuals or established apomicts, though this was only apparent in some seasons. Should this be attributed to general developmental problems, or is this a polyploidy effect on which selection subsequently acts towards more leaves?

Similarly, hybrids flowered earlier than sexuals or established apomicts. It has been shown before that the cue to which plants react for timing of flowering is probably complex and sensitive, because changes in conditions can reverse the order of flowering of different groups of plants (Segraves & Thompson 1999;De Kovel & De Jong 1999). Therefore, it is difficult to conclude about the direction of ploidy effect or selection for the field situation, though both may play a role.

Hybrid Traits in-between Sexuals and Apomicts, Selection on Additive Traits

Hybrid leaf length was in-between sexuals and established apomicts in winter, when leaves are longest and light levels are low. In an earlier experiment, it was shown that the hybrids had shorter leaves than established apomicts when grown in the shade, though not in full light (De Kovel & De Jong 2000). Selection for leaf elongation in shade is a likely explanation for this pattern. A comparison of sexuals and apomicts collected from a single field also showed that apomicts had a stronger leaf length response to shading than sexuals (De Kovel & De Jong 1999). In a field survey in Central Europe, sexual *Taraxacum* were found to have smaller and narrower leaves than apomicts in the same field (Den Nijs *et al.* 1990). This suggests that the pattern is widespread.

A funny 'trait', the correlation between number of seeds per capitulum and number of capitula per plant, also, was in-between sexuals and established apomicts for the hybrids. This suggests a genetic component in this correlation that is different in sexuals and apomicts, and possibly under selection. However, the interpretation of this pattern is difficult.

Heritabilities

Selection is only effective in changing trait values, if the trait values are heritable. Since we want to see whether there is selection on hybrids, we are, of course, especially interested in the heritability of specific traits in the hybrids. Selection may have reduced the heritabilities in the established apomicts, and even in the sexuals.

The fraction of developed and germinable seeds had a positive heritability in the hybrids, but not so in established apomicts, and positive selection on these traits probably took place. Seed weight had a positive h^2 in hybrids and a lower h^2 value in the established apomicts. Selection could take place, and probably did, but selection did not clearly act towards heavier seeds, despite the correlation between seed weight and germination ability. Ovule number per capitulum was also a heritable trait with a higher h^2 value in apomicts than in hybrids. Possibly, the weak correlation between ovule number and seed weight caused balancing selection on both traits (Tweney & Mogie 1999).

Leaf length was heritable in the hybrids as well, but in particular in spring, when leaf lengths were the same in established apomicts and hybrids. Leaf number had positive h^2-values in the hybrids in most of the season. Established apomicts had positive h^2 values except in autumn and spring, which tentatively is evidence for selection towards higher leaf numbers in those periods. Heritability values at subsequent dates are, of course, not independent. I have calculated them as if they were, to get a rough indication of the process that is going on.

Niche Differentiation because of Ploidy Differences

Triploidy caused larger cell sizes. Cell size and shape can affect photosynthetic rate per unit leaf area in either direction (Warner & Edwards 1993). The larger taproots in sexuals may be a result of higher photosynthetic rates. Whether the cell size difference causes niche differentiation between sexual and apomictic *Taraxacum* is not clear from this experiment, though it is conceivable. Triploidy probably caused apomicts to have fewer leaves than sexuals, though this pattern was less clear-cut. The ecological significance of fewer leaves is not quite clear. It is possible that having many smaller leaves results in more economic water use (Dudley 1996). This in turn could result in niche differences with sexuals having advantage in localities that are more arid. Surveys of distribution of sexual and apomictic *Taraxacum* also show a more arid preference of sexuals (Roetman *et al.* 1988). It seems likely that polyploidy also influences the onset of flowering, but sexual and apomictic populations still show a large overlap in flowering time. It seems therefore unlikely that this causes enough niche differentiation to allow co-existence of the two types.

Selection on New Apomictic Lineages

Apomictic lineages were selected for higher proportions of developed, germinable seeds. These traits are directly connected to fitness, so this is not surprising. Such selection also does not cause niche differences between the sexuals and apomicts. Despite heritable variation in the hybrids for seed weight and number of ovules per capitulum, selection did not noticeably act towards trait values differing from those in sexuals. Apomicts were probably selected for a more plastic leaf length response to shading. This may enable the apomicts to grow in locations with more shade or with more variable light conditions than the sexuals can. Selection for higher leaf plasticity may be explained as an adaptation to lower genetic variation in the offspring. It is also possible that the effects of triploidy on leaf number and cell size need to be balanced by changes in other leaf characteristics to ensure optimal growth. As a side effect, this may increase niche differences between sexuals and apomicts.

From this study, there is no clear evidence for selection for traits specifically connected with the apomictic mode of reproduction. Differences between sexuals and apomicts in *Taraxacum* in the traits under study are likely to be mainly the consequence of ploidy differences. Repeated formation of new clones, and a short lifetime of clonal lineages may be the reason that differences between sexuals and apomicts are relatively small.

Acknowledgements

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Chapter 6

Selection on apomictic lineages of *Taraxacum* at establishment in a mixed sexual-apomictic population

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Selection on apomictic lineages of *Taraxacum* at establishment in a mixed sexual-apomictic population

Carolien G.F. de Kovel & Gerdien de Jong

Abstract

A species' mode of reproduction, sexual or asexual, will affect its ecology and evolution. In many species asexuality is related to polyploidy. In *Taraxacum,* apomicts are triploid, and sexuals are diploid. To disentangle the effects of ploidy level and reproductive mode on life-history traits, we compared established apomictic *Taraxacum* genotypes with newly synthesised apomictic genotypes, obtained from diploid-triploid crosses. Diploid-triploid crossing is probably the way that most apomictic lineages originate. New genotypes had on average a much lower seed set than established genotypes. Established genotypes differed on average from new genotypes, in particular under shaded conditions: the established genotypes had longer leaves and flowered later. The differences between new and established triploids resembled the differences that have been found between sexual diploids and established apomictic triploids. We conclude that ploidy differences alone are not directly responsible for observed differences between sexual diploid and apomictic triploid dandelions.

Keywords: apomixis; asexuality; evolution; polyploidy; selection; *Taraxacum*

Introduction

Sexual and asexual reproduction

The disparate consequences of sexual versus asexual reproduction for a species' evolution have been discussed many times over the past decades (Williams 1975;Barton & Charlesworth 1998;Bell 1982;Crow 1994). Evolutionary paths of sexual and asexual species are likely to diverge when environmental conditions are heterogeneous in time or space. Theoretically, the faithful reproduction of the genotype in an asexual lineage will allow such a lineage to dominate in its favoured niche, if this niche is constantly available over time (Vrijenhoek 1984). Such conditions are favourable for specialised asexual genotypes. Sexual types will not be able to compete successfully with such asexual lineages, as recombination will break up their genotypes every generation (Case & Taper 1986). In this way, a combination of asexual lineages outcompetes a population of sexual conspecifics in a spatially heterogeneous, but constant environment (Weeks 1993). The breaking-up of genotypic combinations by recombination may also hinder sexual species in extending into new niches (Kirkpatrick & Barton 1997;García-Ramos & Kirkpatrick 1997;García-Ramos & Kirkpatrick 1997). Asexuals do not have this limitation (Roughgarden 1972). When conditions vary over time at an intermediate time scale, a combination of recombination and changes in allele frequencies can create new genotypes in sexual species so that the majority of the population consists of adapted specialists (Bürger 1999;Crow 1994), provided the genetic variation is large enough. Asexual lineages are unable to do so. Selection between asexual lineages, of course, can alter the genetic composition of an asexual community, but new, better-adapted genotypes do not arise by mutation alone at a sufficient rate (Bürger 1999). Under these conditions, selection will favour generalist asexual genotypes over specialist genotypes. Such genotypes have been called general-purpose genotypes (Lynch 1984).

Two reproductively isolated species, occupying the same niches, can diverge during evolution (Abrams 1986). Such displacement will reduce competition. Competing sexual and asexual types of species are reproductively isolated and competition between them may lead to niche divergence. As they experience different constraints on evolution, the sexual and asexual types might, therefore, well diverge in directions that are not random. Sexuality, or the lack of it, may also 'pre-adapt' species for certain conditions, affecting further evolution. So, for several reasons sexual and asexual forms of a species may diverge with time.

Theoretical concepts about sex are well developed, but empirical data are still scarce. In laboratory experiments, directional selection on geotaxis caused an increase in recombination (Korol & Iliadi 1994). The expectation of faster adaptation of sexuals to changing conditions was confirmed in an experiment with yeast (Greig *et al.* 1998), but was not borne out in some other experiments (Da Silva & Bell 1996;Turner & Chao 1998). Field experiments also have shown mixed results (Antonovics & Ellstrand 1984;Ellstrand & Antonovics 1985;Kelley *et al.* 1988). The type and extent of environmental heterogeneity that species encounter in the field, however, cannot easily be quantified and compared to theoretical models. One approach to study the significance of sexual reproduction is to compare sexually and asexually reproducing types of the same species. We would like to understand the niche relationships of sexuals and asexuals. If no different opportunities or constraints apply to the different types, asexual phenotypes can be a random subset of those present in the sexual population, so-called 'frozen niches' (*sensu* Vrijenhoek 1984). Non-random subsets could be wide-niched clones, i.e. general-purpose-genotypes' (Lynch 1984). Alternatively, asexuals could have niches that are shifted away from the sexuals by competition (Weeks 1993) or because their mode of reproduction makes them better suited to certain niches. In this study, we investigate for what traits asexual clones are selected on establishment in a mixed sexual-asexual population.

Taraxacum as a model system

In many species, asexuality is related to polyploidy (Bierzychudek 1985); this is also the case with *Taraxacum. Taraxacum* forms an agamic complex comprising sexually reproducing diploids and apomictic polyploids. The most common polyploid is triploid. The species shows geographic parthenogenesis over Europe. Diploid sexual genotypes are common in France, but their relative frequency declines towards the north. In the northern parts of Europe, diploids are virtually absent (Den Nijs & Sterk 1984b;Den Nijs & Sterk 1984a). Another area of diploid occurrence is in Central Europe with its focus in Slovakia (Den Nijs *et al.* 1990).

The origin of the triploid apomictic genotypes is largely unknown (Richards 1973). They may result from ancient hybridisations. However, triploids are able to backcross with diploids in mixed populations. Female meiosis in apomicts is usually restitutional and produces unreduced, parthenogenetically developing seeds. Male meiosis is irregular, producing pollen containing from only a few chromosomes up to a complete triploid set of chromosomes (Jenniskens *et al.* 1985). Unreduced pollen or pollen accidentally containing one or two viable set of chromosomes can fertilise reduced ovules in sexual diploids. Crosses with diploids acting as mothers and triploids as fathers have low success. Such crosses usually result in triploid and tetraploid offspring (Morita *et al.* 1990). The frequency of such hybridisations in nature is likely to be low. From an extensive study on allozyme variation in mixed diploid/triploids *Taraxacum* populations, Menken *et al.*(Menken *et al.* 1995) concluded that gene flow between the two ploidy levels is common. This conclusion was based on the facts that allozyme polymorphisms were shared within mixed populations, and that population-unique alleles occurred at both ploidy levels. It is therefore possible that most extant triploid lineages are of recent origin.

If most extant triploid lineages have been created through backcrossing, comparing established and newly formed triploid apomicts allows us to identify the traits for which apomictic triploids are selected. Triploids formed by backcrossing under controlled conditions have experienced little selection. Established lineages are expected to be a subset of those genotypes that are formed.

In addition, such backcrosses that produce new apomicts can give insight in the nature of the observed differences between sexuals and apomicts. Some differences found so far between diploid sexuals and triploid apomicts (De Kovel & De Jong 1999;De Kovel & De Jong 1999;Elzinga *et al.* 1987) may be solely the result of the higher ploidy level in apomicts, whereas other differences potentially reflect different responses to selection pressures. If newly formed triploids are on average more similar to diploids than established triploids are, this indicates that differences between established triploids and the sexual diploids are the result of selection. We studied growth, morphological and life-history traits that have been shown to differ between diploid sexuals and triploid apomicts.

Material and Methods

Seeds

New triploids

Diploid plants were collected in a mixed diploid/triploid population, called Bovenste Polder in Wageningen, the Netherlands (52°N, 5°E). Diploidy of the collected plants was established by flow-cytometry (Ulrich & Ulrich 1991), which measures the amount of DNA per nucleus. Seeds from these plants were grown at the Nederlands Instituut voor Oecologisch Onderzoek (NIOO-CTO, Heteren) and mature diploid plants were put into a field containing only triploid dandelions. This field was a moist meadow used for haymaking. Seeds recovered from these diploid mother plants were grown in the greenhouse at the institute and screened for ploidy level. Triploid offspring plants that produced triploid seeds apomictically were selected for the current experiment. Seed set percentage varied hugely among these new genotypes. For practical reasons we selected twelve plants with seed set above 50% for this experiment.

Established triploids

In the same all-triploids field that had provided the fathers for the new triploids, a number of plants were screened with the intergenic spacer of ribosomal DNA as a marker (Van Dijk 1997). Eleven plants with different genotypes were selected and seeds from these plants were used in this experiment. One capitulum per mother was randomly selected to provide the seeds for the experiment.

Pilot experiment

From each mother ten mature-looking seeds were weighed. Per mother 20 seeds were put in a petri-dish and stored in an incubator with16h light per 24h and temperature 17°C during daytime and 14°C during night hours. After 21 days, the number of germinated seeds was counted.

Treatments

The experiment was carried out on benches in an open greenhouse. On 29 June 1998, seeds were sown in 12x12 cm pots, filled with a 1:1 mixture of sieved black soil and sand. Per pot, three mature-looking seeds were sown. On 1 September, plants were removed until one per pot was left. Two treatments were applied and each genotype was replicated within the treatment, so a total number of 96 pots were employed. Treatments were either shaded or unshaded. Shade was provided by a cage covered by green foil, whereas the unshaded treatments were carried out in cages covered with transparent foil. The treatments received enough water to saturate the soil. Water application was decreased when temperatures dropped and was stopped altogether when frost set in. In spring, water application was resumed.

Measurements

Germination was recorded regularly. The length of the longest leaf per plant was recorded on 25 August, 17 September, and 12 October 1998 and on 27 May 1999; the number of leaves per plant was recorded on the same dates as well as on 31 July 1998 and 17 March 1999. Flowers were counted when fully open, every other day during the flowering period. Seeds were collected in paper bags. From the first capitulum of each plant, ten seeds were weighed collectively. Twenty seeds from each of the first three capitula per plant were placed on a wet filter paper in a petri-dish right after harvesting under conditions as mentioned above and the number of germinated seeds was counted after 14 days.

Harvest

All plants were harvested on 7 and 8 June 1999, when flowering appeared to have ended. Plants were separated into leaves, tap root and fine roots. All plant parts were dried at 70°C for at least 48h and subsequently weighed.

Data analysis

Pilot experiment

Weights of the seeds were compared between the new and established genotypes with ANOVA. To test whether germination ability of the seeds used for the experiment differed between new and established genotypes, a non-parametric Mann Whitney U test (MWU) was used.

Growth

Data from the same genotypes were pooled before further analysis. The effects of origin and treatment on leaf length and leaf number were analysed with ANOVA with treatment and origin (established *vs* newly synthesised) as fixed factors and date as a covariant.

Plasticity was calculated per genotype, after averaging over duplicates, as the leaf length or leaf number in the shaded treatment minus that value in the unshaded treatment. Effect of origin on plasticity was analysed using ANOVA with origin as fixed factor and date as covariate.

Flowering

The number of capitula per plant was analysed with the MWU-test, as this value was not normally distributed. The analysis was carried out, either with origin as the grouping factor or with treatment as the grouping factor. The number of days until flowering was also tested with the MWU-test. The effect of origin was tested separately for the different treatments. The effects of origin and treatment on the number of seeds per capitulum were tested with ANOVA, as were the effects on weight of mature-looking seeds. The proportion of seeds that germinated was compared between plants from different origin with an MWU-test. The same test was used for the proportion of seeds per capitulum that had not developed. A Spearman rank correlation was used to analyse the relation between parental and offspring seed weight, as well as for the relation between seed weight and germination proportion. Plasticity in seed weight was analysed as plasticity in morphological traits.

Harvest

Dry weights were analysed with ANOVA with origin and treatment as fixed effects.

Results

Seed weight and germination in pilot experiment

Mature-looking seeds from established triploids were on average 0.716 ± 0.169 mg, whereas those from the new genotypes weighed only 0.568 ± 0.154 mg ($p=0.039$). Germination ability differed also between the two sets: significantly more seeds from the established genotypes germinated than from the new genotypes: 13.7 ± 5.2 and 9.8 ± 4.1 out of 20 respectively ($p=0.032$).

Germination

In the unshaded treatment, 19 out of 22 established and 14 out of 24 new plants survived until census, whereas 20 established and 13 new plants, out of 22 and 24, respectively, were counted in the shaded treatment. In both of the treatments, this made 11 established genotypes and 9 new genotypes.

Growth

Leaf length increased during the summer. In winter, most plants had few leaves. During flowering in spring few new leaves emerged. The leaves measured on 27 May 1999 were the new leaves that sprouted after flowering and these were smaller than the earlier leaves. Treatment had a significant effect on leaf length on all four measurement dates: shaded leaves were longer ($p<0.05$ for all dates). In addition, leaf number was lower in the shaded than in the unshaded treatment on all dates except 27

May 1999 ($p<0.05$, 27 May $p=0.525$). In the shaded treatment, the leaves of the new genotypes were shorter than those of established genotypes ($p<0.05$), but in the unshaded treatment, no difference was found ($p=0.355$) (Fig. 1). Origin of the genotypes did not affect the number of leaves ($p=0.549$).

With respect to leaf length, the established genotypes were more plastic than the new genotypes ($p<0.05$). Leaves of established triploids were, on average, 8.3 cm longer under shade conditions, whereas the new triploids were 4.6 cm longer. Plasticity in leaf number did not differ between plants from different origin ($p=0.327$).

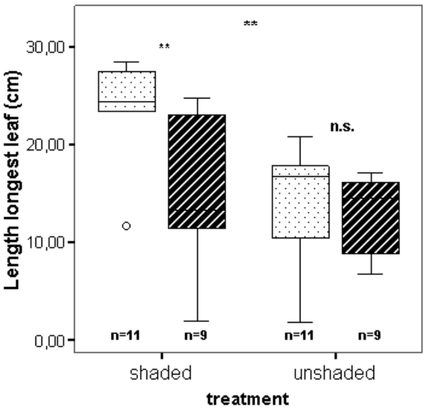


Figure 1. Lengths (cm) of the longest leaf per genotype of established (dotted) and new (hatched) triploid *Taraxacum* genotypes under shaded and unshaded conditions on 17 September 1998. The box contains 50% of the data; the whiskers extend from lowest to highest values, excluding outliers (o).

Flowering and seed set

The plants in the experiment flowered some days before the wild populations. The average number of capitula per plant over two treatments was 3.9 (±1.9). Shading decreased the number of capitula significantly from 5.1 to 2.8 ($p<0.05$, MWU). New and established genotypes produced the same number of capitula on average ($p=0.261$ MWU). Flowering was delayed by shading. New genotypes flowered on average almost two days earlier ($p=0.03$, MWU). This effect was only due to the shaded treatment. (Fig. 2). The number of ovules produced per capitulum was on average 187±35. This was not affected by shading ($p=0.788$), nor by origin of the genotypes ($p=0.560$). Some seeds in each capitulum were small and white instead of brown. We considered them undeveloped. The fraction of seeds that looked mature was lower in the new genotypes than in the established ($p<0.05$): 71% and 88% respectively. A lower fraction of the mature-looking seeds germinated in the new genotypes ($p<0.05$), also after correction for seed weight ($p<0.05$). The average probability that an ovule of a new genotype would produce a viable seed was, therefore, 46%, while in the established genotypes the average was 71% ($p<0.05$). Germination probability ranged from 12%-73% in the new genotypes and 20%-89% in the established genotypes. A treatment effect on germination of mature-looking seeds was not found ($p=0.242$) (Fig. 3). The weight of apparently mature- seeds was on average 29% lower in the shaded treatment than in the unshaded treatment ($p<0.05$) (Fig. 4). Plasticity in seed weight did not differ significantly between genotypes from different origin (p=0.605). Origin of the genotypes did not affect the weight of these seeds significantly (p=0.056). Seed weight of the mature seeds was positively correlated with germination percentage (r=0.346, p<0.01).

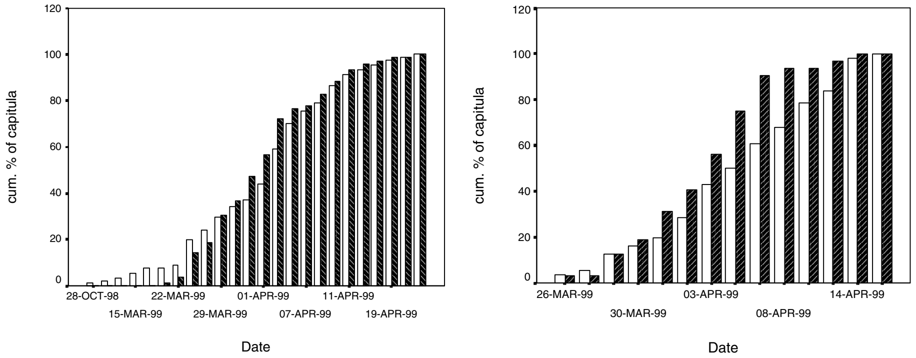
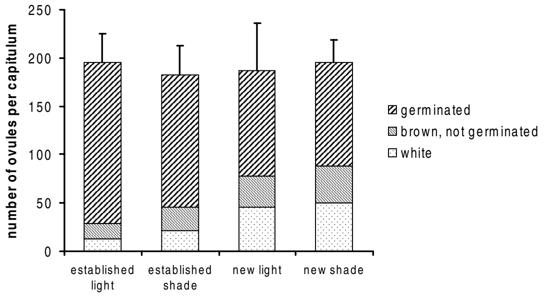


Figure 2. Cumulative proportion of flowering capitula on established (white) and new (hatched) genotypes of *Taraxacum*. *a* shaded treatment; *b* unshaded treatment. Note different X-axes.



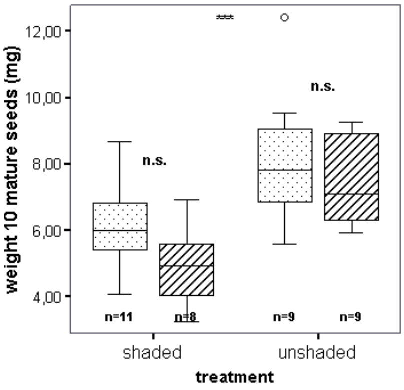


Fig 3. Total number of ovules per capitulum of established and new *Taraxacum* genotypes under shaded and unshaded conditions. Data are shown for the first capitulum per plant. Error bars represent one standard deviation. Patterns show the fractions that looked undeveloped and that looked mature. The fractions of mature-looking seeds that germinated are extrapolated from the germination experiment.

Fig 4. Weight of 10 apparently mature seeds (mg) of new and established *Taraxacum* genotypes under shaded and unshaded conditions. The box contains 50% of the data; the whiskers extend from lowest to highest values, excluding outliers.

The weight of the seeds produced by the plants in the experiment did not correlate with the weight of the original seeds (r=-0.088, p=0.554).

Harvest

Total dry weights were 2.62g (±1.12) in the shaded and 4.44g (±1.66) in the unshaded treatment (p<0.05), and a smaller proportion of biomass was present in the leaves in the unshaded treatment (p<0.05). Between new and established genotypes, no differences were found in total dry weight (p=0.861), nor in the ratio of leaf weight:total weight (p=0.182).

Correlation coefficients between leaf length and germination probability in the newly synthesised triploids were positive for some dates and negative for others, but not significant for any date (p=0.084, p=0.210, p=0.143, p=0.146).

The seeds used for the experiment of the new genotypes were lighter than those of the established genotypes were. The origin of the seeds differed: seeds from new genotypes had been collected in the greenhouse, whereas seeds from established genotypes were collected in the field. As seed weight is plastic with respect to environmental conditions, this difference may have been environmental, rather than genetic. These differences may conceivably have affected subsequent growth as a maternal effect (Andalo *et al.* 1999). Against this, it can be argued that dry weight at harvest did not differ between plants from different origin.

Discussion

Differences between sexual diploid and apomictic triploid *Taraxacum* have been found previously; diploids had shorter leaves than triploids, in particular in shaded conditions (De Kovel & De Jong 1999). In another study, diploids had lighter seeds than triploids (unpublished). Shifts in flowering time between diploids and triploids have been found in both directions, depending on the conditions (De Kovel & De Jong 1999), unpublished). In these observations the effects of ploidy level and mode of reproduction were confounded. To study the consequences of reproductive mode, it is necessary to distinguish between any effects of asexuality and ploidy level. We therefore compared newly synthesised triploids with established triploids. If ploidy level by itself leads to a difference with diploids, it should be visible in the new triploids. If ploidy is the only cause of any difference, new and established triploids should be identical. On the other hand, if the new triploids differ from established triploids, a range of alternative hypotheses about the ecological relation between sexuals and asexuals comes into view.

In this study, we found that established triploids were not a random selection from the new triploids. New triploids had shorter leaves on average than established triploids, especially under shaded conditions, and they seemed to be less plastic in leaf size response to shading. In addition, new triploids flowered slightly earlier, again mainly under shaded conditions. Mature seeds of new triploids tended to be lighter in shade than those of established triploids. The longest leaves and the heaviest seeds belonged to the established triploids; shortest leaves and lightest seeds belonged to the new triploids. So, an explanation beyond the effects of polyploidy is needed.

The most obvious difference between new and established triploids was in the production of germinating seeds. Though the number of capitula and the number of ovules per capitulum were the same in both types of dandelions, the number of germinating seeds was much lower in the new triploids. For this experiment, only those new genotypes had been used that had had above average seed set in the previous generation (see Material & Methods). If seed set characteristics in these genotypes are heritable, the difference in production of germinating seeds between new and established triploids will be much larger than showing in this experiment. The same low fecundity of new asexuals was found in an comparable experiment with *Poeciliopsis* fish (Wetherington *et al.* 1987).

In a weedy species like dandelion, selection for high fecundity will be strong. If other traits are correlated with fecundity, they will hitchhike along with it. In our study, no correlation between morphological traits and fecundity was found in the new triploids, which means that a response to selection on those traits will be independent from selection on fecundity.

If conditions have not changed directionally over time, the new triploids represent the pool of genotypes from which triploids are recruited. The differences in trait values between new and established triploids then correspond to selection for those traits. The conclusion would be that asexuals are selected for longer and more plastic leaves than sexuals. If this interpretation is correct, the question is: why would the two reproductive types have different optima? It is curious that the differences seem larger under shaded conditions. A first explanation is that ploidy level causes differences in physiology that define different optimal values. It has been shown that for a number of species polyploidization changes photosynthetic rates, because it affects cell size (Warner & Edwards 1993). This in turn may influence the optimal shape and positioning of leaves and possibly other traits. There are other possibilities, though.

The difference between new and established triploids could be a case of niche shift and character displacement through competition with the sexuals (Abrams 1986). Because of this competition between sexuals and apomicts, apomictic genotypes that have less niche overlap with the sexual population are more likely to establish (Weeks 1993). This view assumes that asexual genotypes are specialised to some sub-niche that is available at all times. Many different asexual lineages can establish as long as they have limited overlap with niches of other asexual lineages or with the sexual population. This corresponds to the frozen niche variation hypothesis (Vrijenhoek 1979). In the above hypothesis, new triploids will be similar to diploids, but established lineages will show traits that enable them to exploit niches that are not fully exploited by the sexuals. In the current study, established triploids had longer leaves than the average of the new genotypes: a trait that probably enables them to grow in high vegetation (Van Hinsberg 1997). In a field survey in the Netherlands, diploids had a more xerothermic distribution than triploids (Roetman *et al.* 1988). This supports the idea of a niche shift between the reproductive modes, though its cause is not clear.

It is also possible that the new triploids do not resemble the pool from which the majority of the established triploids have been recruited. The diploid mothers had been collected in a field at about 5 km distance from the field where the established triploids and the triploid fathers had been collected. It is therefore possible that the diploid mothers were adapted to some local conditions and for that reason had alleles for e.g. shorter, less plastic leaves than the established triploids. They would have transmitted these alleles to the hybrid offspring. It is intriguing, though, that the differences observed between new and established triploids were in the same direction as those observed between sexual diploids and apomictic triploids from the same field (De Kovel & De Jong 1999). For this last study, diploids and triploids had been collected in the same field as where the diploids had been collected for the current study. This suggests that the pattern goes beyond local adaptation. A replicate experiment using plants from different localities may be able to make this more clear.

In summary, the data show that established apomictic triploids differ in a number of traits from triploids created by backcrosses between sexual diploids and apomictic triploids. New triploids have shorter leaves under shaded conditions as well as delayed flowering, and their leaf morphology responds less plastic to shading. In these respects, they are more similar to sexual diploids than the established triploids are. This suggests that differences found in previous experiments between sexual diploids and apomictic triploids are not solely the direct consequence of polyploidization, but that selection also plays a role.

The majority of the triploids created by backcrossing produced fewer viable seeds than the established triploids. Establishment chances of backcross offspring created under natural conditions are considered to be very low, but existing.

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Chapter 7

The effect of intra-specific competition on seedlings of sexual and apomictic *Taraxacum officinale*

Accepted OIKOS, 2001

The effect of intra-specific competition on seedlings of sexual and apomictic *Taraxacum officinale*

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Abstract

Because of their higher evolvability, sexuals may have an advantage relative to asexual organisms in a competitive environment with many biotic interactions. We tested this idea using sexual and apomictic *Taraxacum*, dandelions. *Taraxacum* seedlings were grown without competition and in different competing combinations in a greenhouse. Apomicts had more and longer leaves than sexuals, but the same dry weight at harvest as sexuals. Competition reduced growth to the same extent in both apomicts and sexuals. Therefore, we conclude that sexual dandelions are no superior competitors relative to apomicts. In *Taraxacum*, new apomictic lineages spin off from the sexual population with some unknown frequency. This may enable the apomictic community to keep up with the sexual population.

Keywords: apomixis, competition, Red Queen, sexual reproduction, *Taraxacum*

Introduction

When interactions between species are competitive or parasitic, continuous genetic changes in the populations are necessary for maintaining position against other evolving organisms (Glesener & Tilman 1978;West *et al.* 1999b). This continuous genetic change has been called Red Queen dynamics. When genetic variation is limited and new genotypes appear only rarely, as in most asexual organisms, the dynamics will slow down or grind to a halt. Sex and recombination can furnish a more rapid and extensive response to such selection pressures than asexual reproduction can (McPhee & Robertson 1970;Bürger 1999). In a species with many interactions with other species, sex may therefore present an advantage (Burt 2000;Glesener & Tilman 1978). Similar arms races can also go on within a species, if e.g. competitive ability varies among genotypes. This has been demonstrated in experimental evolution studies (Sole *et al.* 1999).

Some taxons comprise both sexual and asexual forms. With many interactions within the sexual part of the species, the whole sexual gene pool co-evolves. Asexual conspecifics are potentially 'left behind'. Competitive relations among asexuals cannot evolve as rapidly as is possible in sexuals, though these relations within the asexual community are not necessarily different from those among sexuals. From the above considerations, we hypothesised that sexuals will be better competitors than asexuals of the same species, particularly when competing against asexuals.

To test this hypothesis we set up a competition experiment with seedlings of sexual and asexual *Taraxacum*, dandelion. In *Taraxacum,* sexual and apomictic forms are known. In contrast to the unicellular organisms used in previous experiments, *Taraxacum* genotypes reproduce either obligatory sexually or obligatory asexually (Den Nijs & Menken 1994). These different forms can co-occur in the same locality. For this experiment, we collected sexual and asexual plants from the same field. Sexual forms are diploid, whereas apomictic forms are generally triploid. Ploidy levels may affect growth and development (Tal 1980). We, therefore, defined competitive ability as the difference in biomass sequestration between plants growing alone and plants of the same genotype growing in a pot with another plant. The experiment was carried out in pots containing a poor soil mixture, to make the plants compete for nutrients.

Materials and Methods

Seeds were collected in a mixed sexual/apomictic population in Slijk-Ewijk, the Netherlands, in spring 1999. Ploidy level of the motherplant was established with a flow-cytometer. Seeds from 6 triploid, i.e. apomictic, and from 5 diploid sexual plants were used for the experiment. These mothers were randomly selected from the field.

Motherplants will hereafter be referred to as 'genotypes', though, of course, seeds from a sexual mother are sibs. Seed weight was measured on 10 seeds per genotype. Seeds from all plants were set to germinate in petri dishes. Pots (∅ 5 cm, depth 15 cm) were filled with a poor soil mixture consisting of 5:1 sand:black soil. Pots were placed in a tray containing tap water to prevent desiccation. In the treatments referred to as *competition,* two germinated seeds were placed in a pot in a full factorial design. Thus, every genotype (the *target*) was tested against each other genotype (the *competitor*), including its own. Every combination except those containing two identical genotypes occurred twice. *No-competition* treatments consisted of a germinated seed occupying a pot by itself. The no-competition treatments were replicated three-four times per genotype, depending on available seeds. The experiment was started 11 August 1999 in a greenhouse in which temperature was set at 20°C, and additional lighting was provided. Leaf number and length of the longest leaf were scored every week. All plants were harvested 27 October 1999, separated into leaves and roots, dried at 70°C for 48 hours, and weighed. The plants were 11 weeks old.

Data analysis

Seed weights of sexuals and apomicts were compared with One-Way ANOVA.

Number of leaves and length of the longest leaf were analysed with a repeated measurements analysis. First, the effect of competition on leaf length and number of apomictic and sexual plants was tested. For the competing plants, the effect of the type of competitor on apomicts and sexuals was tested. Because assumptions for the structure of variance-covariance matrix were not met, the growth pattern was analysed with the Huynh-Feldt method. Overall differences between groups were analysed with analysis of variance between subjects tests (SPSS software).

Leaf height was compared with ANOVA, with genotype nested within type of plant.

Dry weights at harvest were log-transformed before further analysis to improve normal distribution. Differences between sexuals and apomicts in dry weight with and without competition were tested with an ANOVA with no competition, competition with a sexual, and competition with an asexual as levels of the fixed factor treatment, as well as with reproductive mode as a fixed factor. Genotypes (of the mother) were nested within reproductive mode. Contrasts were calculated between competition and no competition, as well as between competition against sexual and against apomict.

Dry weights per pot were compared with Oneway ANOVA.

Results

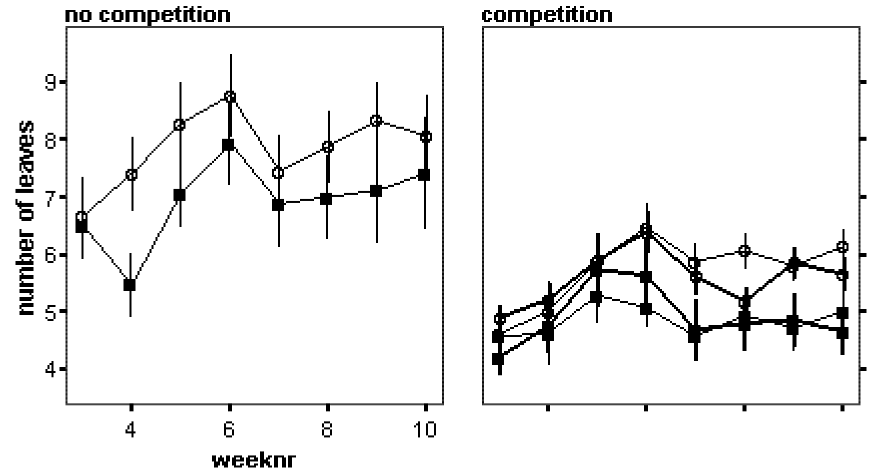

Fig. 1. Average number of leaves of sexual (squares) and apomictic (circles) plants over time *a* without competition, and *b* with competition against sexuals (thin lines) or against apomicts (thick lines). Error bars represent one standard error of the mean.

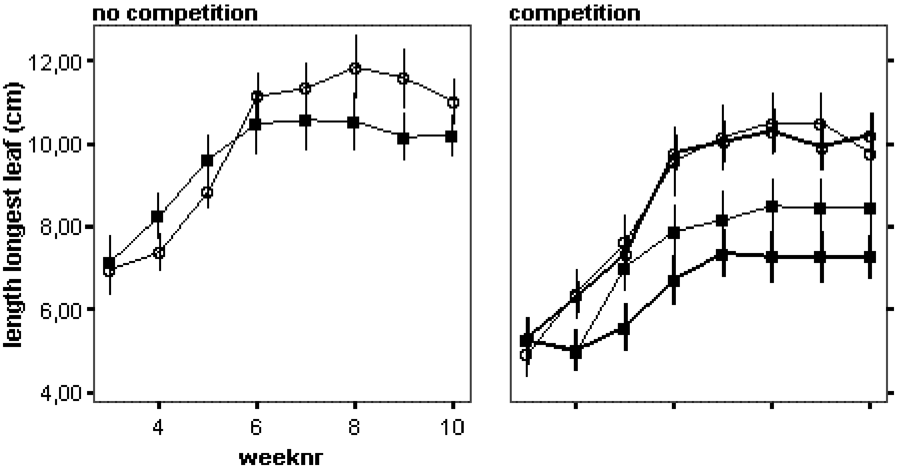

Fig. 2. Average length of longest leaves of sexual and apomictic plants over time *a* without competition, and *b* with competition against sexuals or against apomicts. Error bars represent one standard error of the mean. Symbols as Fig.1.

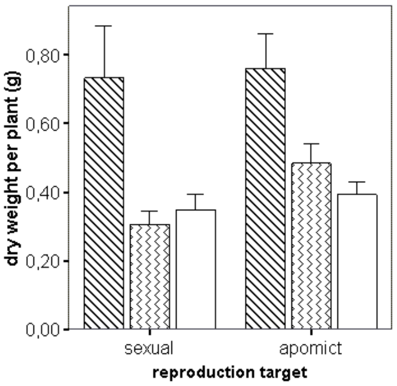

Fig. 3. Dry weight of sexual and apomictic plants without a competitor (hatched), with a sexual competitor (zigzag) or with an apomictic competitor (white). Error bars represent one standard error of the mean.

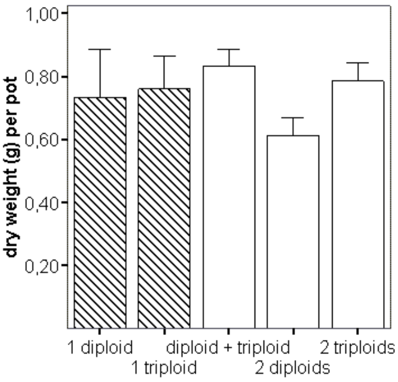

Fig. 4. Average dry biomass per pot of pots containing different combinations of sexuals and apomicts. Hatched: no competition; white: competition. Error bars represent one standard error of the mean.

Seed weight

Average seed weights of sexuals and apomicts were not significantly different (p=0.836).

Leaf length and number

Leaf length and leaf number both seemed to have reached a plateau after about seven weeks (Fig 1,2). Over all treatments, the leaf length growth curve differed between sexuals and apomicts (Huynh-Feldt p=0.017): apomicts grew longer leaves. Competition had a significant negative effect on leaf length during the whole period (p<0.001), but no significant interaction between mode of reproduction and the effect of competition was found (p=0.344). Sexual and apomictic plants without competition did not differ significantly in leaf length (p=0.617) or growth pattern (p=0.512). For the plants growing with competition, the apomicts had longer leaves (p=0.003), but no effect of the type of competitor was found (type of competitor p=0.643; interaction target x competitor p=0.376). Sexuals had leaves of 10.2 ± 2.1 cm at harvest without and 8.5±2.8 with competition; apomicts had leaves of 11.0±2.4 cm without and 9.4 ± 2.8 with competition. The growth curve of leaf number was not affected by the reproductive mode of the plant (p=0.067), but it was affected by competition (p=0.047). The two different types did not have a significantly different reaction on competition (interaction p=0.099). The total number of leaves was higher in the apomicts over all treatments (p=0.004) with a significant negative effect of competition (p=0.001). No interaction between the type of reproduction and competition was found on overall leaf number (p=0.091). Apomicts had 6.27±2.13 leaves with and 8.05±3.04 leaves without competition at harvest; sexuals had 5.66±2.39 leaves with, and 7.41±4.05 without competition. In the treatments with competition, no effect was found of the type of competitor (p=0.241), nor an interaction between target and competitor (p=0.117).

Harvest

Ninety-six of the 128 sexuals, and 126 of the 156 apomicts survived until harvest. Only pots of the competition treatment that contained both plants were used for analysis. Over all treatments, sexuals and apomicts did not differ in dry weight (p=0.228). Plants of a given genotype growing without competition were heavier than plants of the same genotype growing with competition (p<0.001) by, on average, a factor 1.9 (Fig 3). There was no interaction between reproductive mode of the target plant and the presence of competition with either a sexual or an apomict, which means that sexuals and apomicts did not differ in their response to competition (interaction p=0.583). However, when only plants growing in competition were considered, apomicts were significantly heavier than sexuals (ANOVA p=0.013), whereas this was not found without competition (p=0.984) (Fig. 3). The ploidy level of the competitor had no significant effect on the dry weight of the target plant at harvest (p=0.463), nor was a significant interaction found between the ploidy levels of the competing plants (p=0.331).

To see whether different combinations of competing plants acted differently, we summed the dry mass of the two competing plants per pot. The total amount of biomass in a pot with two plants was 0.83 (± 0.45) g in pots containing one apomict and one sexual, 0.79 (± 0.42) g in pots containing two apomicts, and 0.61 (± 0.30) g in pots containing two diploids (p=0.062) (Fig. 4).

Root:shoot ratios did not differ between plants of different types or plants experiencing different treatments (Kruskall-Wallis, p=0.262)

Discussion

We studied the effect of competition by conspecifics on sexual and apomictic genotypes of *Taraxacum* in the seedling stage. We expect dandelions to compete with each other mainly during seedling stages, as many seedlings often appear together in open patches, whereas adult plants often grow farther apart. Individual plant dry weight, leaf number and leaf length were reduced when plants were grown in competition treatments, so competition took place. Both with and without competition, leaf number and leaf size levelled off after about seven weeks, which probably means that resources were being depleted. Dry weight has been shown to be strongly correlated with the number of capitula that will be produced (Van Loenhoud & Van der Heijden 1980) and, therefore, provides a good measure for competition outcome and fitness.

Effects of competition

We expected that, if sexuals were better competitors than apomicts, they would have captured a larger part of the resources in a sexual-apomictic combination. Consequently, their dry weight would have been less reduced by competition relative to without competition than that of apomicts. In sexual-sexual combinations and apomictic-apomictic combinations, the dry weight of both competing plants would be expected to have been reduced to the same extent, relative to no competition. No significant interaction was found for total dry weight between the presence of competition and the mode of reproduction. This leads to the conclusion that sexuals and apomicts responded in the same manner to competition; they were equally good or equally tolerant.

However, when only competing plants were considered, apomictic genotypes were significantly heavier than sexuals, whereas this was not the case for non-competing plants. A similar pattern was found for leaf length. So, if anything, apomicts appeared more tolerant to competition than sexuals. More tolerant to competition means that the plants could either obtain more resources when growing in competition or lost less to non-growth functions.

In the competition treatments, the type of competitor did not affect dry weight of the target at harvest, nor the length or number of leaves. Again, this shows that the two types did not differ much in their handling of competition.

Consequences for co-existence

When sexuals are no better or even worse competitors than apomicts, the implication is that, in a mixed field of sexual and apomictic dandelions, the advantage that apomicts often have because of reproductive assurance (Sterk 1987a) is not offset by a competitive advantage of the sexuals in the seedling stage. Sexuals must either be sufficiently different from apomicts or must have some unknown advantage; otherwise, they will be driven to extinction by their apomictic conspecifics.

Coexistence would be possible if niche differentiation between sexuals and apomicts might result in lower competition between reproductive modes than within reproductive modes (Doncaster *et al.* 2000). The results do not show this clearly, but some indication may be found in Fig 3: dissimilar types seem to affect each other less than plants with the same reproductive mode. Two sexuals together are least productive, possibly because of strong competition between them. Differences between sexual and apomictic dandelions have earlier been shown to exist in leaf length and leaf length response to shading (De Kovel & De Jong 1999;De Kovel & De Jong 2000). Niche differences can themselves be the result of competition (Abrams 1986). It is also possible that some relevant differences causing niche separation are the consequence of the ploidy differences between the sexuals and apomicts. In the current experiment, relevant niche differences could, for instance, have existed in the ratio of the minerals that the plants can or must acquire (Tilman 1985).

Explanations for equal competitiveness

A reason why apomictic dandelions would be equally good competitors as sexuals is the system's particular dynamics. In *Taraxacum,* sexual and apomictic forms are not completely genetically isolated. The apomictic genotypes can form diploid pollen, though with very low success. This pollen can father new apomictic triploids when fertilizing a haploid ovum in a sexual plant (Tas & Van Dijk 1999a). Apomicts can, in this way, incorporate alleles from the sexual population. If this occurs often enough –which is unknown- apomicts can evolve at a rate similar to the sexuals. Older apomictic lineages may go extinct, either because they are left behind in evolution or because they suffer from accumulation of mutations (Crow 1994). The surviving apomicts are well-adapted genotypes that can quickly multiply in the population.

Another reason could be that competition with other plant species, e.g. grasses, requires strategies different from those required for intra-specific competition. If competition with other species is a stronger selective force than intra-specific competition, the results of the current study will not be conclusive.

Competition studies in other species

Competition between sexuals and asexuals has been studied in a (limited) number of other species. The results from these studies are inconclusive. It was shown in *Saccharomyces* that the evolvability generated by sexual reproduction can create a competitive advantage of sex over asexuality (Birdsell & Wills 1996;Greig *et al.* 1998), though in *Chlamydomonas* and RNA viruses such sex advantage was not found (Turner & Chao 1998;Da Silva & Bell 1996). A single parthenogenetic clone of the freshwater snail *Potamopyrgus antipodarum* was able to outcompete a sexual population in a laboratory environment in a multi-generation experiment. This result may well have been the consequence of a cost of males in the dioecious sexual snails

(Jokela *et al.* 1997a). Sexual, dioecious *Artemia salina* (brine shrimp), though, eliminated parthenogenetic strains in a multi-generation experiment (Browne 1980). In the fish species *Poeciliopsis,* sexuals had a higher growth rate and fecundity averaged over all combinations than either of the two parthenogenetic lineages that were used. Competition reduced growth rate and fecundity in both types. However, the *relative* effect of competition, either from sexual or from parthenogenetic competitors, appeared to be stronger in the sexuals than in the parthenogenetic genotypes (Weeks 1995).

Both the snails and the fish have a system with recurrent formation of clonal genotypes (Dybdahl & Lively 1995a;Vrijenhoek 1993), like dandelions. In such systems, asexuals may hitchhike along with the sexual species, picking up currently advantageous alleles from the sexual population. Without sexual conspecifics in the neighbourhood, they will have a lower evolvability. The sexual and parthenogenetic strains of *Artemia salina* originated from different continents and, thus, evolved in isolation from each other (Browne 1980). In this case, the sexuals may have evolved to become better competitors and have ‘left behind’ the parthenogens, but many factors may have contributed to the differences between the reproductive modes, as they have evolved under very different conditions.

Biotic interactions and the advantage of sex

Our expectations about the competitive relationships between sexual and apomictic dandelions were not borne out. In other systems, the results have not been clear-cut either. There is no evidence, so far, that asexuals are left behind in a continuous evolutionary race. The particular system with recurrent formation of clones that occurs in dandelions, may be present in a number of the cases where sexuals co-exist with asexual conspecifics. In these systems, differences between sexuals and asexuals may be small, because of hitchhiking. Though the distribution of parthenogenesis suggests a relationship between the frequency of biotic interactions and reproductive mode (Glesener & Tilman 1978;Bierzychudek 1985), no direct evidence is availabe yet.

Acknowledgements

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Chapter 8

Genetic variation in sexual and apomictic *Taraxacum officinale* and growth in heterogeneous environments

Genetic variation in sexual and apomictic *Taraxacum officinale* and growth in heterogeneous environments

Carolien G.F. de Kovel

Abstract

Producing genetically variable offspring could be advantageous in a heterogeneous environment, given a number of assumptions are met about the nature of this environmental heterogeneity, the mode of selection, and the way in which different genotypes differ from each other. In this study, I grew sexual and apomictic *Taraxacum* under four different conditions to test whether these assumptions are likely to be met. In the first experiment, pots containing 12 seeds from a sexual mother or 12 seeds from an apomictic mother were grown under four light-moisture conditions. In the second experiment, mixtures as well as pure cultures of apomictic genotypes were grown under the same set of four conditions. Sexual as well as apomictic *Taraxacum* were genetically variable. Different genotypes responded in different ways to the treatments. Asexual families reacted in the same way to treatments as mixtures of genotypes. Selection seemed a mixture of soft and hard selection. The consequences of these results for theories about the consequences of sexual reproduction are discussed.

Keywords: apomixis, genetic variation, heterogeneous environment, sexual reproduction

Introduction

A popular argument for the advantage of sexual reproduction has often been that it will be profitable to have genetically variable offspring in a variable environment. More detailed models have shown that this argument will only hold under specific conditions (Bell 1982;Williams 1975). Different genotypes must differ from each other in such a way that in different environmental conditions, the rank ordering of their fitnesses alters (Bierzychudek 1987;Bell 1987). This means that every genotype is specialised to certain environmental conditions. Other restrictions depend on the nature of the environmental variability and the nature of interactions between individuals.

Two specific models predict that the production of a genetically variable clutch will be profitable under certain conditions. The first model argues that if environmental conditions vary from one generation to the next, the fitness of an asexual lineage will be close to its geometric mean fitness over time (Robson *et al.* 1999). If a sexual mother were to produce offspring specialised to each of the possible conditions, then the fitness of the sexual lineage over time would be closer to the arithmetic mean fitness (Robson *et al.* 1999). The arithmetic mean fitness will be higher than the geometric mean, because the geometric mean is strongly influenced by very low values. This model assumes that the fitness of a genotype solely depends on the external conditions and not on the presence or identity of conspecifics. This is called hard selection. The idea that asexual lineages would have a better chance to survive many generations if they were tolerant to environmental variation, that is, if they had General Purpose genotypes (Lynch 1984), is based upon this model.

The second model has been called a lottery model, and focuses on spatial heterogeneity in the environment. In a spatially heterogeneous environment, the situation is more complicated than in a temporally fluctuating environment. Suppose a sexual mother produced offspring adapted to different conditions in the same proportion as the availability of those conditions. If distribution of offspring were random, then the sexual mother's fitness would be equal to that of asexual mothers that produce the same number of offspring adapted to one of the available conditions (Williams 1975). This is because all seeds have an equal chance of landing in patches to which they are not well adapted.

Usually, a given site contains more than one seed, and seedlings will compete with each other. In *Taraxacum*, for example, a species with sexual and asexual forms, seed production can be over 50.000 m^{-2} (Sterk 1987b), so many seeds will fall close to each other, and only few will survive to adulthood. Under competition the situation becomes different from the simple models. Possibly, the best adapted genotype in a patch will acquire a disproportionate part of the available resources and have the highest fitness. When competition is very severe, only the best competitor will survive or reproduce. In a spatially variable environment, the sexuals will have an advantage if the number of competitors per site is high, relative to the number of survivors (Williams 1975;Bell 1982). This is because if the number of seeds produced for each patch is large enough, the sexual mother has a good chance that she also produces the best adapted genotype for that patch. In this model, the fitness of an individual depends strongly on the density and identity of conspecifics in the patch. This is called soft selection.

The other combinations, fluctuations in time combined with soft selection and spatial heterogeneity with hard selection have not been discussed as much in the literature. Spatial variation in the environment, combined with hard selection, as argued above, will result in equal fitness for sexual and apomictic mothers. Environmental fluctuations in time, combined with soft selection, will benefit sexual mothers under about the same conditions as for spatial heterogeneity, though again the geometric mean fitness over generations rather than the arithmetic fitness is the right fitness measure.

So, in summary, producing genetically variable offspring can be advantageous under different models. One option is when the environment is heterogeneous in space, dispersal of seeds or juveniles is random, and very strong soft selection acts. The other option is if the environmental conditions fluctuate randomly over time and selection is hard. Environmental fluctuations over time with strong enough soft selection will also benefit sexual mothers.

In *Taraxacum*, dandelion, two reproductive types exist: sexual and apomictic. This makes it a convenient species to study the consequences of producing genetically variable offspring. Seed production can be very high, so in the field, competition among seedlings is likely to occur. Whether under natural conditions the assumptions of any of the models are met, though, is not known. We set up experiments with *Taraxacum*, simulating natural conditions, to test some of the assumptions and predictions of both models. We compared the performance of genetically diverse arrays of *Taraxacum officinale* Wigg. with the performance of genetically identical arrays under four different environmental conditions. Two set-ups were employed: in the first set-up sibships from sexual mothers were compared to those of asexual mothers under a number of conditions. The progeny of a sexual mother are probably half-sibs; the progeny of an apomict are all genetically identical. In the second set-up, we compared genetically diverse arrays of apomicts with genetically identical arrays. In this last set-up no confounding effects of sexuality, other than genetic variation among offspring, would disturb the effect of genetic variation.

We assumed that biomass production per patch would be correlated with the average fitness in a patch, since previous studies have shown a relation between dry weight, and survival and seed production (Welham & Setter 1998;Van Loenhoud & Van der Heijden 1980).

In the extreme case, selection is purely soft; that is, in any patch the best genotypes survive, no matter how good or bad this best genotype is. After selection, the number of surviving individuals in each patch is the same and their fitness as well, no matter whether the initial group were genetically variable or genetically identical. Within each patch, one would expect larger variation when different genotypes are present than when all individuals have the same genotype, and thinning would probably be more rapid.

On the other end is purely hard selection. This means that the fitness of a genotype depends solely on the environmental conditions and not on the genetic identity of conspecifics within the patch. In this case, genetically variable combinations will show less variation in fitness between conditions than genetically identical combinations. If conditions vary from one generation to the next, the appropriate fitness measure would be the geometric mean fitness over all conditions. In that case, genetically monomorphic groups, which assumedly react more strongly to variation in environmental conditions, would have a lower fitness, and mothers that reproduce asexually would be at a disadvantage.

So, we measured growth of the individual plants in the pots containing many seeds, and the total biomass they produced together. From these data we calculated the variation within pots, the arithmetic and geometric mean biomass production over treatments. For each group we calculated Levin's B (Levins 1968), a measure showing how strongly treatments, or environmental conditions, will affect growth.

Material and methods

Taraxacum officinale Wigg (Asteraceae), dandelion, is a perennial, herbaceous species, common to open habitats. Triploid cytotypes reproduce apomictically, whereas diploid cytotypes reproduce sexually. In the Netherlands sexuals are rare and most populations consist solely of apomictic, triploid types, but a few mixed sexual/apomictic population are known.

Seeds from sexual and apomictic plants from a mixed population situated on the banks of the river Rhine, near Wageningen, the Netherlands (5°40' E, 52°00' N) were collected by the NIOO in Heteren. The ploidy level of the mother was determined with a flowcytometer. For the second experiment seeds from triploid, apomictic dandelions were collected in an all-triploid population situated in a wet meadow in Utrecht, the Netherlands (5°10' E, 52°05' N). With the use of microsatellite primers (Falque *et al.* 1998) 11 microsatellite-phenotypes (further called genotypes) were selected from this collection for use in the second experiment.

Comparison sexual and apomictic sibships

On 14 October 1997 twelve seeds from each of twelve sexual and twelve apomictic mothers were sown in 11x11x12 cm pots, filled with potting soil. Each family was represented once per treatment. Four treatments were applied, simulating variation in natural conditions that can occur in the field: shading-wet, shading-dry, light-wet and light-dry. The shaded treatments consisted of a cage covered with green foil that was put over the pots. The light treatments had a cage covered with transparent foil. In the green cage PAR was about 40% of that in the transparent cage. Red:far red ratios were 1:4.2 in the green cage, and 1:1.6 in the transparent cage, varying somewhat with the time of day. Wet treatments received 90 ml tap water three times a week, which was enough to saturate the soil; dry treatments received 20 ml twice a week and 30 ml once a week. Temperature in the greenhouse was 15°C during daytime for the first 6 weeks of the experiment and 20°C during the remainder of the experiment; night temperatures were 14°C (day: night=16:8). Germinated seeds were counted every other day; shoot length was measured once a week. After 17 weeks, February 1998, the experiment was harvested. Shoots were harvested individually, whereas roots were collected per pot as they could not be disentangled. Leaf area was measured on all fresh leaves per pot. Shoots and roots were dried at 70°C for at least 48h and weighed.

Monocultures and mixtures of all-apomictic genotypes

Seeds from eleven apomictic mothers were sown in pots of 11x11x12 cm, filled with a 1:1 mixture potting soil: sand mixture. Either 30 seeds from the same genotype were sown (monoculture) or a mixture of 30 seeds was sown in which each genotype was represented 3 or 2 times. Monocultures were represented twice per treatment for each genotype, whereas the mixture was represented seven times per treatment. The pots were placed on benches under a transparent roof, where light and temperature conditions followed the outside. Pots with seeds were subjected to one of four treatments: light-wet, light-dry, shade-wet, shade-dry. In the 'light' treatments pots were placed under a cage covered with transparent foil. This foil reduced PAR to 70% and red: far red ratio was 1:1.5±0.1. In the shade treatment the cages were covered with green foil. This reduced PAR to 45% and red: far red ratio in the cage was on average 1:4.9±1.3. In the wet treatments the pots received 390 ml water per week; this was enough to saturate the pots. In the dry treatments the pots received around 135 ml a week. Because the green foil reduced evapotransparation, the soil in the shaded treatments frequently was wetter than in the light treatments. Nutrients were added as N:P:K = 25:10:20 plus micro-elements at a dose of 0.9 g N m^{-2} wk^{-1}.

The number of plants per pot was counted regularly: three times per week at the beginning of the experiment, once every two weeks near the end of the experiment. During the experiment plants were attacked by aphids, thrips and mites, which were controlled with natural enemies, and by fungus, which was controlled by chemical means. The experiment lasted from 12 March 1998 until harvest in November 1998. At this time, autumn had begun and leaves had begun to die back. At harvest, the plants were removed from their pot and separated into taproot and leaves. The fine roots from the individual plants could not be disentangled, so these were harvested per pot. Plant parts were dried for at least 48h at 70°C and weighed.

Data analysis

sexuals vs apomicts– To test whether germination, infection with fungus, and survival of germinated seeds were independent of reproductive mode, a Chi-square test of independence was used. Repeated measurements analysis was used to study effect of ploidy and treatment on leaf length.

The total leaf area per pot and the total dry weight per pot were log-transformed before analysis as this improved normal distribution of the values. The effect of reproductive mode and treatment on these values were analysed with ANOVA.

The equability measure Levin's B (Levins 1968) was calculated for total dry weight per pot as $B = \frac{1}{S * \sum_{i=1}^{S} p_i^2}$ with $p_i = \frac{dw_i}{\sum_{j=1}^{S} dw_j}$. in which *dw* is the total dry weight per pot. This value will be 1 if the performance of the family is the same in all (S) conditions and close to 0 if performance varies hugely with treatment. B-values for sexual and apomictic families were compared with the Mann-Whitney U-test.

Arithmetic and geometric mean dry weight over treatments were calculated from the data and compared with ANOVA with reproductive mode as a fixed factor.

Variation within pots was examined after log-transformation of individual shoot weights. Because variation depended on the mean, the coefficient of variation (s.d. / mean), based on these log-transformed shoot weights, was used as a measure of variation. These c.v. values were compared with ANOVA with treatment and reproductive mode as fixed factors.

Whether families within a reproductive mode differed from each other in germination probability, growth and other traits was tested with ANOVA separately for both reproductive modes, treatment as a fixed factor and family as a random factor.

All apomicts

Most tests were similar to those described above with monocultures and mixtures substituted for asexual and sexual families.

Differences between the germination rates of the different genotypes were analysed with a Kruskall-Wallis test. Differences in number of germinated seeds were analysed with ANOVA, as the data seemed to meet the requirements.

Dry weights per whole pot in the second experiment were not log-transformed before analysis as this transformation did not improve normal distribution. Because each family was represented twice per treatment, not just main effects of family, but also interaction with treatment could be tested with ANOVA. Differences between genotypes in shoot, taproot, fine root, and total dry weights per pot were analysed using ANOVA with treatments as fixed factor and genotype as random factor.

Dry weights of individual plants were log-transformed prior to analysis, as this improved normal distribution. Variation within pots was expressed as coefficient of variation, as described above and tested with ANOVA.

Linear Pearson regression was used to analyse whether there was a relation between the biomass per pot and the number of surviving plants.

Values for Levin's B, based on total dry weight per pot, were calculated after randomly assigning replicates to the same series.

Results

Sexuals and apomicts

Treatment effects– In total, 63% of the seeds germinated. Treatment influenced the number of germinated seeds significantly (Kruskall-Wallis, $p<0.05$ for apomicts and sexuals). More seeds germinated in the wet treatments than in the dry treatments. Leaf length and leaf area were affected by treatment ($p<0.05$). The highest leaf area was obtained in the light-wet treatment. Dry weights per pot were similarly influenced by treatment ($p<0.05$). In the light-wet treatment dry weights were about ten times as high as in the most stressful dark-wet treatment (Fig. 1).

*Reproductive modes—*At the end of the experiment significantly fewer apomictic than sexual seeds had germinated ($p<0.05$), 5.87 vs 9.25 seeds out of twelve respectively. However, one of the apomictic genotypes had not germinated at all. After exclusion of this genotype, the germination of apomicts was 6.44, still significantly lower than in sexuals ($p<0.05$). More apomictic seeds suffered from fungus infections than sexual seeds ($p<0.05$), but it is unclear whether this was cause

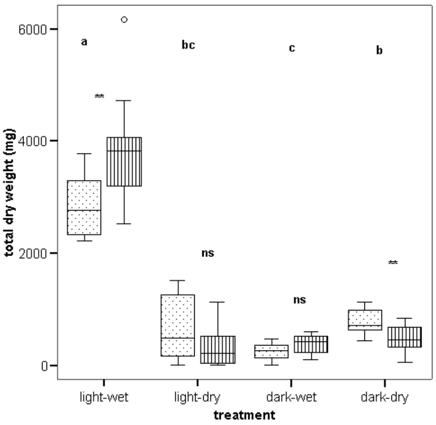

Figure 1. Dry weight per pot (g) of sexual (dotted) and apomictic (striped) *Taraxacum* families at harvest. Boxplots contain 50% of the data, horizontal line shows the median, and whiskers extend from minimum to maximum except for outliers.

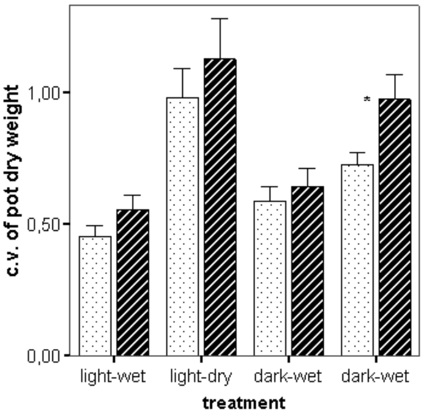

Figure 2. Average coefficient of variation (s.d. / mean) of shoot dry weight within pots containing sexual (dotted) or apomictic (hatched) *Taraxacum* families at four treatments. Error bars show 1 s.e. of mean. * : $p<0.01$.

or effect of worse germination. Out of 726 germinated seeds, 50 plants died in the course of the experiment, but survival did not differ between sexuals and apomicts ($p=0.229$).

The apomictic plants had longer leaves than sexual plants during the course of the experiment in the wet treatments ($p<0.05$), but no significant differences were found in the dry treatments (treatment x reproduction interaction: $p<0.05$). On average sexuals had a higher leaf area at harvest than apomicts ($p<0.05$), but this was due only to the dry treatments as there was a significant interaction between reproductive mode and treatment ($p<0.05$). Total dry weight at harvest did not differ significantly between sexuals and apomicts ($p=0.265$). Again, though, a significant interaction was found between reproductive mode and treatment ($p<0.05$) with the sexuals having a higher dry weight than the apomicts in the dry treatments (Fig. 1).

Family differences– Total dry weight per pot differed significantly among sexual families ($p<0.01$), but not among apomictic families ($p=0.243$).

Within pot variation– The within pot variation for shoot dry weight, expressed as coefficient of variation, was higher in apomicts than in sexuals by 10-15% ($p<0.01$) (Fig. 2).

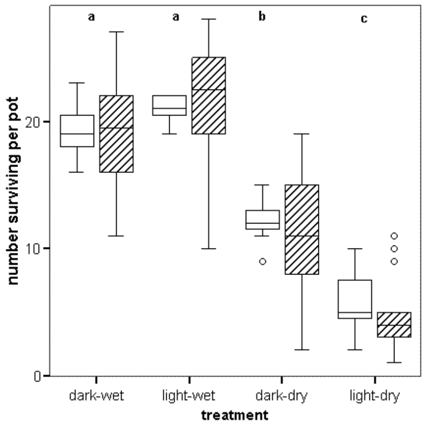

Figure 3. Average number of surviving plants per pot. Pots were sown with either 30 seeds of the same genotype (clear) or 30 seeds from 12 different genotypes (hatched) of apomictic *Taraxacum* and subjected to four treatments. Boxplots contain 50% of the data, horizontal line shows the median, and whiskers extend from minimum to maximum except for outliers.

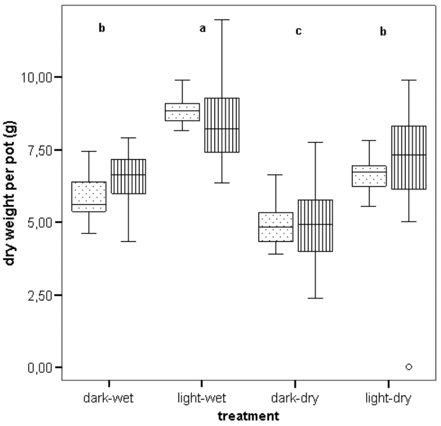

Figure 4. Average biomass per pot at harvest. Pots were sown with either 30 seeds of the same genotype (dotted) or 30 seeds from 12 different genotypes (striped) of apomictic *Taraxacum* and subjected to four treatments. Boxplots contain 50% of the data, horizontal line shows the median, and whiskers extend from minimum to maximum except for outliers.

Treatments combined– As could be expected from the fact that total dry weights did not differ between sexuals and apomicts, the arithmetic mean performance over the treatments did not differ between the reproductive modes (p=0.427). Though the apomicts showed a stronger response to treatments than the sexuals, the geometric mean of the total dry weight did not differ between sexuals and apomicts (p=0.265). The ratio between geometric and artihmetic mean was, however, higher in sexuals (i.e. closer to 1) than in apomicts, suggesting the sexuals had a more even biomass production over treatments. The equability measure Levin's B for total dry weight was on average 0.54 for the sexuals and 0.42 for the apomicts. The sexuals had a significantly more even performance than the apomicts (MWU, p<0.05).

All-apomictic mixtures and monocultures

Treatment effects— The first seeds germinated six days after sowing, and seeds were still germinating in the week of the harvest. Of all the seeds in the experiment, 58% germinated, ranging from all 30 in a pot with a mixture in treatment light-wet, to only 1 in one of the pots of genotype 41 in treatment light-dry. Nineteen per cent of the seeds that had germinated, died during the experiment, usually rather quickly after germination. Survival depended on treatment ($p<0.05$), being around 90% in the wet treatments and around 64% in the dry treatments.

The numbers of plants at harvest varied significantly between treatments ($p<0.05$). In the wet-shaded treatment the average was 19±4, in wet-light 21±5, in dry-shade 11±4 and in dry-light only 5±3 (Fig 3.).

The total dry weights per pot were influenced by the treatment, with the highest average dry weight in treatment light-wet and the lowest in treatment shade-dry. Treatment also affected the distribution of biomass over taproots, fine roots and shoots (treatment effect on leaf weight ratio: $p<0.001$, on taproot weight ratio $p<0.001$). Most biomass was present in roots in the light-wet treatment, least in the dark-dry treatment (Fig 4).

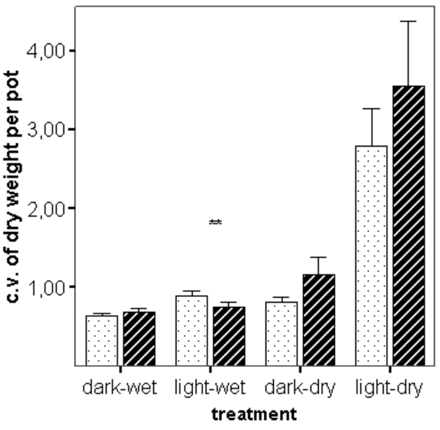

Figure 5. Average coefficient of variation (s.d. / mean) of plant dry weight within pots. Pots were sown with either 30 seeds of the same genotype (dotted) or 30 seeds from 12 different genotypes (hatched) of apomictic *Taraxacum* and subjected to four treatments at four treatments. Error bars show 1 s.e. of mean. * : $p<0.01$.

Monocultures vs mixtures— Germination and survival did not differ between mixtures and monocultures ($p=0.916$ and $p=0.20$). Germination rates also were not affected ($p=0.754$). Neither did the factor mixture/monoculture affect total dry weight per individual surviving plants ($p=0.829$) or per pot at harvest ($p=0.890$). Number of surviving plants did not differ between monocultures and mixtures either ($p=0.776$).

Genotype differences— The total number of seeds that germinated differed between genotypes ($p<0.05$). Germination rates also differed among genotypes (Kruskall-Wallis, $p<0.05$), but survival did not ($p=0.265$). At harvest, therefore, the number of surviving plants differed between genotypes. There were no significant genotype x treatment interactions.

No overall differences in total dry weight were found between genotypes ($p=0.071$), but biomass distribution over roots and shoots varied among genotypes. A significant genotype effect was found for the ratio of tap-root: total dry weight and for fine root: total dry weight at harvest (both $p<0.05$). Though average biomass production was not different, genotypes differed in their response to the different treatments, as was indicated by a significant genotype x treatment interaction

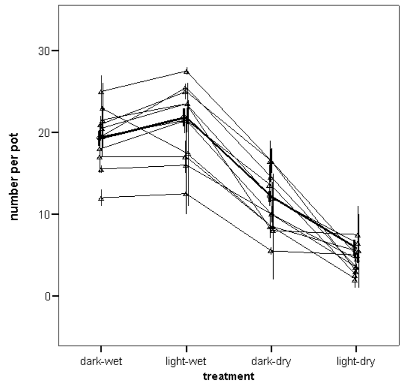

Figure 6. Average dry weight (g) per pot at harvest of pots containing plants of one single genotype. Lines connect pots containing the same genotype at four different treatments. Fat line with circles connects pots containing a mixture of genotypes. Error bars show 1. s.e. (n per genotype = 2).

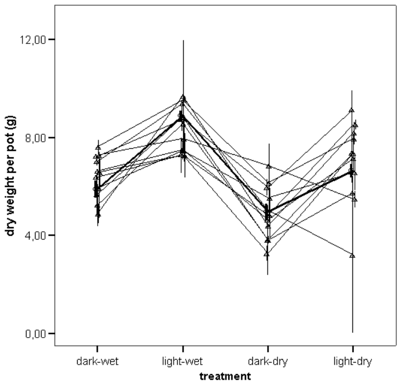

Figure 7. Average number of surviving plants per pot at harvest of pots containing plants of one single genotype. Lines connect pots containing the same genotype at four different treatments. Fat line with circles connects pots containing a mixture of genotypes. Error bars show 1. s.e. (n per genotype = 2).

for total dry weight ($p<0.05$). Also for the ratio shoot: total dry weight (LWR) a significant genotype x treatment interaction was found ($p<0.05$), showing differences in plasticity for this ratio.

Within pot variation–The variation among plants within the same pot did not significantly differ between monocultures and mixtures, except for the treatment wet-light, where the monocultures showed significantly less within-pot variation than the mixtures (Fig 5).

The total dry weights per pot did not significantly depend upon the number of surviving plants per pot, suggesting that the available resources were limiting growth ($p>0.05$ in all treatments)

Treatments combined— Both the arithmetic mean and the geometric mean over all treatments for total dry weight per pot did not differ between mixtures and monocultures (MWU, $p=0.308$ and $p=0.285$ resp.) indicating that mixtures did not react differently relative to single genotype arrays under the conditions of this experiment. Geometric and artihmetric mean for total pot dry weight did not differ much from each other. The average value for the equability measure Levin's B for total dry weight was 0.83 for the mixtures and 0.84 for the monocultures. This B-value varied from 0.74 to 0.92 for the different genotypes. So, mixtures and monocultures did not differ in evenness of performance, and some of the genotypes had a more even performance than the mixtures (Fig 6 & 7).

Discussion

Comparison sexuals and apomicts

Our first experiment showed interesting differences between sexual and apomictic sibships. Apomicts had fewer germinating seeds and longer leaves, but the most interesting difference within the framework of this experiment, was in their response to the treatments. The apomicts were more highly specialized, as was shown by their lower Levin's B value. As a group, they achieved a higher biomass than sexuals under wet conditions, but lower under dry conditions. This pattern, as well as the results from the second experiment, suggests that these differences were not due to the fact that sexual sibships are genetically variable, but to other differences between sexuals and apomicts. Possibly the differences were due to the difference in ploidy level. Surveys of the distribution of apomictic and sexual dandelions in the Netherlands have shown that sexuals have a slight (xero)thermic affinity relative to apomicts (Elzinga *et al.* 1987;Roetman *et al.* 1988). Yet, apomicts in these surveys had a wider ecological range than sexuals (Elzinga *et al.* 1987); they occurred in the same sites as sexuals as well as in additional sites.

Though interesting, these results cannot answer whether in the field one or the other models described in the introduction applies. We will therefore focus on the second experiment.

Mixtures and monocultures of apomicts

Whether sexual or asexual mothers will be better at using a heterogeneous environment, depends on whether the environment is variable in space or in time, and on whether selection is soft or hard. In the current experiments, the mode of selection was not clearly one type or the other. Number and biomass of surviving plants differed between treatments, so selection was not purely soft. Total biomass per pot was independent of the number of surviving plants, which is indicative of competition. The variation within pots was, however, significantly larger in mixtures than in monocultures for only one out of four treatments. Thinning as a consequence of competition was not very severe: overall mortality was around 19%. The main process seemed to be that the more individuals survived hard selection, the less resources each individual survivor could obtain, without much variation among survivors.

For sexual mothers to perform absolutely better than individual asexual mothers in a spatially variable environment, the best-adapted genotype within a patch (or treatment) would have to profit disproportionally from its well-adaptedness. The highest advantage would be obtained if this genotype were the sole survivor in the patch and able to monopolize all the resources (Bell 1982). Such severe selection did not occur within the timespan of our experiment. In an experiment with plant species *Anthoxanthum odoratum,* specifically designed to test this lottery model, severe selection did take place, but not down to one individual per patch, and no differences in total fitness between sexually and asexually produced offspring were found (Kelley 1989).

When conditions fluctuate in time, mothers producing variable offspring will have an advantage if the geometric mean fitness of mixed offspring is higher than that of genetically identical offspring. This requires that genotypes specialise on certain environmental conditions, and perform worse at others. Different genotypes should specialise on different environmental conditions, and the differences between the performances of genotypes under any condition should be relatively large. An other assumption of the model was that selection is hard. Under the conditions of the experiment, we observed genotype*environment interactions for dry weight, which suggests that genotypes specialised on different conditions, but all genotypes produced more biomass in the light-wet than in the dark-dry treatment. Levin's B values varied, which shows that, with respect to the conditions of the experiment, some genotypes were probably more specialised than others. Different treatments had different numbers of surviving plants and different amounts of biomass at harvest for each genotype. This means that hard selection acted, but not exactly in the way required by the model. As argued above, selection also had a 'soft' component. Mixtures did not have a significantly higher geometric mean fitness than monocultures. The reason most likely was, that the differences between genotypes within treatments, though significant, were not very large: maximally 3-fold.

These results show that conditions for a sexual organism to perform better than an asexual organism in a heterogeneous environment are not so easily met. The different genotypes of apomictic dandelions in the second experiment were ecologically different. The same was true for the sexual families in the first experiment. The maintenance of such genetic variation, and with this the maintenance of sex and recombination remains puzzling. From our experiments, no clear advantage for genetic variation emerged.

Dandelion life-span, however, is much longer than the usual duration of experiments. As a perennial species, *Taraxacum* will flower a number of times, while rooted in the same spot. The disproportional advantage needed in the lottery models may not result from viability selection in early life stages, but may accumulate over time. The variation in seed output in *Taraxacum* from one individual to the next can be a factor twenty or more in a single season (Sterk 1987b).

Also, the models assume random environmental variation in the environment. This may not be true from the point of view of the plants. Dispersal of seeds in space occurs, even in dandelions, over very limited distances (Sheldon & Burrows 1973). Seeds will land close to their parents, so probably in conditions similar to their parents' environmental conditions. From one generation to the next, the advantage of being well-adapted to the local conditions can multiply. This is the process that is assumed in models as 'the Frozen niche variation' that predicts co-existence of clones (Vrijenhoek 1984;Weeks 1993). Environmental fluctuations in time may also, from the point of view of dandelions, not fluctuate randomly from one generation to the next, also because these are perennial plants.

Acknowledgements

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Chapter 9
General Discussion

General Discussion

Introduction

Aim of the thesis

This thesis is meant to contribute to our understanding of the significance of sex for evolution of eukaryote organisms. The main question of this thesis has been whether the different ways in which genetic variation is generated and maintained in sexual and apomictic *Taraxacum* has caused those two different types to adapt to local conditions in different ways. It was suggested in the *Introduction* that reproductive assurance and independence of pollinators in the apomicts might also add to evolution of different strategies in the two types. Because sexual dandelions are diploid, whereas apomictic genotypes are triploid, an effort was made to show which differences between the two dandelion types can clearly be attributed to their differences in reproductive modes and which differences can also be the consequence of the ploidy differences.

Outline of discussion

This general discussion will first explore the genetic variation in sexual and apomictic dandelions. I will try to deduce what processes can be responsible for the origin and maintenance of genetic variation, in particular the type of selection that has acted on the sexual and apomictic population. Next, differences and similarities in mean trait values and plasticity between sexual and apomictic genotypes will be treated. I will consider whether differences are the result of selection or of other processes. If they are the result of selection, the question is whether they represent different degrees of 'adaptedness', or different strategies of the reproductive systems to cope with the environment, or whether they have other causes. One possible cause, polyploidy, is treated in more detail. So far, the discussion has tried to answer whether the mode of reproduction has an effect on microevolution of dandelions.

The last part of this discussion is my personal interpretation of the dynamics of this system, and is necessarily more speculative.

Genetic variation

Origin of genetic variation and possibility for evolution

The main question of this project has been whether asexual genotypes adapt to local conditions in different ways than sexuals do. For evolution to proceed, a population must generate sufficient heritable variation in the traits that matter. In sexual populations, usually, a large amount of genetic variation is maintained, though by what mechanism is still not wholly understood. In apomictic dandelions, genetic variation can be created through backcrossing events between sexual and apomictic plants. New genotypes contain a diploid chromosome set from their apomictic father and a haploid set from their sexual mother. Mutation can also create variation. Studies with neutral AFLP markers indicate that most of the genetic variation among apomictic dandelions is attributable to recombination events rather than to mutation (Van der Hulst *et al.* 2000). This suggests that most new genotypes result from backcrossing, either recently or some time ago.

Backcrossing occurs in mixed populations. Chapters 5 and 6 describe the variation that can be naturally produced by such crosses. The range of phenotypes was in the same order in those newly originated apomicts as in the sexuals, and the variation had a large heritable component. Selection could, thus, readily choose from among the variation that was generated, but the appearance of more extreme phenotypes is probably a rare event.

Some proportion of the new triploid, apomictic genotypes that are generated by backcrossing have developmental problems in apomictic seed production (Chapter 5*)*. These will not establish in the field. Among the genotypes that produce seeds well, genotypes exist with different leaf length, leaf number, biomass allocation, phenology, seed weight, ovules per capitulum, speed of germination, and the degree of plasticity in those traits (Chapters 5*,* 8*)*.

Standing genetic and phenotypic variation in sexual and apomictic dandelion populations

Theoretical expectations

Selection in a static environment will most likely select for a single optimal value of any phenotypic trait that is related to fitness. In sexual populations, the additive genetic variation for this trait will be reduced by selection. Genetic variation in the population can be maintained by other components of the genetic variation, such as dominance variance or pleiotropic effects among loci, as well as by mutation. In equilibrium, a mutation-selection balance will be established, the width of which is determined by the mutation rate, the effects of mutations on the phenotypic value, and the relative fitness of deviant phenotypes.

In asexual populations, selection does not solely act upon the additive component of variation, but on all genetic variation, and this may have different outcomes for the mean trait value as well as the distribution (Templeton 1982). Since the non-additive part of genetic variation can be eroded as well, and eliminated genotypes will not readily be recreated by recombination, the phenotype distribution in equilibrium is likely to be narrower in asexual communities, if all other things are equal.

In a spatially heterogeneous environment, with different optima for phenotypic traits, variation can be maintained by immigration of genotypes if heterogeneity is on a smaller scale than dispersal. Heterogeneity in space can also induce purely phenotypic variation in a community, because of the plastic response of many plant traits to environmental conditions (Schmid 1992), even if no genetic variation is present.

Models, such as the frozen niche model and models based upon it, predict that the distribution of asexuals will more closely follow the distribution of available niches than does the distribution of a sexual population (Roughgarden 1972). Transient dynamics may show a distribution with asexuals better represented than sexuals in the more extreme classes (Case & Taper 1986;Weeks 1993).

Models of temporal variation in the environment have predicted that a directional moving optimum, such as for instance climate change can induce, can result in a sexual populations with trait distributions that have an average closer to the optimum than asexuals as well as a higher variation (Bürger 1999).

Genetic and phenotypic variation in sexual and apomictic dandelions

In chapters 3 and 5 it was shown that asexual and sexual *Taraxacum* populations from the same locality had similar broad-sense heritability values for morphological traits such as biomass distribution and leaf length, as well as for life-history traits such as seed weight or seed number per capitulum. For leaf number and total dry weight in spring the apomicts even seemed to have higher h^2 values than sexuals (Chapter 3*)*. The amounts of phenotypic variation for those traits, measured as coefficients of variance, were also very similar for sexuals and apomicts. Visual inspection of histograms of phenotypic variation from the experiments described in chapters 4 and 6 also revealed no striking differences between sexual and asexual samples. Possibly apomictic samples showed a slightly narrower distribution, but not for all traits.

Explanations for the variation pattern

The patterns of phenotypic variation and its heritable component in sexual and asexual samples of *Taraxacum* from the same locality suggest that there is no strong selection for a single optimum for the traits that were measured. The high heritability values for some traits and the about equally wide trait distributions in sexuals and apomicts might be explained if the traits that were measured were close to selectively neutral.

Earlier studies, though, have shown that root:shoot ratio, leaf length, specific leaf area (SLA) etcetera in dandelions and other plants are associated with occurrence or success in certain habitats (Hommels *et al.* 1991;Hommels 1991;Solbrig & Simpson 1977;Solbrig & Simpson 1974;Vavrek 1998;Sterk 1987b;Mølgaard 1977). A change of management of a field will bring in genotypes with different values for those traits (Sterk 1987b). In the experiment described in chapter 8*,* for example, genotypes with a low leaf weight ratio (lwr) produced a high biomass in both unshaded treatments, but not in the shaded treatments. Neutrality of those morphological traits seems therefore unlikely.

Neutrality of life-history traits such as capitulum number, seed number, and seed weight is hard to accept. Natural selection will strive for the highest possible values of those, and only trade-offs with other functions will prevent them from ever increasing. Trade-offs may have different outcomes in different conditions, and thus may maintain heritable variation for these life-history traits. These traits are highly plastic (Sukatschew 1928) and may have different optimal combinations under different conditions. Though phenotypic variation for all life-history traits was present, h^2 values for capitulum number were very low (Chapter 5*)*.

Comparison of newly generated with established apomictic genotypes (Chapters 5 & 6*)* also showed that on most of the measured morphological and life-history traits little selection acts at establishment of apomicts in the field. Selection was more apparent under shaded conditions. Longer leaves and heavier seeds, as well as a small shift in flowering time, all under shaded conditions, were selected for in apomicts (Chapter 6*)*. Yet, considerable variation was maintained among the established apomicts.

The patterns described above suggest to me that neither sexuals nor apomicts experience, or have recently experienced, strong selection for a single trait value for most of the measured traits, though the traits are unlikely to be neutral. Rather, environmental heterogeneity, most likely spatial heterogeneity (Hedrick 1986)& Chapter 2*,*selects for different genotypes and thus maintains genetic variation in sexual and apomictic *Taraxacum* communities. Directional change in the environment is likely to eliminate variation, similar to the way balancing selection for a single optimum does. Random fluctuations in the environment in time are unlikely to maintain genetic variation, unless additional variation in space is present (Chapter 2*)*.

Therefore, I think spatial variation is more important for the evolution and maintenance of dandelion diversity within a single site than other types of environmental heterogeneity.

Differences in traits between sexual and apomictic dandelions

Differences between sexuals and apomicts

Sexual and apomictic *Taraxacum* differed from each other in a few characteristics. Sexuals had smaller cells than apomicts (Chapter 5*).* In most conditions, that is, during the greater part of the season, sexuals have more leaves than apomicts (Chapter 5*).* All *Taraxacum* elongate their leaves when shaded and when days grow shorter, but apomicts did this much stronger than sexuals (Chapters 3*,* 4 and 5 and 8*).* In spring, when days grow longer, apomicts have shorter leaves than sexuals (Chapters 3 and 5*).* Combined, this means that apomictic genotypes had a more plastic leaf response to shading (Chapter 3*).* Apomicts also positioned their leaves more upright when shaded than sexuals do (Chapters 3 and 4*).* Under wet conditions, sexuals had a higher proportion of their biomass in leaves than apomicts, whereas under dry conditions, they had a lower proportion of their biomass in leaves (Chapter 8*).* Probably associated with the differences in leaf characteristics, sexuals produced more biomass than apomicts under dry conditions, though not under wet conditions (Chapter 8*).* When less biomass is present in leaves, the respiring leaf surface is smaller, and water loss is reduced. Water deficits, even short-term, have detrimental effects on growth processes (Larcher 1995). In addition, plants with many small leaves, such as the sexuals, instead of a few large leaves are likely to have a better water use efficiency (Dudley 1996).

Apomicts, on the other hand, produced more biomass in one of the common garden experiments (Chapter 4*).*

In the field, as well as in most experiments, sexuals and apomicts differed a few days in flowering. In the field, sexuals usually are a few days earlier than apomicts, but in the experiments it varied. This probably means the two types of dandelions differ in the way they interpret environmental cues.

Apomicts had heavier seeds than sexuals in one of the common garden experiments (Chapter 3*)*, though not in other experiments (Chapter 4 and 5*).* The results of one of the experiments suggested that seeds of apomicts and sexuals had different plastic responses to environmental conditions (Chapter 4*).* Weight is usually positively correlated with the probability of a seed to germinate (Tweney & Mogie 1999), Chapter 5.

The size of the average differences was maximally around 60% of the mean trait value (for leaf length in shade) and the overlap of the distributions between sexuals and apomicts was around 30% (for cell size) or more.

No differences on the other hand were found in the number of capitula per plant, or the number of ovules per capitulum, though these traits are highly plastic and varied with treatments (Chapter 3, 4 and 5).

Summarizing, sexuals and apomicts differ in some of the studied traits, in particular morphological traits; the differences seem more pronounced under shaded conditions. Some of these morphological differences enable sexuals to grow better in dry conditions than apomicts. Possibly, the apomicts grow better in shaded environments.

Coping with environmental heterogeneity

In the previous sections, some differences between sexuals and apomicts were discussed. Now I will review whether these differences imply different ways of coping with heterogeneous environments~~.~~ In a previous section, I have argued that the most important source of environmental heterogeneity for the studied dandelion populations, on the short-term, is variation in space. Growth of sexual phenotypes was less affected by differences in soil moisture content than was growth of apomictic genotypes. On the other hand, apomictic genotypes reacted more strongly than sexual genotypes to shading with changes in leaf morphology. This, however, did not result in significantly higher biomass or higher seed production than that of sexual genotypes under shaded conditions rather than under full light conditions, though the trend was in this direction. Tentatively we could say that all the sexuals were more tolerant to drought than the apomicts, whereas they were less tolerant to shading. I suppose that shade and soil moisture content would vary on similar scales in time and space, so no general answer can be given about environmental tolerance of sexual and apomictic genotypes.

When the main component of environmental variation is variation in space, the theoretical expectation was that sexual and apomictic genotypes would be equally tolerant. If the environmental conditions show some correlation over space and dispersal is limited, a version of the Frozen Niche Model could apply. In that case more specialised apomicts are possible under certain soft selection regimes (Chapter 2, but see discussion in Chapter 8), but are not the inevitable result of the dynamics. Equal degrees of specialisation in sexuals and apomicts are therefore not surprising.

So far, we have seen that sexual and apomictic dandelions are not entirely identical. The main source of environmental heterogeneity seems variation in space.

This type of variation is not expected to lead to differences between sexual and asexual genotypes, except possibly with respect to niche width. Exceptions are when the system is not in equilibrium, but when apomicts are invading (Weeks 1993), but for this particular system, this does not seem the most likely scenario. Directional selection could lead to differences between sexuals and apomicts, but the variation patterns do not carry the hallmark of such selection.

Sexual and apomictic dandelions differ not just in their mode of reproduction, but also in ploidy level. Before considering more complicated scenarios, I will discuss whether this ploidy level difference accounts for the differences we found between sexuals and apomicts.

The effect of ploidy level

Sexuals are diploid and apomicts are triploid. It has been shown many times before that ploidy level can significantly affect cell size, enzyme activity and many other traits (Tal 1980;1979;Levin 1983). Two of the experiments described in this thesis were aimed at disentangling ploidy effects from selection. The conclusions from these experiments were that the difference in ploidy level was responsible for differences in cell size, as expected, and probably at least in part for differences in leaf number and leaf length, as well as timing of flowering. Selection on apomicts was responsible for leaf elongation in shade, phenology differences in shade, and possibly selection acted on apomicts to have more leaves, so to have a trait value closer to the sexuals.

Why sexuals and apomicts differ

For many of the measured traits, the distributions of sexuals and apomicts overlapped. If one would assume that sexuals are as well adapted as possible, one could wonder why selection does not drive apomicts towards the same values. Even when ploidy level differences cause differences between the average morphology and life history of sexuals and apomicts, selection could drive the apomicts back. Several explanations can be thought of why this does not happen.

a) Competitive interactions between sexuals and apomicts favour niche divergence. In a special case of this, the frozen niche variation model (Vrijenhoek 1984) could apply: more extreme clones have a higher probability of establishing. Competitive interactions between dandelions are not unlikely. High numbers of seeds are produced every year. These perennial plants can produce several hundred seeds per season per individual, of which on average, obviously, less than one will survive to reproduction. Apomictic clones that show little overlap in niche with established clones or with the sexual population may have a higher probability of establishment. If the conditions of the frozen niche variation model apply, these are likely to be clones at the edge of the sexuals' distribution (Weeks 1993).

b) Selection acts slower than the generation of new apomictic genotypes. New apomictic genotypes are formed by backcrossing, or, more rarely, by mutation. If survival and fecundity under local conditions are high enough, they will, with some luck, establish themselves in the population. If they do better than older lineages, then their numbers will increase, the numbers of the older lineages will decrease, if population numbers are constant, and the older lineages will eventually go extinct. The differential rates of origin, expansion, and extinction will determine the number of genotypes as well as their average phenotype. The rate of origin of new apomictic genotypes is unknown. Preliminary studies in the same populations as were used for this project have shown that sexual mothers produced around 2% triploid offspring (pers. comm. P.J. van Dijk) in this mixed population. Rates of extinction are unknown.

c) Selection does not favour individual traits, but suites of characters. If some of the traits are out of range and cannot be moved by selection into the realm of the sexuals, other traits must be in accordance with those to form a co-adapted complex. Some combinations of, for example, leaf size, specific leaf area, root: shoot ratio, and timing may specify a local fitness optimum, whereas other combinations may not. Whereas most of the traits studied in this thesis showed considerable overlap between sexuals and apomicts, for some traits this overlap was rather small. For stomatal cell length, for instance, new apomictic genotypes showed only 30% overlap with sexuals. Cell size has been shown to affect photosynthetic rates, though whether the effect is positive or negative depends on cell shape (Warner & Edwards 1993). Because triploids have three copies of every gene in their genome rather than two, gene expression can be higher in triploids (Levin 1983). So triploidy may change some traits in such a way that the population is moved into the attraction of a fitness peak different from the one the diploid sexuals are on.

d) As mentioned before, apomicts have on average a higher seed set than sexuals. In addition, they do not have to invest in male function. Both factors could lead to a surplus of resources that could be invested in a different way than in sexuals. However, this seems not the case. Apomictic *Taraxacum* still produces the male gametophytes and pollen, so these resources cannot be reallocated towards the production of seeds or otherwise, but are simply lost. Production of insect-luring devices, such as yellow petals, and nectar (and pollen) are not reduced relative to sexual *Taraxacum* in most apomictic genotypes (Jenniskens *et al.* 1984), though clones without either of those features have been reported (Gustafsson 1946). Apomictic genotypes, in the experiments in this thesis, produced the same number of capitula per plant and the same number of seeds per capitulum, as did the sexual genotypes. Neither did they always grow larger than sexuals. Reallocation of surplus investment in seeds or male function is no cause for differences between sexuals and apomicts.

The last possibility, as mentioned, seems unlikely to be of much importance. A sort of mutation-selection balance as described under option *b,* would mean that selection in very weak, so the fitness loss because of polyploidisation is mild. This option would not account for found selection on e.g. leaf plasticity away from the sexuals. The strongest force keeping sexuals and apomicts apart is, in my opinion, the fact that polyploidisation moves the apomicts in the attraction of a different fitness optimum, as described under c. Competition may play an additional role, but is, to my opinion, also in view of results and discussion in Chapters 7 and 8*,* less important.

A little bit of sex will go a long way

Sexual reproduction, with recombination creating new variants, seems to give an advantage to the organisms that use it, though the nature of this advantage is still obscure. In the species *Taraxacum* described in this thesis, apomictic genotypes are very similar to their sexual conspecifics in many respects: they have similar morphology and plasticity, similar phenology, and similar within population variation in those traits. They also share DNA markers and alleles with local sexual populations. There are no indications that they evolve their own strategies based on their reproductive assurance and their faithful duplication of genotypes in the next generation. What differences exist between sexuals and apomicts can mainly be explained by the physiological effects of polyploidy. In the (relatively) short run, apomictic genotypes that were created by backcrossing are often enough superior to older genotypes and can replace them. Why they would be superior is difficult to find out. Two main explanations can be thought of. The first is that older lineages deteriorate because they accumulate mutations. The second explanation is that local conditions continuously change slightly and require the local population to adapt. Biotic and abiotic changes in the environment can exert selection pressures that are directional, or frequency dependent. Both types of selection pressure can be met by creating new genotypes.

The amount of 'sex' in apomicts

The striking observation is that the apomicts are so close to the sexuals. They obviously have been able in the recent past to keep up with any adaptation to (changing) local conditions as well as the sexuals. In sexual reproduction, two sets of chromosomes come from different parents. The self-incompatibility system prevents too close inbreeding. Crossing-over during meiosis can create large variation among the gametes. In a backcross that creates a new apomictic genotype, one of three chromosome sets of the apomictic father is replaced by a new chromosome set from the sexual mother. During the meiotic division that produces the diploid pollen grains in the apomictic father, crossing-over can take place, though at lower frequency than in sexual dandelions (Van Baarlen *et al.* 2000). The peculiarities of the system ensure that one chromosome is always retained because it contains the apomixis region. The region on this chromosome coding for apomixis is dominant and always seems to appear in single copy (Aaa) in the apomicts (pers. comm. P.J. van Dijk). In sexuals, this dominant marker is not found (aa). Because crossing-over is possible in apomicts when pollen are produced, other regions of this chromosome can be switched with homologous chromosomes present in the apomict in the next round of gametes. Thus, new apomictic genotypes are produced by a fair amount of "sex". The frequency with which new genotypes are produced is, however, much lower than in sexuals. Yet, this seems enough to enable the apomicts to keep up with the sexuals.

Equally adapted with less sex?

If the apomicts are content with as little sex as they have, why would the sexuals do it so often: why do they have sex every reproduction and have such high crossing-over rates? Are the apomicts only able to do without because sexuals do it so often, or do the sexuals have sex more often than strictly necessary?

The question why diploid sexuals have sex every reproduction is probably irrelevant. Their physiology may not allow partial or facultative sexuality. Reasons why partial sexuality may be unstable are given by Hurst & Peck (1996). The question why sexual organisms have such high recombination rates has been considered in a number of models. Genetic variation for crossing-over rates is present in natural populations. Strong directional selection on a quantitative trait can select for higher recombination rates (Korol & Iliadi 1994). Possibly short episodes of strong directional selection maintain high recombination rates in sexual populations. The data in this thesis did not show a recent episode of strong directional selection in the studied populations, but these probably occur with some frequency. If such a change in conditions occurs and moves the optimum outside the current range of standing variation, recombination will enable a sexual population to move closer to it than an apomictic population.

Apomictic dandelions have on average a higher production of offspring than sexual dandelions, because of their independence from pollinators. This means that they probably can subsist even when they are not as well adapted to local conditions as the sexuals. Catching up with jumps when new well-adapted apomictic genotypes appear from crosses with well-adapted sexuals may be good enough for them not to go extinct. They may 'parasitize' on the high recombination rate of the sexuals.

Alternatively, the local apomictic population could go extinct, while the sexual population adapts to the changed conditions. Recolonisation by adapted apomictic genotypes from other regions is possible, if the pool of available apomictic genotypes is large enough. While sexuals may have difficulty invading an apomictic population, because they are swamped with incompatible pollen, apomicts invading a sexual population may meet no obstructions. Chance effects will eventually erode the genetic variation in apomicts, so occasional new input from backcrosses may be necessary to replete the pool.

Purely apomictic populations

Apomictic communities that occur in mixed sexual-apomictic populations can probably continue to be adapted to local conditions because they exchange genes with the local sexual population. In large parts of the world: Northern Europe, North America, Australia, apomictic *Taraxacum* occur far from any sexual population. Yet, these populations survive very well, and contain high levels of genetic variation. In most studies, those all-apomictic populations contain many genotypes that appear unique to that locality, and possibly a few that are more common and widely spread (Lyman & Ellstrand 1984;Richards 1996;Wittzell 1999;Menken *et al.* 1989;King 1993;Ford & Richards 1985;Battjes *et al.* 1992;Akhter *et al.* 1993).

It seems possible that this pattern is maintained by an ongoing game of extinction and colonisation of genotypes when conditions change, a so-called meta-population system. An experiment by Mølgaard (Mølgaard 1977) showed how easily *Taraxacum* would colonise new areas, a fact known to every gardener and farmer. It also showed how the characteristics of the colonising plants depend on the conditions in the colonised field. This probably indicates that different genotypes were available for colonisation. Though the majority of dandelion seeds will land within a meter from the mother (Bostock 1978), enough seeds will travel larger distances. Well-adapted communities are maintained by the seeds that land close to the mother, but some seeds travel far enough to cross inhospitable regions and reach new areas where they can prosper. New apomictic genotypes that are produced by backcrossing in mixed-reproduction areas can travel wide and far and survive for many generations in areas that are favourable, even when only temporarily so. Genetic variation is maintained by spatial heterogeneity on a large scale as much as on a small scale. The weedy nature of dandelions –high seed production and wide dispersal– is part of their success as asexuals.

The role of sex in micro-evolution

This study on dandelions detected little difference between sexuals and asexuals. Under natural conditions of environmental variation, no short-term effect of the mode of reproduction on adaptation could be found. Other detailed studies of differences between sexual/asexual conspecifics under natural conditions have been made on the fresh-water planarian *Schmidtea polychroa* and on the fresh-water snail *Potamopyrgus antipodarum,* as well as on the cladoceran *Daphnia pulex*. Both *Schmidtea* and *Potamopyrgus* have diploid sexuals and triploid parthenogens (Beukeboom *et al.* 1996). Parthenogenetic *Daphnia* are polyploid as well. In all these species, gene flow between parthenogens and sexuals is possible with unknown frequency (Pongratz *et al.* 1998). The studies have shown high genetic variation among parthenogens (Pongratz *et al.* 1998;Dybdahl & Lively 1995a). The snails showed local adaptation to water depth (Jokela *et al.* 1997b). *Daphnia* and *Schmidtea* showed reduced investment in males or male function (Weinzierl *et al.* 1998;Innes *et al.* 2000). Parthenogenetic clones of *Schmidtea* and *Potamopyrgus* have shown to be more vulnerable to attack by certain parasites than their sexual conspecifics, possibly because the parasites can more easily adapt to the stable clonal resistance genotype (Dybdahl & Lively 1995b;Dybdahl & Lively 1998). Otherwise, in these species, sexuals and apomicts are rather similar to each other (Jokela *et al.* 1997a;Weinzierl *et al.* 1999), like sexual and apomictic dandelions. Other differences that were found between sexual and parthenogenetic types of these animals can as readily be attributed to their ploidy level differences as to their differences in reproductive mode. From the studies to date, it seems as though the short-term evolution of secondarily asexual organisms that still have some residual sex does not differ considerably from that of their sexual conspecifics. The number of studied species, though, is still small. It is still possible that a short-term significance of sex shows during brief and infrequent bursts of directional selection or that continuous selection such as by parasites makes sex a useful strategy. However, gene flow between the two reproductive types together with the high colonising abilities of asexual forms makes any short-term significance of sex hard to detect.

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Summary

Summary

Introduction

Most eukaryote multicellular organisms reproduce sexually. This means they produce gametes, e.g. sperm and egg cells, through meiosis. Each gamete contains a random choice from the maternal and paternal chromosomes. In addition, crossing-over between the parental chromosomes is very common. Crossing over means that pieces DNA are exchanged between the two parental chromosomes. During fertilisation, two gametes fuse, and the resulting individual contains a unique set of genetic material. This method of reproduction is very costly, yet has been highly successful. All complex organisms have evolved through repeated cycles of sexual reproduction. Lineages that have reverted to asexuality seem to be all, but for a few noteworthy exceptions, of recent origin. This means, such lineages probably don't live long and don't split up into many new species.

This raises the question whether sex is important for evolution. An old argument has been that variation in offspring provides material for selection to act upon. Subsequent theoretical work has elucidated the conditions under which sexual populations will evolve differently from asexual populations, assuming that the mode of reproduction is the only difference. Though models can give insight into which processes are possible and which are unlikely, empirical studies have to show what really happens in nature. Detailed studies with different species are necessary to give a general answer about the role of sexual reproduction in adaptation and evolution. This thesis is a report of such a study into the evolution of sexual and asexual (or apomictic) dandelions (*Taraxacum*).

Sexual and asexual (apomictic) dandelions

Dandelions are a convenient species to use for the study of the role of sexual reproduction. Both sexual and asexual types are common and they occur in mixed populations, as well as purely asexual populations. Sexual dandelions are normal sexual organisms. They are diploid, containing one set of chromosomes from each parent. Each individual dandelion produces both pollen and egg cells, but usually cannot fertilise itself. Asexual dandelions contain, however, three copies of each chromosome instead of two. They produce seeds without meiosis and, except for mutations, all offspring are genetically identical to the mother. Any difference found between sexual and asexual dandelions can be the consequence of the mode of reproduction or of this difference in chromosome copy number, which is called ploidy level. Careful experiments must disentangle those two factors.

Between sexual and asexual dandelions some DNA can still be exchanged. Though asexual dandelions do not need pollen themselves, they still produce it. This pollen is of very bad quality, because the plants try during meiosis to divide three sets of chromosomes into two portions. However, sometimes a pollen grain contains the right combination of chromosomes and can fertilise a sexual plant. The offspring is sometimes a new sexual diploid, more often a new asexual triploid. This so-called gene flow is responsible for most of the genetic variation that is found in asexual dandelions.

Local adaptation in sexuals and asexuals

Plants can become adapted to local conditions with respect to for instance light, temperature, nutrient availability, water availability and many other aspects of the environment. They also can become adapted to the way these factors vary over the season, from one year to the next, and from the spot where the parent grows to the spot where the seeds fall. This study focuses on whether sexual and asexual dandelions differ in the speed with which they become adapted, the degree to which they become adapted, and whether they find different solutions to the problems that the environment poses to them. In short, it focuses on the significance of sex for short-term evolution. In addition, some attention is paid to how sexual and asexual dandelions coexist without one of them outcompeting the other.

The theoretical work from the last decades predicts a number of differences between the way sexuals and asexuals can adapt to local conditions. I will treat them one by one, discussing the insights gained from this study.

Random fluctuations in time

Local conditions can fluctuate, within certain limits, from one year to the next. Each year, the sexual mothers produce a variety of offspring, some of them will be able to cope well with that year's conditions, and some of them won't. Asexual mothers produce all identical offspring that are either all well adapted or not so well adapted. Some years with less favourable conditions could wipe out the whole asexual lineage. The sexual mother will probably loose some percentage of her less well-adapted offspring every year, but never looses all of them and therefore can persist. If selection eliminates all mal-adapted genotypes, rather than eliminating the worst adapted of those that are present, theory predicts that asexual genotypes that are tolerant to variation in the environment will survive, so called jacks-of-all-trades, and specialist asexual genotypes will go extinct. Simulation models described in chapter 2 show that this applies to sexuals as well, if they cannot maintain sufficient genetic variation. If sexual populations are large or exchange genes with other populations through migration, so they maintain sufficient genetic variation, the optimal sexual genotypes may be more specialised than optimal asexual genotypes. The studies described in this thesis show no indication that this is a general pattern in dandelions. Asexual (or apomictic) genotypes were not in general more tolerant to environmental variation than sexual genotypes (chapters 4,8). This means that some of the assumptions of the model are not met, or that one or the other type is not optimally adapted. Probably the environment does not vary in such a way as the model expects, neither does selection (chapter 8).

Directional change in time

If the environment does not fluctuate randomly, but changes directionally, populations have to adapt. Because sexual populations can produce new genotypes every generation by reshuffling existing genetic information, by combining mutations that arose in different individuals into one new individual, sexual populations can usually adapt faster and to a greater extent to changes than asexual populations. Under such conditions, asexual populations will loose more genetic variation than sexual populations and they will lag behind in adaptation, because they have to wait for mutations in the right combination. Consequently, they can go extinct, unless they can make up for mal-adaptedness by being much more fertile. In the studied populations, asexual dandelions do not seem to be more depleted of genetic variation than the sexual population (chapters 3, 5). It seems therefore unlikely that the dandelions have recently experienced strong directional selection.

Random variation in space

Local conditions can also vary in space. Because seeds will land some distance from the mother, the offspring can experience conditions that are different from those the mother has experienced, even if nothing changes over time. If this variation in space is completely random, life is a sort of lottery. A sexual mother, who produces genetically variable offspring, has as much chance that her children will end up in a favourable or unfavourable spot as an asexual mother has. Variation among offspring is not necessarily better in a variable environment, if you cannot choose where you want to live.

If the variation in space is random, or at least seems random to the dandelions because of the distance at which they disperse, producing genetically variable offspring can be advantageous under some strict conditions. If many seeds land at every site, but eventually only one per site can survive, you must assure that your offspring is the one that will survive. If there are differences between sites in what genotype is the best, the best option for the mother is to produce many different seeds, 'hoping' the right one is among them. In these circumstances, mothers who produce many identical seeds will be at a disadvantage. This has been compared to a lottery for which the sexual mother buys many different lottery tickets for each site, while the asexual mother buys many tickets with the same number. If they play in many lotteries, the sexual mothers have a higher chance of winning if the percentage of excess seeds that are produced for every site – that is the number of tickets bought per prize– is high 'enough'. This model assumes that competition is the main selective force, and that in each site many young plants compete to the death. Dandelions produce a lot of seeds for each available site. Yet, competition seems not the sole selective force, and competition to the death has not been observed (chapter 8).

This means that, at least for these dandelions, adaptation to random variation in space is probably not a significant consequence of sexual reproduction.

Structured variation in space

If you can choose where you want to live, such as many animals can, numerous asexual lineages can co-exist with each other and with sexual conspecifics as well, if all genotypes are relatively specialist. This has been termed the 'Frozen Niche Variation'-model. The more different asexual lineages are present, the more difficult things become for the sexual population, but usually the sexuals, with their large variation, will find some empty 'niches' where they can persist.

Plants cannot choose where they want to live, but the majority of their seeds will land relatively close to the mother. In dandelions, the majority of the seeds will land within one meter from the mother. So, if the environment does not change randomly, but gradually, offspring will experience conditions that are similar to their mother's. The simulations of chapter 2 show that under some combinations of dispersal distance and environmental change, the Frozen Niche Variation model can apply to plants as well.

The data collected for this thesis show that in both sexual and asexual dandelions many genotypes coexist in the same field. At least some of these different genotypes can be considered as different specialists, because the experiments (chapter 8) show that under different conditions, different genotypes grow best. The range of different genotypes is of the same order in both sexuals and apomicts (chapter 3, 5). Probably spatial variation is important for maintaining genetic variation in both sexuals and apomicts. It does not necessarily work much differently in sexuals and asexuals.

Differences between sexuals and apomicts

This study has detected no differences in the way a genotype cope with environmental fluctuations in time because of its mode of reproduction. Neither seems sexuality in dandelions to lead to a better adaptedness to random variation in space. It could also not been shown that directional selection is acting or has recently been acting in such a way that sexuals have been better able to respond to it.

The high genetic variation in both types rather suggests that the environment is heterogeneous in space, probably in such a way that it seems structured to the dandelions. With this is meant that the current conditions of the parents give some indication of the conditions the offspring will encounter. Many clones and sexual genotypes can co-exist, each best adapted to its own set of environmental conditions.

Yet, sexual and asexual genotypes from the same field were not completely identical. The sexuals differed from the apomicts in the following respects. Sexuals had smaller cells than apomicts (chapter 5). In most conditions, that is, during the greater part of the season, sexuals had more leaves than apomicts (chapter 5). All *Taraxacum* elongate their leaves when shaded and when days grow shorter, but apomicts did this much more strongly than sexuals (chapters 3, 4 and 5 and 8). In spring, when days grow longer, apomicts had shorter leaves than sexuals (chapters 3 and 5). Combined, this means that asexual genotypes had a more plastic leaf response to shading (chapter 3). Apomicts also put their leaves more upright when shaded than sexuals did (chapters 3 and 4). Under wet conditions, sexuals had a higher proportion of their biomass in leaves than apomicts, whereas under dry conditions, they had a lower proportion of their biomass in leaves (chapter 8). Probably associated with the differences in leaf characteristics and biomass allocation, sexuals produced more biomass than apomicts under dry conditions, though not under wet conditions (chapter 8). When less biomass is present in leaves, the respiring leaf surface is smaller, and water loss is reduced. In the field, as well as in most experiments, sexuals and apomicts differed a few days in flowering. In the field, sexuals usually are a few days earlier than apomicts, but in the experiments it varied. Apomicts had heavier seeds than sexuals in one of the commongarden experiments (chapter 3), though not in other experiments (chapter 3 and 5). Usually, weight is positively correlated with the probability of a seed to germinate (chapter 5). Under some conditions, apomicts probably produce better germinating seeds than sexuals, under other conditions probably not (chapter 5).

Ploidy level effects

If different responses to selection of sexual and asexual types cannot explain the differences between sexuals and apomicts, what can? There are several possibilities, but a likely option that can be investigated is that the difference in ploidy level is responsible. Sexuals contain two sets of chromosomes; they are diploid. Apomicts contain three sets of chromosomes: they are triploid. Having more DNA in the cell and having three copies of every gene instead of two can have large consequences for physiology and development.

In chapter 5 and 6, experiments are described that try to separate the effects of ploidy level from those of the mode of reproduction. The experiment compared diploids with triploids that had not yet experienced much selection and triploids that were collected in the field. This experiment found that the effect of a higher ploidy level was that the plants had larger cells. This has often been found in plants with higher ploidy levels. The higher ploidy level was also –at least in part- responsible for fewer leaves, longer leaves and different timing of flowering. Differences that were – at least partly– the result of selection were the stronger elongation of leaves in shade, and a shift in timing of flowering under shaded conditions.

Why different?

If sexuals are considered optimally adapted to their local environment, we might expect selection to push apomicts in the direction of the sexuals, even if ploidy level causes some differences. However, this seems not the case for all traits. Both the distribution patterns of traits, and the comparisons described in chapters 5 and 6 show this. For instance, apomicts are probably selected for a more plastic response to shading than sexuals. The question is now why selection would favour different traits in sexuals and apomicts if it is not an adaptation to environmental variation.

In the discussion (chapter 9), several possibilities are considered. The most likely explanation seems to me that the effect of triploidy, on for example cell size, is such that a different combination of morphological and other traits works best physiologically. As a result, the triploid apomicts prefer slightly different ecological conditions that sexuals, but such conditions are obviously common enough for the apomicts to survive.

What are the consequences of the differences?

Though the differences between sexuals and apomicts probably are not caused by a different response to selection because of their different reproductive systems, they may have consequences for the stability of the system. Sexuals and apomicts seem to occupy somewhat different ecological niches: the sexuals probably prefer dryer and sunnier sites than the apomicts on average. Some studies also report that apomicts are more common on disturbed sites. The competition experiment (chapter 7) also gave some indications that sexuals and apomicts are more strongly hindered by their own type than by the opposite reproductive type. If sexuals and apomicts do not occupy exactly the same ecological niches, then they can coexist ‘peacefully’ without the apomicts with their higher reproductive assurance ousting the sexuals.

The role of sexual reproduction for short-term evolution

The results in this thesis show no evidence that the dandelions of the studied dandelion populations have recently encountered any challenges that were met in different ways by the sexuals and the apomicts. Maybe such events are rare, and the traces are quickly eliminated by backcrossing and by colonisation. The results do not mean that sexual reproduction has no significance for short-term evolution and local adaptation under natural conditions, but it is hard to detect. The regular addition of new genotypes to the pool of asexual dandelions by backcrossing may be essential for long-time survival of the asexual dandelions.

Samenvatting

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Inleiding

De meeste eukaryote meercellige organismen planten zich seksueel voort. Dit betekent dat ze gameten produceren, bijvoorbeeld eicellen en sperma, via meiose. Elke gameet bevat een willekeurige selectie uit de chromosomen van de moeder en de vader. Bovendien is overkruising tussen de ouderlijke chromosomen veelvoorkomend. Overkruising is het uitwisselen van stukjes DNA tussen de beide ouderlijke chromosomen. Bij de bevruchting smelten twee gameten samen en het individu dat daaruit groeit bezit een unieke set genetisch materiaal. Deze wijze van voortplanten is erg kostbaar, maar is zeer succesvol gebleken. Alle complexe organismen zijn geëvolueerd via herhaalde cycli van seksuele voortplanting. Afstammingslijnen die teruggegaan zijn naar aseksuele voortplanting lijken allemaal, op een paar opvallende uitzonderingen na, van recente oorsprong. Dit betekent dat zulke lijnen waarschijnlijk niet oud worden en zich ook niet in een waaier van soorten opsplitsen.

Dit werpt de vraag op of seks van belang is voor evolutie. Een oud argument is dat de variatie tussen nakomelingen materiaal verschaft waar selectie op kan aangrijpen. Theoretisch werk heeft sindsdien opgehelderd onder welke condities een seksuele populatie anders zal evolueren dan een aseksuele populatie, indien de manier van voortplanten het enige verschil tussen de beide populatie. Hoewel modellen inzicht kunnen verschaffen in welke processen mogelijk zijn in de natuur en welke onwaarschijnlijk, zijn er praktijkstudies nodig om te laten zien wat er echt in het veld gebeurt. Gedetailleerde studies aan verschillende soorten zijn nodig om een algemeen antwoord te geven op de vraag welke rol seksuele voortplanting speelt in aanpassing en evolutie. Dit proefschrift is het verslag van zo'n studie naar de evolutie van seksuele en aseksuele (of apomictische) paardebloemen (*Taraxacum*).

Seksuele en aseksuele paardebloemen

Paardebloemen zijn geschikte organismen voor een studie naar de rol van seksuele reproductie, omdat zowel seksuele als aseksuele vormen bestaan, die ook in gemengde populaties voorkomen. Seksuele paardebloemen zijn normale seksuele organismen. Ze zijn diploïde en bevatten dus één set chromosomen van elk van de ouders, samen twee sets. Elke paardebloem produceert zowel stuifmeel (pollen) als eicellen, maar kan normaalgesproken niet zichzelf bevruchten. Aseksuele paardebloemen bevatten echter drie kopieën van elk chromosoom in plaats van twee; dit wordt triploïdie genoemd. Aseksuele paardebloemen produceren zaden zonder meiose of bevruchting en alle nakomelingen zijn genetisch identiek aan de moeder, afgezien van mutaties. Verschillen tussen seksuele en aseksuele paardebloemen kunnen dus het gevolg zijn van het verschil in de manier van voortplanten, maar ook van het verschil in het aantal kopieën per chromosoom, het ploïdieniveau. Zorgvuldige experimenten zijn nodig om de effecten van deze factoren uit elkaar te halen.

Uitwisseling van DNA tussen seksuele en aseksuele paardebloemen is tot op zekere hoogte mogelijk. Hoewel aseksuele paardebloemen zelf geen stuifmeel nodig hebben, produceren ze het wel. Dit stuifmeel is van slechte kwaliteit, omdat de planten proberen om in de meiose drie sets chromosomen in tweeën te verdelen. Soms bevat een stuifmeelkorrel echter bij toeval de juiste combinatie chromosomen en dan kan deze korrel een seksuele plant bevruchten. De nakomeling is soms een diploïde seksuele plant, maar vaker een triploïde aseksuele plant. Deze genuitwisseling is verantwoordelijk voor het grootste deel van de genetische variatie die we bij aseksuele paardebloemen vinden.

Lokale aanpassing in seksuele en aseksuele organismen

Planten kunnen door evolutie aangepast raken aan de lokale condities met betrekking tot bijvoorbeeld licht, temperatuur, nutriëntenbeschikbaarheid, waterbeschikbaarheid en allerlei andere aspecten van het milieu. Ze kunnen ook aangepast raken aan de manier waarop deze factoren variëren over het seizoen, van het ene jaar op het andere, en van de plek waar de ouders staan tot de plek waar de zaden terechtkomen. Deze studie concentreert zich op de vraag of seksuele en aseksuele paardebloemen verschillen in de snelheid waarmee ze zich aanpassen, de mate waarin ze in aangepast raken en of ze verschillende oplossingen vinden voor de problemen waarvoor de buitenwereld hen stelt. Kortweg, de studie richt zich op het belang van seksuele voortplanting voor korte-termijn evolutie. Daarnaast wordt enige aandacht besteed aan hoe de seksuele en aseksuele paardbloemen naast elkaar kunnen voorkomen zonder dat de ene de andere wegconcurreert.

Het theoretische werk van de laatste decennia voorspelt een aantal verschillen tussen de manieren waarop seksuele en aseksuele organismen zich aanpassen aan de lokale omstandigheden. Door seksuele en aseksuele paardebloemen onder verschillende omstandigheden op te kweken en hun groei en ontwikkeling te volgen, heb ik data verkregen die inzicht geven in mate van aanpassing en de variatie daarin bij beide groepen paardebloemen. Ik zal de verschillende theoretische ideeën één voor één behandelen en de inzichten bespreken die deze studie heeft opgeleverd.

Willekeurige milieufluctuaties in de tijd

Lokale omstandigheden kunnen binnen bepaalde grenzen fluctueren van het ene jaar op het andere. Elk jaar produceren de seksuele moeders een aantal genetisch verschillende nakomelingen en sommige van hen zullen goed in staat zijn zich te redden onder de dan heersende omstandigheden en sommige minder. Aseksuele moeders maken nakomelingen die allemaal identiek zijn en deze zijn ofwel allemaal goed aangepast ofwel allemaal niet. Een aantal jaren achter elkaar met ongunstige condities kan een hele aseksuele familie elimineren. De seksuele moeder verliest waarschijnlijk elk jaar een aantal nakomelingen dat slecht aangepast is, maar ze verliest ze niet allemaal en daarom kan de seksuele familie blijven voortbestaan.

Als selectie alle slecht aangepaste individuen verwijdert in plaats van de minst goed aangepaste van alle die aanwezig zijn, dan voorspelt de theorie dat aseksuele genotypen die tolerant zijn voor variatie in het milieu, een soort manusjes-van-alles, zullen overleven, terwijl specialisten uit zullen sterven. De simulatiemodellen van hoofdstuk 2 laten zien dat hetzelfde gebeurt in seksuele populaties als ze niet in staat zijn voldoende genetische variatie te handhaven. Kunnen ze dat wel, doordat bijvoorbeeld de populaties zeer groot zijn of doordat er geregeld nieuwe genen binnenkomen door immigratie, dan zouden seksuele genotypen meer gespecialiseerd kunnen blijken dan aseksuele genotypen.

De studies in dit proefschrift laten geen aanwijzingen zien dat dit een algemeen patroon is in paardebloemen. Aseksuele genotypen bleken niet in het algemeen beter bestand tegen verschillende milieuomstandigheden dan seksuele genotypen (hoofdstuk 4, 8). Dit betekent dat aan enkele aannamen van het model niet wordt voldaan in de praktijk of dat het ene of andere type paardebloem niet optimaal is aangepast. Het lijkt waarschijnlijk dat het milieu niet fluctueert op de manier die het model aanneemt en dat selectie ook iets anders werkt (hoofdstuk 8).

Gerichte milieuverandering

Als het milieu niet op een willekeurige manier fluctueert, maar op een gerichte manier verandert, bijvoorbeeld alsmaar droger wordt, dan moeten de organismen in dat gebied zich aanpassen om te kunnen overleven. Omdat seksuele populaties elke generatie nieuwe genotypen kunnen produceren door bestaande genetische variatie te herverdelen, door mutaties die in verschillende families zijn opgetreden te combineren in een nieuw individu, kunnen seksuele populaties zich meestal sneller en vooral beter aanpassen aan veranderingen dan aseksuele populaties. Onder zulke omstandigheden verliezen aseksuele populaties meer genetische variatie dan seksuele populaties en ze zullen een achterstand vertonen in de mate van aanpassing aan het milieu, omdat ze moeten wachten op het optreden van mutaties in de juiste combinaties. Ze kunnen dan uitsterven tenzij ze een ander voordeel hebben, bijvoorbeeld als ze veel meer zaad zetten. In de populaties die voor dit proefschrift bestudeerd zijn leken de aseksuele populaties niet armer aan genetische variatie dan de seksuele populaties (hoofdstuk 3, 5). Het lijkt er daarom niet op dat deze paardebloemen onlangs sterke gerichte selectie hebben meegemaakt.

Willekeurige milieuvariatie in de ruimte

Lokale omstandigheden kunnen natuurlijk ook in de ruimte variëren. Omdat zaden op enige afstand van de moeder terechtkomen kunnen de nakomelingen omstandigheden meemaken die anders zijn dan die van de moeder. Als er geen systeem zit in hoe het milieu van plek tot plek varieert, dan lijkt het leven op een loterij. Een seksuele moeder die genetisch gevarieerde nakomelingen produceert heeft evenveel kans dat haar kinderen op een gunstige of ongunstige plek terechtkomen als een aseksuele moeder die allemaal identieke nakomelingen produceert. Variatie in nakomelingen is niet noodzakelijkerwijs beter in een variabel milieu, als je niet kunt kiezen waar je gaat leven.

Als de milieuvariatie dus geheel zonder systeem is, of zo lijkt voor de paardebloemen door de manier waarop ze zich verspreiden, dan zijn er toch omstandigheden waaronder het theoretisch gunstig is om gevarieerde nakomelingen te produceren, maar hiervoor gelden strikte voorwaarden. Als er op elke plek heel veel zaden terechtkomen, maar er kan maar één zaad per plek overleven, dan moet de moeder proberen ervoor te zorgen dat die ene overlevende haar kind is. Het beste wat een seksuele moeder nu kan doen is voor elke plek een heleboel verschillende nakomelingen produceren en ‘hopen’ dat de juiste ertussen zit. Dit wordt wel vergeleken met een loterij waarvoor de seksuele moeder lootjes koopt met allemaal verschillende nummers en de aseksuele moeder lootjes met allemaal hetzelfde nummer. Als ze voor heel veel trekkingen meespelen, dan hebben de seksuele moeders betere kansen als het aantal boventallige zaden dat voor elke plek geproduceerd wordt, –dat is het aantal kaartjes dat per trekking wordt gekocht–, hoog ‘genoeg’ is. Dit model veronderstelt dat competitie de belangrijkste selectieve kracht is en dat veel jonge planten concurreren tot de dood erop volgt. Paardebloemen produceren inderdaad een enorme overmaat aan zaden, maar concurrentie lijkt in de experimenten in dit proefschrift (hoofdstuk 8) niet de belangrijkste selectiekracht en competitie tot de dood is niet waargenomen.

Dit betekent dat, in ieder geval voor deze paardebloemen, aanpassing aan ongestructureerde milieuvariatie niet een belangrijk aspect is van seksuele reproductie.

Gestructureerde milieuvariatie

Als je wel kunt kiezen waar je wilt leven, zoals veel dieren dat kunnen, dan kunnen talrijke aseksuele families naast elkaar bestaan en ook samenleven met seksuele soortgenoten, wanneer alle genotypen specialisten op verschillende gebieden zijn. Dit systeem wordt het ‘Frozen Niche Variation’-model genoemd. Hoe meer verschillende aseksuele families er zijn, hoe lastiger het wordt voor de seksuele populatie, maar door de grote genetische variatie in seksuele populaties zullen die meestal wel in staat zijn om wat lege ‘niches’ te vinden waarin ze kunnen voortbestaan.

Planten kunnen niet kiezen waar ze willen wortelschieten, maar de meeste zaden komen in de buurt van hun ouders terecht. Als het milieu niet geheel willekeurig varieert, maar geleidelijk, dan ondervinden de meeste nakomelingen omstandigheden die lijken op die van de moeder. De simulaties van hoofdstuk 2 laten zien dat onder bepaalde combinaties van milieuverandering met afstand en dispersie van de zaden, dit ‘Frozen Niche Variation’-model ook voor planten kan opgaan.

De data die voor dit proefschrift werden verzameld laten zien dat er vele seksuele en aseksuele genotypen naast elkaar leven in hetzelfde veld. Tenminste sommige van deze genotypen kunnen beschouwd worden als verschillende specialisten, omdat de experimenten laten zien dat het afhangt van de condities welk genotype het beste groeit. De spreiding van verschillende genotypen was in dezelfde orde van grootte in seksuele en aseksuele paardebloemen (hoofdstuk 3,5). Waarschijnlijk draagt ruimtelijke variatie bij aan het handhaven van genetische variatie in zowel seksuele als aseksuele paardebloemen. Dit werkt waarschijnlijk op ongeveer dezelfde manier voor beide groepen.

Verschillen tussen seksuele en aseksuele paardebloemen

Deze studies hebben geen algemeen verschil gevonden in de manier waarop seksuele en aseksuele genotypen omgaan met milieufluctuaties van jaar tot jaar. Het lijkt er ook niet op, dat seksuele paardebloemen beter aangepast zijn aan willekeurige variatie in de ruimte. Noch leek het erop dat er sterke gerichte selectie optreedt of onlangs is opgetreden waarop de seksuele populatie beter heeft kunnen reageren dan de aseksuele populatie.

De grote hoeveelheid genetische variatie in beide typen paardebloemen suggereert dat het milieu heterogeen is in de ruimte op een manier die de paardebloemen als gestructureerd ervaren. De huidige omstandigheden van de ouders geven dus enige indicatie van de condities waaronder de nakomelingen zullen opgroeien. Veel aseksuele families en seksuele genotypen kunnen dus samenleven, elk het best aangepast aan zijn eigen specifieke set van milieuomstandigheden.

Toch bleken seksuele en aseksuele paardebloemen uit hetzelfde veld niet geheel identiek. De seksuele verschilden van de aseksuele in de volgende kenmerken: Seksuele paardbloemen hadden kleinere cellen (hoofdstuk 5). Gedurende het grootste deel van het jaar hadden de seksuele meer bladeren (hoofdstuk 5). Paardebloemen maken langere bladeren als het donkerder is of als de dagen korter worden, maar de seksuele deden dit minder sterk dan de aseksuele paardebloemen (hoofdstuk 3,4,5,8). Als de dagen weer langer worden, worden de bladeren korter, maar minder sterk bij de seksuele dan de aseksuele genotypen (hoofdstuk 3,5). De aseksuele plaatsten hun bladeren ook rechterop als het donkerder was (hoofdstuk 3,4). Onder natte omstandigheden hadden de seksuele een groter deel van hun biomassa in bladeren dan de aseksuele, onder droge omstandigheden andersom (hoofdstuk 8). Waarschijnlijk als een gevolg van de verschillen in bladkenmerken en biomassaverdeling groeiden de seksuele paardebloemen beter onder droge omstandigheden dan de aseksuele paardebloemen. De aseksuele genotypen groeiden daarentegen beter in een van de proeftuinexperimenten (hoofdstuk 4). In het wild en ook in de meeste experimenten verschilden de beide typen enkele dagen in bloeitijd, hoewel de bloeiperiode sterk overlapte (hoofdstuk 3, 4, 5). De aseksuele planten hadden in een van de experimenten zwaardere zaden (hoofdstuk 3), maar niet in andere experimenten (hoofdstuk 4, 5). Het gewicht van zaden is meestal positief gecorreleerd met de kiemkans (hoofdstuk 5), dus onder sommige omstandigheden produceren aseksuele paardebloemen waarschijnlijk betere zaden dan seksuele (hoofdstuk 5).

Effecten van het ploïdieniveau

Als de verschillen tussen seksuelen en aseksuelen niet verklaard kunnen worden door verschillende responsen op selectie vanwege de verschillen in de manier van reproduceren, waardoor dan wel? Er zijn verscheidene mogelijkheden, waarvan het verschil in ploïdieniveau een goede kandidaat is. Zoals gezegd, seksuele paardebloemen bevatten twee kopieën van elk chromosoom, aseksuele bevatten er drie. Meer DNA per cel, zowel als meer kopieën van elk gen per cel kan ingrijpende gevolgen hebben voor de fysiologie en de ontwikkeling van het organisme.

In hoofdstuk 5 en 6 worden experimenten beschreven waarin gepoogd wordt een onderscheid te maken tussen de effecten van ploïdieniveau en de effecten van selectie. Diploïde seksuele planten werden hiervoor vergeleken met triploïde aseksuele planten uit het veld en triploïde aseksuele planten uit het lab die nog weinig selectie hadden meegemaakt. In deze experimenten werd gevonden dat een hoger ploïdieniveau een grotere cel tot gevolg had. Dit effect van ploïdieniveau wordt algemeen in planten gevonden. Verder was het hogere ploïdieniveau van de aseksuele planten, in ieder geval gedeeltelijk, verantwoordelijk voor minder bladeren, langere bladeren en een verschil in bloeiperiode. Verschillen die, in ieder geval gedeeltelijk, toe te schrijven waren aan selectie waren een sterkere verlenging van de bladeren in de schaduw en een extra verschuiving van de bloeitijd in de schaduw.

Waarom verschillen?

Als men zou aannemen dat de seksuele paardebloemen optimaal zijn aangepast aan de lokale condities, dan zou me kunnen verwachten dat selectie de aseksuele dezelfde kant op drijft, zelfs al veroorzaakt triploïdie een aantal verschillen. Dit lijkt echter zeker niet voor alle kenmerken het geval. Zowel het patroon van variatie als de vergelijkingen in hoofdstuk 5 en 6 laten dit zien. De aseksuele paardebloemen werden bijvoorbeeld geselecteerd voor een sterkere reactie op beschaduwing dan de seksuele. De vraag is dan waarom selectie verschillende kenmerken zou begunstigen in seksuele en aseksuele paardebloemen als dit niet een verschil is in aanpassing aan milieuvariatie.

In de discussie (hoofdstuk 9) wordt een aantal mogelijkheden besproken. De meest waarschijnlijke lijkt me dat het effect van ploïdieniveau, op bijvoorbeeld celgrootte, dusdanig is dat een andere combinatie van morfologische en andere kenmerken fysiologisch het beste werkt. De aseksuele paardebloemen hebben als gevolg daarvan een voorkeur voor iets andere ecologische omstandigheden dan de seksuele, maar deze omstandigheden zijn zo algemeen dat dit verder geen probleem vormt.

De gevolgen van de verschillen tussen seksuele en aseksuele paardebloemen

Hoewel de verschillen tussen seksuele en aseksuele paardebloemen niet het directe gevolg zijn van verschillen in de manier van voortplanten, kunnen deze verschillen wel gevolgen hebben voor de stabiliteit van het systeem. Seksuele en aseksuele paardebloemen lijken iets verschillende niches te bezetten: de seksuele geven de voorkeur aan wat drogere, zonnigere condities dan de aseksuele. Sommige studies melden ook dat aseksuele paardebloemen algemener zijn op verstoorde plekken. Het competitie-experiment (hoofdstuk 7) gaf ook aanwijzingen dat de seksuele en aseksuele paardebloemen meer hinder ondervinden van concurrenten van hun eigen type dan van het andere type. Als seksuele en aseksuele paardebloemen verschillende ecologische niches bezetten, dan kunnen ze naast elkaar bestaan zonder dat de aseksuele vorm de seksuele wegconcurreert.

De rol van seksualiteit in korte-termijn evolutie

De resultaten die gepresenteerd worden in dit proefschrift geven niet aan dat de paardebloemen in de bestudeerde populaties in het nabije verleden geconfronteerd zijn met een situatie waarop de seksuele en aseksuele vormen verschillend hebben gereageerd, of met condities waarin een duidelijk voordeel of nadeel van seksuele voortplanting optreedt. Hoewel daar theoretisch diverse mogelijkheden voor zijn, zoals besproken, heb ik weinig aanwijzingen gevonden dat in het veld de omstandigheden tot verschillende reacties van seksuele en aseksuele paardebloemen leiden. Mogelijk treden zulke situaties onregelmatig op en worden de sporen ervan snel uitgewist door het terugkruisen tussen de seksuele en de aseksuele planten en door kolonisatie, zoals beschreven in hoofdstuk 9. Deze resultaten betekenen niet dat seksuele voortplanting totaal geen betekenis heeft voor korte-termijn evolutie en aanpassing aan lokale situaties onder natuurlijke omstandigheden, maar het is moeilijk vast te stellen. Regelmatige aanvulling van de voorraad aseksuele genotypen door middel van genoemde terugkruisingen zou essentieel kunnen zijn voor de lange-termijn overleving van de aseksuele vorm.

Levensloop

Ik ben geboren op 9 mei 1969 in Dordrecht. Daar heb ik ook de middelbare school doorlopen: het Christelijk Lyceum. Mijn vakkenpakket was voornamelijk beta-gericht met daaraan toegevoegd Duits. Na mijn eindexamen in 1987 ben ik naar Eindhoven verhuisd en daar ben ik begonnen met de studie bouwkunde aan de TU Eindhoven. Na een kleine twee jaar heb ik deze studie afgebroken. In 1989 ben ik begonnen met de studie biologie aan de Rijksuniversiteit Groningen. Gedurende mijn studie heb ik een aantal malen als student-assistant gewerkt in de cursussen mathematica, methodologie en statistiek. Ik heb doctoraalonderwerpen gedaan bij Franjo Weissing in het Laboratorium voor Populatiegenetica en bij Manja Kwak in het Laboratorium voor Plantenoecologie. Mijn diploma behaalde ik in 1993. Hierna werkte ik aan een project binnen het Nationaal OnderzoeksProgramma 'Global Air Pollution and Climate Change', eerst bij het (toenmalige) CABO-DLO, later aan de Landbouwuniversiteit Wageningen, in beide gevallen onder leiding van Dr Frank Berendse. In 1996 begon ik als OiO aan de Universiteit Utrecht aan een project getiteld 'Fitness, phenotypic plasticity and genetic variation in sexual and asexual *Taraxacum officinale*'. Dit werk werd uitgevoerd bij de afdeling Evolutionaire Populatiebiologie onder begeleiding van Dr Gerdien de Jong.