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ECOLOGICAL AND GENETIC INTERACTIONS BETWEEN DIPLOID SEXUAL AND
TRIPLOID APOMICTIC DANDELIONS

ECOLOGICAL AND GENETIC INTERACTIONS BETWEEN DIPLOID
SEXUAL AND TRIPLOID APOMICTIC DANDELIONS

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Summary

The paradox of sex

For evolutionary biologists, explaining the existence of sexual reproduction is rather problematic. Compared with its alternative, asexual reproduction, sex seems overly complicated and costly, for a wide variety of reasons. The most important reason why sex is considered costly compared to asexual reproduction is that it necessitates the existence of two (or more) sexes, instead of just one. A typical population of asexually reproducing organisms consists of only female individuals that reproduce by giving birth without any need for fertilisation. The offspring of asexuals are genetically identical to their mother; the offspring are all “clones” of their mother. To see why making males is considered costly, imagine a population that consists of one hundred sexual organisms. Assuming there are as many males as females, this population will initially consist of fifty males and fifty females. If every female produces on average exactly two offspring, the size of the population will be stable. In contrast, in a population consisting of asexually reproducing individuals the population size is not stable, but doubles every generation: here, the population consists entirely of females and if every female produces two offspring, the hundred females together produce two hundred offspring, all of which are female. In the next generation, the population should have grown to four hundred individuals. In general, this cost is called the “two-fold cost of producing males”.

There are several other reasons why sexual reproduction is thought to be costly compared to asexual reproduction. Sexual reproduction also involves finding a mate, which can cost a lot of energy and can lead to extra exposure to predators. Think for example of the showy flowers many flowering plants make to attract pollinators: flowers cost a lot of energy to produce (including the sugar needed to produce nectar) and they make the plants not only attractive to pollinators but also to herbivores. In addition, biologists have recognized other costs of sex, such as the cost of genome dilution, the cost of recombination, the cost of sexual selection and others. Considering all these costs, the existence of sexual reproduction indeed seems quite paradoxical. Why then is the great majority of species on earth reproducing sexually?

Many hypotheses have been proposed to explain the ubiquity of sex in nature, most of which are centred around one crucial difference between sexual and asexual reproduction: the offspring of a sexually reproducing female are genetically diverse while the offspring of an asexual female are genetically identical. Genetic variation is generally deemed evolutionarily advantageous. According to some of the hypotheses that have been proposed to maintain sexual reproduction, it helps to fend off diseases and parasites, to cope with the huge ecological diversity present in nature and to prevent the accumulation of deleterious mutations.

Dandelions (*Taraxacum* sect. *Ruderalia*) provide a good model system to study hypotheses on the maintenance of sex, as there are both sexual and asexual dandelions.

The two types are morphologically indistinguishable but differ in the number of chromosomes; sexuals are diploid (they have two copies of every chromosome), whereas asexuals are triploid (they have three copies of every chromosome). In Southern and Eastern Europe, sexual and asexual dandelions can live side by side in a single population, a situation which is impossible if the asexuals indeed have a reproductive advantage over the sexuals.

This thesis focuses on the seemingly paradoxical coexistence of sexual and asexual dandelions. I specified the nature and size of the cost of sex in dandelions, and whether the coexistence can be explained by a difference in ecological preferences between the sexuals and asexuals. Furthermore, I investigated the consequences of the coexistence on the genetic variation in and between the sexual and asexual dandelions.

The cost of sex in dandelions

The above explanation of the cost of males is only generally valid for dioecious species, where the two sexes are found in separate individuals. However, most plants, including dandelions, are hermaphrodites: they are a male and a female packaged in a single individual. This has important consequences for the cost of males because then the asexuals can also be hermaphrodites. In that case, asexuals may still have their male function (the production of pollen in plants, sperm in animals), and then there is no cost of males for the sexuals (note that the other costs of sex are still applicable, however). In dandelions, this is exactly the case as the majority of asexual dandelions still produce pollen, even though they do not need to be pollinated to produce seeds. The pollen that they produce is largely infertile and their production can therefore be considered a waste of resources. It is therefore puzzling to see that selection has not worked to stop pollen production in asexuals; these resources could be used for the production of more, or better, seeds.

In this thesis, I have compared seed production of pollen producing and male sterile asexual dandelions and found that such resource reallocation indeed takes place. Male sterile asexuals produced 38% more seed heads than pollen producing asexuals, though there was no difference in the number of seeds per head, seed weight and germination rate. Therefore, if all asexual dandelions would have been male sterile, there would have been a high cost of males for the sexual dandelions. However, this potentially high cost is not fully realised as only a small percentage of asexuals are male sterile, consequently, the cost of males in dandelions is low.

To see whether there is indeed a paradox of sex in dandelions, I have reviewed all costs of sex that have been suggested in the literature and investigated whether and how they are realised in dandelions. I found that the total cost of sex in dandelions is likely to be much lower than the two-fold cost of sex that is often assumed in studies on the maintenance of sex. Because of this relatively low cost of sex, the mechanism enabling the coexistence of sexual and asexual dandelions does not have to be very strong.

Ecological differentiation

Sexuals and asexuals may be able to coexist over a long evolutionary timescale when there is some form of ecological differentiation between them; when the habitats occupied by the sexuals are different from those occupied by the asexuals. Investigating such habitat differentiation is the subject of several of the chapters included in this thesis. I started by studying habitat differentiation on a regional geographic scale, comparing the vegetation of different dandelion populations around Neuchâtel, Switzerland, to see whether populations with a high frequency of sexuals have different vegetations than populations with a high frequency of asexuals. I found that asexual dandelions are more common in habitats where the vegetation is indicative for a large amount of human disturbance, whereas sexual dandelions are mainly found in more stable habitats.

I then continued to test whether such ecological differentiation can also be found within a population, and indeed found that also on a very small geographical scale, sexuals occur in slightly more stable spots than asexuals. Furthermore, a decrease of the amount of disturbance in this population over a 14-year period resulted in a relative increase in the frequency of sexuals, also indicating that sexuals prefer more stable habitats than the asexuals. Such ecological differentiation could possibly be explained through the “Tangled Bank” hypothesis for the maintenance of sex. Under this hypothesis, sexuals and asexuals compete for scarce resources, and sexuality provides a benefit since it creates more variable offspring which should be able to use a wider array of resources than the genetically less variable asexuals. This can cause selection to move the ecological niche of the sexuals away from the asexuals. An assumption of the Tangled Bank hypothesis is that each clonal lineage present in the population has its own ecological niche. I used DNA markers to assign individuals to clonal lineages and found that there is no ecological difference between the different clones. Therefore, Tangled Bank mechanisms are unlikely to cause the observed ecological difference between sexual and asexual dandelions. Furthermore, the Tangled Bank hypothesis assumes a difference between the sexuals and asexuals in the amount of *intraspecific* competition (competition between individuals belonging to the same species), while my results indicate that the sexuals are better at *interspecific* competition (competition between individuals belonging to different species). Possibly, the observed ecological differentiation is caused by the difference in ploidy level between the two reproductive types rather than the mode of reproduction per se.

Genetic differentiation

Looking at the distribution of genetic variation in sexual and asexual dandelions can tell us important things about their evolutionary dynamics. For example, the amount of ecological differentiation that can evolve between the two reproductive types is determined by the amount of exchange of genetic variation between them. Such gene flow is possible because not all pollen produced by the asexuals is sterile and the pollen

can be used to pollinate sexuals, although with a low rate of success. Such pollinations have indeed been shown to take place in natural populations. If the two types exchange genetic variation at a certain level, a genetically based difference between them will not be possible.

In this thesis, I used several types of genetic markers to look at genetic diversity in sexual and asexual dandelions and found that almost all genetic variation is shared between the two types. I also found that sexuals and asexuals that grew close together were in general genetically more similar than sexuals and asexuals that grew further apart. These results indicate a high rate of gene flow between the sexual and asexual dandelions, which has important consequences for their spatial genetic structure. Gene flow between sexuals and asexuals will lead to a high diversity of clonal lineages within the asexuals. As many theories that have been proposed to explain the maintenance of sexual reproduction critically depend on a relatively low amount of variation in the asexuals when compared to the sexuals, clonal diversity is a key concept in studies on sex. It is therefore important to be able to distinguish between clonal lineages, to assign individuals to clonal lineages, and to have the statistical tools to estimate clonal diversity. As standardised methods for these tasks were missing in the research field, I wrote two computer programs to perform these tasks, and made them available to the scientific community. Using these programs for analyzing my own data, I confirmed earlier studies indicating that clonal diversity is generally very high in dandelions.

Conclusions

The cost of sex is relatively low in dandelions, but high enough to make the coexistence of sexuals and asexuals problematic to understand from an evolutionary point of view. However, the relatively low cost of sex means that a not so very powerful mechanism is needed to explain the coexistence of sexual and asexual dandelions. The consistently found difference between sexual and asexual dandelions in the preferred amount of human-induced disturbance may therefore explain for a large part the coexistence of the two reproductive types. The high amount of gene flow between the sexual and asexual dandelions makes a genetically based ecological difference between them unlikely. Possibly, the observed difference is caused by the difference in ploidy level between the sexual and asexual dandelions rather than mode of reproduction per se.

Samenvatting

Het paradoxale bestaan van seks

Voor evolutionair biologen is het bestaan van seksuele voortplanting niet vanzelfsprekend. In vergelijking met het alternatief, asexuele voortplanting, is seks nodeloos ingewikkeld en kostbaar. Er zijn verschillende redenen waarom seks zo kostbaar is vergeleken met asexuele voortplanting, maar de belangrijkste reden is dat het bestaan van meer dan één sekse noodzakelijk maakt. Een populatie van asexuele organismen bestaat gewoonlijk namelijk alleen uit vrouwtjes. Deze vrouwtjes planten zich voort zonder dat daar bevruchting voor nodig is, en de nakomelingen die daaruit voortkomen zijn genetisch identiek aan de moeder. Anders gezegd, de nakomelingen zijn allemaal “klonen” van hun moeder. Om te begrijpen waarom de productie van mannetjes als evolutionaire onkosten wordt gezien kun je je een populatie voorstellen van honderd seksuele organismen. Ervan uitgaande dat er evenveel mannetjes als vrouwtjes in de populatie zijn, zal deze populatie dus bestaan uit vijftig mannetjes en vijftig vrouwtjes. Als elk vrouwtje gemiddeld twee nakomelingen produceert, één mannetje en één vrouwtje, zal de populatiegrootte stabiel zijn. Een populatie van asexuele organismen daarentegen heeft geen stabiele grootte maar zal elke generatie verdubbelen. Dit komt omdat een asexuele populatie geheel uit vrouwtjes bestaat; als de honderd asexuele vrouwtjes in de populatie, net als de seksuele vrouwtjes, elk gemiddeld twee nakomelingen krijgen, produceren ze in totaal tweehonderd nakomelingen. Al deze nakomelingen zijn zelf ook asexuele vrouwtjes, dus nog een generatie later zal de populatie bestaan uit vierhonderd individuen. Dit verschil in groeisnelheid tussen seksuele en asexuele populaties wordt ook wel de “tweevoudige prijs van mannetjes” genoemd.

Er zijn verschillende andere redenen waarom seksuele voortplanting kostbaar is in vergelijking met asexuele voortplanting. Zo is het bijvoorbeeld noodzakelijk om een partner te vinden, wat veel energie kan kosten en wat een organisme kan blootstellen aan allerlei gevaren. Denk bijvoorbeeld aan de bloemstructuren die door planten gevormd worden om bestuivers te lokken: die kosten de plant veel grondstoffen, zoals suikers voor de productie van nectar, en maken de plant niet alleen aantrekkelijk voor bestuivers, maar ook extra opvallend voor herbivoren. Andere kosten die verbonden zijn aan seksuele voortplanting zijn wat abstracter, zoals de kosten van genoomverdunding, de kosten van recombinatie en de kosten van seksuele selectie. Al deze verschillende onkosten in ogenschouw nemend, lijkt het bestaan van seksuele voortplanting tamelijk paradoxaal. Waarom planten de meeste organismen zich dan toch voort door middel van seks?

Er zijn veel verschillende hypothesen voorgesteld om de aanwezigheid van seksuele voortplanting te verklaren, en bijna al deze hypothesen richten zich op een belangrijk verschil tussen seksuele en asexuele voortplanting: de nakomelingen van een zich seksueel voorplantend vrouwtje zijn genetisch allemaal verschillend van elkaar, terwijl de nakomelingen van een asexueel vrouwtje allemaal identiek zijn. Genetische variatie

wordt over het algemeen verondersteld een evolutionair voordeel te geven. Volgens sommige van de voorgestelde hypothesen helpt seksuele voortplanting en de daaruit voortkomende genetische variatie in het afweren van ziekten en parasieten, in het omgaan met de ecologische variatie die aanwezig is in de natuur en tegen de accumulatie van schadelijke mutaties.

Paardebloemen (*Taraxacum* sect. *Ruderalia*) vormen een goed onderzoeksobject om de verschillende hypothesen rond de evolutie van seks te bestuderen. Dit is omdat er zowel seksuele als asexuele paardebloemen zijn. De twee typen zijn morfologisch niet van elkaar te onderscheiden maar verschillen in ploëdie niveau ofwel het aantal chromosomen dat ze hebben: seksuele zijn diploïd (met twee kopieën van elk chromosoom) en asexuele zijn triploïd (met drie kopieën van elk chromosoom). In Zuidwest- en Zuidoost-Europa kunnen deze twee typen paardebloemen in gemengde populaties gevonden worden, een situatie die niet mogelijk zou zijn als de asexuelen daadwerkelijk een voortplantingsvoordeel zouden hebben.

In dit proefschrift kijk ik naar het ogenschijnlijk onmogelijke (naast elkaar) voortbestaan van zowel seksuele als asexuele paardebloemen. Daarvoor heb ik gekeken hoe groot de kosten van seksuele reproductie daadwerkelijk zijn in paardebloemen en vervolgens of de coëxistentie verklaard kan worden doordat er een verschil in ecologische voorkeur bestaat tussen de seksuele en asexuele planten. Verder heb ik de consequenties onderzocht die de coëxistentie heeft op de verdeling van genetische variatie binnen en tussen de twee typen paardebloemen.

De kosten van seksuele voortplanting in paardebloemen

De beschrijving die ik hierboven heb gegeven van de evolutionaire kosten die verbonden zijn aan het produceren van mannetjes, vormt de basis van bijna al het onderzoek naar de evolutie van seksuele voortplanting. Deze beschrijving is echter enigszins kort door de bocht; hij is namelijk alleen geldig in tweehuizige soorten waar alle individuen ofwel mannelijk zijn ofwel vrouwelijk. De meeste planten, waaronder paardebloemen, zijn daarentegen hermafrodiet: alle individuen zijn zowel mannelijk als vrouwelijk. Dit heeft belangrijke consequenties voor de kosten van seksuele voortplanting, want als seksuele planten hermafrodiet zijn, kunnen hun asexuele soortgenoten dat ook zijn. In dat geval hebben de asexuelen naast hun vrouwelijke functie (het produceren van eicellen en de daaruit voortkomende zaden) ook nog hun mannelijke functie (het produceren van stuifmeelkorrels) en zijn er voor de seksuelen dus geen méerkosten verbonden aan hun mannelijke functie. De andere kosten van seksuele voortplanting kunnen echter nog steeds van toepassing zijn. De meeste asexuele paardebloemen zijn hermafrodiet en produceren dus stuifmeel, hoewel ze zelf geen bestuiving nodig hebben om zaad te produceren. Het door asexuelen geproduceerde stuifmeel is voor het grootste deel onvruchtbaar en de productie ervan kan dus worden gezien als een verspilling van grondstoffen en energie. Het is daarom verbazingwekkend dat natuurlijke selectie de productie van stuifmeel

in asexuelen niet gestopt heeft, want de verspilde grondstoffen zouden ook gebruikt kunnen worden voor de productie van meer of beter zaad.

Ik heb daarom de zaadproductie vergeleken van paardebloemen die stuifmeel produceren en paardebloemen die geen stuifmeel produceren en heb gevonden dat er inderdaad een dergelijke verplaatsing van grondstoffen plaatsvindt. Mannelijk steriele paardebloemen produceerden gemiddeld 38% meer zaadhoofdjes (“pluizenbollen”) dan paardebloemen die wel stuifmeel produceerden. Er was echter geen verschil in het aantal zaden per hoofdje, zaadgewicht en het percentage gekiemde zaden. Gezien het verschil in het aantal geproduceerde hoofdjes zou er potentieel een grote kostenpost zijn voor de seksuele paardebloemen als alle asexuele mannelijk steriel zouden zijn geweest. Deze potentiële kosten zijn echter niet gerealiseerd omdat maar een klein percentage van de paardebloemen mannelijk steriel is. Daarom zijn de kosten van de mannelijke functie in seksuele paardebloemen laag.

Om te kijken of de coëxistentie van seksuele en asexuele paardebloemen inderdaad zo paradoxaal is, heb ik ook alle andere kosten van seksuele voortplanting bij paardebloemen bekeken. Ik heb gevonden dat de totale kosten van seks in paardebloemen waarschijnlijk een stuk lager zijn dan vaak wordt aangenomen in studies over de evolutie van seks. De totale kosten zijn echter hoog genoeg om de coëxistentie “paradoxaal” te maken, maar omdat ze relatief laag zijn, hoeft het mechanisme dat de coëxistentie veroorzaakt niet erg sterk te zijn.

Ecologische differentiatie

Langdurige coëxistentie, op een evolutionaire tijdschaal, van seksuele en asexuele planten is mogelijk als er een bepaalde mate van ecologische differentiatie is tussen de twee typen, als de habitats van de seksuele enigszins afwijkt van die van de asexuele planten. In verschillende hoofdstukken van dit proefschrift heb ik het bestaan van een dergelijke habitatdifferentiatie tussen de seksuele en asexuele paardebloemen onderzocht. Eerst heb ik habitatdifferentiatie op regionale schaal bekeken door de vegetatie van verschillende paardebloempopulaties rond Neuchâtel, Zwitserland, met elkaar te vergelijken. Het bleek dat populaties met een hoog percentage aan seksuelen in andere vegetaties voorkwamen dan populaties met een hoog percentage aan asexuelen. De vegetaties met veel asexuele paardebloemen vertoonden aanwijzingen voor veel verstoring en de vegetaties met veel seksuele paardebloemen vertoonden aanwijzingen voor relatief weinig verstoring.

Vervolgens heb ik getest of een dergelijke ecologische differentiatie ook binnen een enkele populatie te vinden is en vond dat ook op een zeer kleine geografische schaal de asexuelen op meer verstoorde plekken te vinden zijn dan de seksuelen. Daarnaast heb ik gevonden dat een daling in de hoeveelheid verstoring gedurende een periode van veertien jaar heeft geresulteerd in een stijging van het percentage seksuelen in de paardebloempopulatie. Dit alles wijst er op dat seksuele paardebloemen een voorkeur hebben voor een habitat die relatief stabiel is dan de habitat van asexuele paardebloemen.

De gevonden ecologische differentiatie zou verklaard kunnen worden door de zogenaamde “Tangled Bank”-hypothese. Deze hypothese stelt dat als er competitie is om schaarse grondstoffen, er minder competitie is tussen de seksuele individuen onderling dan tussen de asexuele onderling. Seks resulteert immers in genetisch variabele nakomelingen, die daardoor gezamenlijk een breder ecologisch bereik hebben dan de minder variabele nakomelingen van asexuelen. Natuurlijke selectie kan er vervolgens voor zorgen dat de ecologische niche van de seksuelen zich verwijderd van de niche van de asexuelen. Een aanname van de Tangled Bank-hypothese is dat elke klonale lijn die in de asexuele populatie aanwezig is, een eigen ecologische niche heeft. Ik heb DNA-merkers gebruikt om asexuele paardebloemen in klonale lijnen te groeperen, en vervolgens gevonden dat de verschillende klonale lijnen ecologisch niet van elkaar verschillen. Het is dus onwaarschijnlijk dat Tangled Bank-mechanismen de gevonden ecologische differentiatie tussen seksuelen en asexuelen veroorzaken. Daarnaast veronderstelt de Tangled Bank ook in de seksuele en asexuele individuen een verschil in de hoeveelheid *intraspecifieke* competitie (competitie binnen een soort), terwijl mijn resultaten erop duiden dat de seksuele paardebloemen beter zijn in *interspecifieke* competitie (competitie tussen soorten). Een alternatieve verklaring is dat de gevonden ecologische differentiatie tussen de twee typen paardebloemen direct veroorzaakt wordt door het verschil in ploëdieniveau.

Genetische differentiatie

Het bestuderen van de verdeling van genetische variatie binnen en tussen seksuele en asexuele paardebloemen kan belangrijke inzichten opleveren in de evolutionaire dynamiek tussen de twee typen. Zo kan bijvoorbeeld natuurlijke selectie alleen tot een ecologische differentiatie tussen de twee typen leiden als er voldoende reproductieve isolatie is tussen de seksuelen en asexuelen. Het blijkt echter dat er genenuitwisseling plaatsvindt tussen de twee typen. Het stuifmeel dat geproduceerd wordt door de asexuelen is weliswaar grotendeels onvruchtbaar, maar toch kan het voorkomen dat seksuele paardebloemen erdoor bestoven worden. Hoewel zulke bestuivingen over het algemeen maar weinig zaden opleveren, is toch gebleken dat ze plaatsvinden in natuurlijke paardebloempopulaties.

Ik heb verschillende typen genetische merkers gebruikt om de genetische variatie in paardebloemen te bekijken. Het bleek dat bijna alle genetische variatie werd gedeeld door de twee planttypen. Ook heb ik gevonden dat seksuelen en asexuelen, die in elkaars nabijheid groeiden, over het algemeen meer verwant aan elkaar waren dan seksuelen en asexuelen die verder van elkaar vandaan stonden. Deze resultaten duiden allemaal op een hoge mate van genenuitwisseling tussen de seksuele en asexuele paardebloemen, met belangrijke gevolgen voor de ruimtelijke genetische structuur binnen paardebloempopulaties. De genenuitwisseling leidt er ook toe dat er constant nieuwe klonale lijnen gevormd worden en er dus een hoge klonale diversiteit is binnen de asexuele paardebloemen. Veel van de hypothesen die de aanwezigheid van seksuele

voorplanting verklaren, gaan uit van een lage klonale diversiteit binnen de asexualen. Klonale diversiteit is dus een belangrijke factor in onderzoek naar asexualen organismen. Desondanks zijn er weinig gestandaardiseerde methoden in gebruik om klonale lijnen te herkennen, en vervolgens klonale diversiteit te meten. Ik heb daarom twee computerprogramma's geschreven om deze taken uit te voeren en ze beschikbaar gesteld aan de wetenschappelijke gemeenschap. Ik heb deze twee programma's ook gebruikt om mijn eigen data te analyseren en zo de resultaten van eerdere studies bevestigd dat de klonale diversiteit in paardebloemen inderdaad erg hoog is.

Conclusies

De evolutionaire kosten van seksuele voortplanting zijn relatief laag in paardebloemen. Dit betekent dat er voor de coëxistentie van seksuele en asexualen paardebloemen ook niet heel erg sterke mechanismen aanwezig hoeven te zijn. De ecologische differentiatie die ik meerdere malen gevonden heb tussen seksuelen en asexualen is inderdaad niet heel erg sterk, maar kan de coëxistentie mogelijk al voor een groot deel verklaren. De hoge mate van genenuitwisseling tussen de seksuele en asexualen paardebloemen maakt het onwaarschijnlijk dat de gevonden ecologische differentiatie een genetische oorzaak heeft. Het is echter mogelijk dat de ecologische differentiatie wordt veroorzaakt door het verschil in ploëdieniveau tussen de seksuele en asexualen paardebloemen.

Introduction: Coexistence of sexual and asexual dandelions

1



Patrick G. Meirmans

Sex and reproduction

Reproduction is a central element in every definition of life on earth. It is therefore probably not surprising that a large array of different modes of reproduction exists in nature. In eukaryotes, these reproductive modes are variations on two main themes: sexual and asexual reproduction. Of these two, sexual reproduction is by far the most common among higher organisms. This ubiquity of sex is, however, quite paradoxical considering that asexual reproduction seems much more efficient. The whole process of going through meiosis, finding a partner and diluting your genetic material seems overly complex compared to just budding off some cells. Indeed, so many costs are thought to be connected to sexual reproduction that it is puzzling that first of all sex has ever evolved, and second that it has been maintained for millions of years. In particular, Williams (1975) and Maynard Smith (1978) pointed to this problem. Numerous theories have been proposed that attempt to explain the dominance of sexual reproduction, and many biologists still think that the so-called “paradox of sex” is “the queen of problems in evolutionary biology” (Bell, 1982).

In this thesis I focus on the coexistence of sexuals and asexuals within a single species complex: dandelions (*Taraxacum* sect. *Ruderalia*). Any explanation for such coexistence is largely dependent on the realised cost of sex in dandelions. If there is a large cost of sex in dandelions, a strong mechanism is needed to explain the coexistence. On the other hand, when the cost of sex is zero, the problem turns into a problem of species coexistence, which would require a less strong mechanism. I therefore start this introduction by reviewing the most important costs of sex that have been recognised (the following overview mainly stems from Crow, 1999; Lewis, 2001; Meirmans, 2005). I will use this review in my overall discussion (Chapter 8) to discuss which of the suggested costs of sex are actually realised in dandelions, using data and insights from my own research and that of others.

After discussing the several costs of sex in this introduction, I discuss some of the many theories that have been proposed to explain the paradox of sex. My own work focuses on possible niche differentiation between the sexual and asexual dandelions, which might be placed under the Tangled Bank hypothesis. For comparison, I nevertheless think it is good to place this hypothesis among some of the other hypotheses for the maintenance of sex. I then go on to discuss two topics that I think are important in studies of sexual versus asexual reproduction: clonal diversity and polyploidy. Though I acknowledge that there are several other topics of equal importance for the study of sex, I have a special interest in these two, and think that they are especially worth exploring when looking at sex in dandelions. After this discussion on the theory around the maintenance of sex, I describe the study species, dandelions, in more detail.

The paradox of sex

The costs of sex

When the cost of sex is addressed, often only one of the many possible costs is mentioned: the cost of males, also known as the “two-fold-cost-of-sex”. The emphasis on a two-fold cost is somewhat awkward as the cost of males is not necessarily always two-fold and, more importantly, there are other costs to sex that may be even more influential. The size of all these costs to sex and their applicability can vary considerably between species. It is therefore preferable that any study on the benefits of sex in a particular species is accompanied by a study of the cost of sex in that species.

1). The cost of males and the cost of genome dilution.

These are the most often mentioned costs of sexual reproduction and, confusingly, both are known as the “two-fold-cost-of-sex”. The cost of males stems from the notion that an asexual population in which females produce daughters asexually has twice the growth rate of a sexual population in which females produces both daughters and sons sexually (Maynard Smith, 1978). The cost of genome dilution (also known as the “cost of meiosis”) is based on the notion that asexuals transfer their complete genome to the next generation, while sexuals “dilute” their genome by going through meiosis and subsequently fusion of gametes. The cost of males and the cost of genome dilution have often been confused, and there has been discussion whether or not they are actually the same (Lewis, 2001). It appears that the two costs are not the same but they are actually interdependent and, in some cases, mutually exclusive (Charlesworth, 1980; Joshi, Moody, 1998). Joshi and Moody (1998) made a model that specifically looked at asexuality in hermaphrodites; this enabled them to tear apart the cost of males and the cost of genome dilution. As their model shows some important aspects of the cost of males and the cost of genome dilution that are very well applicable to dandelions, I will discuss it here in a bit more detail.

Joshi and Moody (1998) showed that there is no cost of genome dilution if sexuals are hermaphrodites and asexuals are females. Imagine a situation in which the male function of the sexual hermaphrodites comes at entirely no cost to the individuals. In that hypothetical case there would be no cost of sex as both types effectively reproduce their whole genome: the asexuals entirely through their female function, the sexuals half through their female function and half through their male function. However, in real life sexual hermaphrodites invest resources in their male function; female asexuals do not have any male reproductive output, and may be able to reallocate these resources into the production of more offspring (Figure 1a). The difference in total reproductive output between the sexuals and asexuals constitutes the cost of males; the observed genome dilution is only a side effect of the cost of males and is, under this scenario, not a cost of sex in itself. There is also only a cost of males in dioecious species, where the sexuals

invest about fifty percent of their resources in the male function with the male function packaged in a separate individual (Roughgarden, 1991).

In the other extreme, the asexuals are hermaphrodites rather than females, i.e. they produce asexual offspring through their female function, but also invest in male function, enabling them to father some of the offspring of the sexuals. In this case, there is no difference in the cost of males if both sexuals and asexuals invest equally in male reproductive output, but there is a cost of genome dilution. The sexuals reproduce their entire genome once, one half through their female function and one half through their male function. The asexuals on the other hand reproduce their genome one-and-a-half times, their whole genome through their female function and half their genome through their male function (Figure 1b). This phenomenon of a “one-and-a-half-fold” cost of sex in hermaphrodites has been observed before by Charlesworth (1980).

Joshi and Moody (1998) furthermore found that, apart from these two extremes,

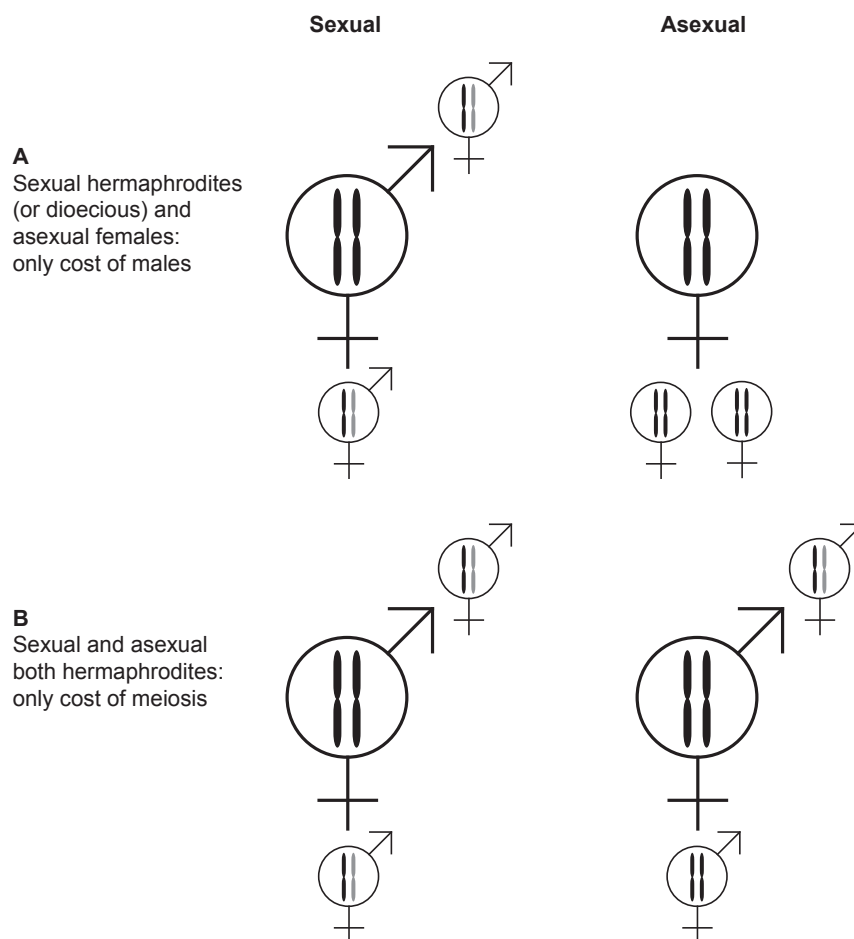


Figure 1: The cost of sex in hermaphrodites under two scenarios. a). The sexuals are hermaphrodites, the asexuals are females. Here the cost of sex stems from the production of males. Though there is genome dilution, the difference between the two modes of reproduction stems only from the reallocation of resources from the male function to the female function by the asexuals (Joshi, Moody, 1998). b). The sexuals and asexuals are all hermaphrodites. In this case, the cost of sex stems from genome dilution as the sexuals reproduce half of their genome through their female function, while the asexuals reproduce their whole genome. Both sexuals and asexuals also reproduce half their genome through the male function.

the total cost of sex is a combination of both the cost of males and the cost of genome dilution. The size of the cost then depends on the proportions of sexuals and asexuals in the population, the relative investment in the male function of sexuals and asexuals and the success with which male gametes (or pollen) produced by asexuals can fertilise sexual eggs. In general, the total cost of sex is highest, and can even exceed two for certain parameter combinations, when the asexuals have an intermediate level of male reproductive output, i.e. when they produce less male gametes than the sexuals, but more than zero. Joshi and Moody (1998) suggested that asexual hermaphrodites might be selected to have such an intermediate level of male reproductive output. In my view, however, the latter argument is difficult as it requires group selection: an asexual that produces less male gametes and instead puts the saved resources into the production of more eggs (this trade-off is one of the main assumptions of Joshi and Moody's model) will have a fitness benefit over the other asexuals, even if it lowers the cost of sex. This fitness benefit will be most pronounced if the male gametes that are produced by the asexuals have little success in fertilising sexual eggs. Asexual hermaphrodites are therefore expected to minimise their male reproductive output.

2). *The cost of sexual selection.*

Sexual selection can lead to costly maladaptive traits, the textbook example is the tail of the peacock; male peacocks are overtly visible to predators and the long tail makes them less agile than the females, and therefore less likely to escape these predators. Sexual selection is not limited to the excessive displays of secondary sexual characters in males, like in the peacock. In many species (e.g. *Drosophila*), males induce harm to their mating partners by inseminating chemicals together with their sperm. These chemicals are supposed to raise the possibility of the sperm fathering the female's eggs, but at the cost of a decreased lifespan of the female. Examples of traits under sexual selection abound, and as sexual selection often opposes natural selection, it is therefore thought to be costly to the male or female.

Related to the cost of sexual selection is the cost of having a combined male/female genome (Rice, 2002). Females might be better at being females if they would not need to have the potential to make males. Certain traits that would be advantageous when expressed in females may be not selected for when they are disadvantageous when expressed in males, a phenomenon that is known as intersexual ontogenetic conflict (Rice, Chippindale, 2001). An interesting example of this is a recent study that shows that homosexuality in males (which in an evolutionary sense is a disadvantageous trait) is associated with a higher fertility in females (Camperio-Ciani *et al.*, 2004).

3). *The cost of finding a mate.*

Sexual species have to spend considerable energy in finding a suitable mate (sperm has to find eggs, pollen has to find a stamen), which can be difficult when densities are low, or when individuals are not very mobile; a potentially very fit individual may not realise

this potential simply because it did not manage to find enough mates. In addition, advertising or looking for a mate often leads to extra costs and, through extra exposure, also to an increased predation risk. Plants produce costly flowers, nectar and odours to attract pollinators, which also make them more noticeable (e.g. to large herbivores eating whole flowers or inflorescences, to insects laying eggs on flowers or ovaries, and to humans picking flowers). A large part of the pollen may get lost through pollen predation and, especially in wind or water pollinated species, large amounts of pollen may simply get lost without reaching a mate. The latter argument also holds for animals that release their sperm and eggs directly in the water (Lewis, 2001).

4). The cellular-mechanistic cost of meiosis and syngamy.

Energetically, meiosis is not a very efficient process, compared to double mitosis (Lewis, 2001); it involves complex molecular machinery that is vulnerable to errors. The same holds for syngamy, the fusion of gametes. These mechanistic costs are mainly important in small organisms as the relative cost of meiosis is rather large in unicellular organisms but negligible in multicellular ones. Both meiosis and syngamy also lead to a time delay, relative to asexual reproduction. This too is mainly important in small organisms, for instance when the speed of reproduction is essential in the colonisation of empty habitat.

5). The cost of recombination and segregation.

Sex and recombination break up linkage between genes, which may be co-adapted or between which there are beneficial epistatic interactions. A different way to put this is to say that in asexuals selection acts on the total genetic variance, whereas in sexuals it acts only on the additive component of the genetic variance. This makes short-term selection more effective in asexuals. Of course, this cost depends on the amount of positive epistatic interactions between loci and on the amount of co-adapted gene complexes that can be broken down by recombination. Another effect of segregation is that in sexuals heterozygosity is constantly broken down, whereas in asexuals fixed heterozygosity is possible. In case of a heterozygote advantage at a locus, the frequency of heterozygotes in a sexual population will at most be 50%; in an asexual population, this can be 100%. The cost of unfavourable homozygotes can be regarded as some form of genetic load. If overdominance takes place at a large number of loci, many individuals will be homozygous for many loci, which may seriously hamper their survival. It remains doubtful therefore, whether there exist indeed many loci at which such overdominance takes place.

6). The cost of a balanced set of chromosomes.

Meiosis makes it impossible for certain cytological types to persist in a sexual population, such as triploids, aneuploids, and certain karyotypes with inversions of translocations.

In some cases, an unbalanced set of chromosomes may provide a fitness benefit to an individual. However, if this individual reproduces sexually it may not be able to pass the set on to the next generation. If, for example, a new beneficial karyotype arises in a sexual population, most likely all possible partners will have another, incompatible, type and the beneficial type therefore has difficulty to spread. In asexuals, fixation of a beneficial karyotype can take place without such difficulties. In mice, many different karyological races are known, most of which have only a very small geographic distribution. Hybrids between such karyological races are usually less fertile, or inviable (Hauffe, Searle, 1998).

7). Cost of hybridisation and inbreeding.

Sexual reproduction has the inherent risk that the mating takes place with an individual from another species, another ploidy level or another reproductive type. In the other extreme, the individual mates with itself, or a close relative. Such matings often lead to less viable or infertile offspring, and can therefore incur a considerable cost. The cost of hybridisation is especially large if the other species or type is dominant in the population (Levin, 1975, also see the part on polyploidy below). Hybridisation may already be detrimental when taking place between individuals from two different populations of the same species if both populations are adapted to local conditions, a phenomenon that is known as outbreeding depression.

8). Sexually transmitted diseases and transposons.

Sexual species are prone to sexually transmitted diseases and harmful transposons, whereas asexual species are not or less vulnerable. The link between sexual reproduction and transposons is suggested by the observation that in bdelloid rotifers, a group of small animals that are supposed to have been asexual for about 100 million years, no traces of any known transposons have been found despite a large research effort to find them (Arkhipova, Meselson, 2000).

Potential and realised costs

Given all of the above-mentioned costs, the persistence of sex is indeed paradoxical. On the other hand, it is remarkable that many of the above phenomena are both mentioned as a cost and as a benefit of sex, depending on the context. In particular, recombination and segregation are not only mentioned as costs but also as benefits since they lead to genetically variable offspring, which is a key element in most theories that have been proposed to explain the maintenance of sex. Mating between individuals from different populations does not necessarily lead to outbreeding depression, but can also lead to heterosis (outbreeding vigour); which of the two is taking place is determined by the degree of local adaptation and the amount of inbreeding.

It is also important to note that all mentioned costs of sex are *potential* costs that are

not necessarily *realised* when a newly arisen asexual lineage goes into competition with its sexual source population. For example, the new asexual lineage would still express all the now maladaptive traits that had previously been used to attract mates or pollinators. It may take a long time for the necessary mutations and selection to remove these traits, and it may even be impossible to remove them completely because of morphological or developmental constraints. Because of such constraints, and because many costs are dependent on the life history of species, the realised total cost of sex is strongly species-dependent (Meirmans, 2005). Nevertheless, only the two-fold cost is mostly used, as a baseline for theories explaining the maintenance of sex, possibly because this is the only cost that is easily quantifiable.

Despite the observation that not all costs of sex are always realised, the ubiquity of sex remains paradoxical, as there are simply so many costs that in every species some of them will be realised. To solve the paradoxical ubiquity of sex, many theories have been proposed. Most of these theories fall into one of two main categories (West *et al.*, 1999): mutational and ecological theories. Most mutational theories suggest that sexual reproduction may provide a fitness benefit as it helps to purge deleterious mutations from populations. Ecological theories focus on the aspect of mixis, emphasising that genetic variation resulting from sexuality helps in dealing with the huge variation in ecological interactions species are engaged in.

Mutational theories

The conceptually simplest mutational theory is “Muller Ratchet” (Muller, 1964), which states that in small populations of asexuals, stochastic processes lead to the accumulation of slightly deleterious mutations. In such small populations, the class of individuals without any deleterious mutations will eventually go extinct due to stochastic processes; the best class then consists of individuals with only one deleterious mutation. This class will ultimately also get lost through stochastic processes, leaving a best class consisting of individuals with two mutations. This process is irreversible and leads to a continuing accumulation of deleterious mutations, to a point where the fitness gets so low that the population goes extinct. Muller’s Ratchet has been criticised to work only for population sizes that are much smaller than usually observed in nature. Rice (2002) argued that this is indeed true for the original description of the Ratchet where all deleterious mutations have the same effect, but that the Ratchet does work for very large populations when a more realistic, leptokurtic, distribution model of mutational effects is used. A more valid argument against the importance of Muller’s Ratchet to maintain sex is that it operates on relatively long evolutionary timescales; in mixed sexual and asexual populations, asexuality will have gone to fixation long before the effects of the Ratchet kick in (West *et al.*, 1999).

A mutational model that does provide a short-term explanation for the maintenance of sex is the “mutation deterministic model” (Kondrashov, 1988). This model provides a deterministic version of Muller’s Ratchet, i.e. it does not depend on population size

and the stochastic loss of mutation free lineages. The two main assumptions that give sexuals an advantage in the model are epistatic interactions between different deleterious mutations and a high input of new deleterious mutations per generation. The epistatic interactions make that the effect of having two deleterious mutations is bigger than twice the effect of the single mutations, which gives sexuals a benefit in producing a progeny that is fitter than that of the asexuals. The evidence that negative epistasis is indeed acting in this way is as yet scarce. Experimental studies show that the distribution of epistatic interactions between mutations is in general symmetric around a mean of zero, meaning that negative epistasis is as frequent as positive epistasis, and in that case the model does not work (Rice, 2002). The second assumption of the model is that, to counteract the two-fold cost of sex, the number of mutations per genome per generation should be more than one, which may not always be the case (Rice, 2002). However, this threshold value of one mutation depends on other parameters in the model and, as discussed above, the cost of sex does not necessarily have to be two-fold. Another type of mutational model works without epistasis, but involves assortative mating for fitness in sexuals (Agrawal, 2001); when the most fit individuals mate with each other, a class of superfit, mutation-free, offspring is constantly produced.

Ecological theories

The best supported ecological theory explaining the maintenance of sex is the “Red Queen” (Jaenike, 1978; Hamilton, 1980). This theory states that sex recreates rare genotypes, which might be advantageous if selection fluctuates over time. Temporarily fluctuating selection can for example be due to interactions with other species that are under selection themselves. The most important of such interactions are thought to be between host and parasites, even though other types of interspecific interactions can also be of importance. Under the simplest scenario, all asexually reproducing individuals are members of the same clone, and therefore have the same genotype for loci that are important for the interspecific interaction (e.g. resistance loci). In that case, it is easy for parasites to adapt to that one genotype. Sexually reproducing individuals would be able to escape parasitism due to variation, generated by mixis, at the same resistance loci. However, the virulence of the parasite needs to be quite high for Red Queen dynamics to counteract a two-fold cost of sex. Furthermore, the Red Queen works best when the asexual population has a low clonal diversity: if there is a high clonal diversity, it is hard for parasites to specialise (West *et al.*, 1999). For example, clonal diversity can be maintained if the host species has a metapopulation structure and in such a case, the Red Queen does not work to maintain sexual reproduction (Judson, 1997). In such metapopulations, the frequency dependent selection resulting from the interactions with parasites can actually help to maintain clonal diversity at an even higher level. Using molecular markers, clonal diversity has indeed been found to be high in a large number of asexually reproducing species (Ellstrand, Roose, 1987), rendering the maintenance of sex through Red Queen dynamics problematic for at least those species.

The Tangled Bank hypothesis for the maintenance of sex (Ghiselin, 1974; Bell, 1982; Case, Taper, 1986) states that sex may be advantageous in cases of intensive competition for multiple, scarce, resources. In asexuals, all members of a clone are thought to have the same ecological niche; therefore, they all compete for the same resources. This competition between clone mates provides an advantage to individuals that can use underexploited resources. Sexual reproduction leads to polymorphic offspring and therefore to the creation of individuals that can use these underexploited resources. The Tangled Bank hypothesis has long been one of the most favored hypotheses for the maintenance of sex, but has lost a lot of its following, mainly due to a single paper. Burt and Bell (1987) looked at the correlation between chiasma frequency (frequency of recombination) and litter size in mammals. Their reasoning was that under the Tangled Bank, chiasma frequency is expected to be positively correlated with litter size, as sib-competition is thought to be more intense for large litters. What they found however was that chiasma frequency was correlated with generation time (which is itself negatively correlated with litter size), which supports the Red Queen hypothesis as when generation time is long, antagonists have more time to adapt, and to counteract this a high frequency of recombination is needed. Burt and Bell (1987) therefore claimed that the Red Queen and not the Tangled Bank best explains the maintenance of sex. Interestingly, when doing the same analysis on plants, Koella (1993) found that the only hypothesis that could explain the found correlations was the Tangled Bank, and not the Red Queen.

An attractive quality of the Tangled Bank hypothesis is that coexistence of sexuals and asexuals is easily obtained (Case, Taper, 1986), while most other models lead to the fixation of just one of the two reproductive types (see below). A problem of the Tangled Bank hypothesis is that it, like the Red Queen, does not work when clonal diversity is high. Under high clonal diversity, it is harder for the sexuals to fill in the gaps between the “frozen” niches of the asexuals, as there are simply less gaps. However, the vulnerability of the Tangled Bank hypothesis to clonal diversity can be solved by adding for every clone an extra dimension to the niche-space (Weeks, 1993).

A pluralist view

Although the theories mentioned above are all able to explain the persistence of sexual reproduction, the conditions under which they are able to do so are often quite restrictive. Therefore West *et al.* (1999) advocated a pluralist approach in which different theories are combined. An example of such a combined model is the work of Howard and Lively (1998), who combined the Red Queen with Muller's Ratchet and discovered that there is a large synergy effect to the combination. In the model, the Red Queen dynamics forces the different clones repeatedly through bottlenecks which lowered their effective population sizes and created more opportunity for the Ratchet to operate. Because of this, sex could be maintained for lower, and therefore more realistic, levels of virulence of the parasite.

Coexistence

The single most important element concerning the maintenance of sex is the arrival of asexually reproducing individuals in a sexually reproducing species. The speed at which this happens determines to a large extent whether the ubiquity of sexual reproduction is paradoxical at all: if mutations to asexuality are very rare, sex will be the dominant mode of reproduction in nature even if asexuals quickly outcompete their sexual relatives (Maynard Smith, 1978). This will be even more so if asexual reproduction is an evolutionary dead-end, e.g. due to the accumulation of deleterious mutations. Such a scenario would explain the observed phylogenetic distribution of asexuality in the tree of life; asexual taxa are usually at the tips of the tree, whereas very few completely asexual families and hardly any asexual orders exist (Burt, 2000). There is a good reason why mutations to asexuality should be very rare: such mutations are likely to be very complex; simply substituting meiosis for mitosis would not work. In dandelions, for example, apomixis is known to consist of three separate mechanisms: unreduced meiosis (diplospory), autonomous embryo development (parthenogenesis) and autonomous endosperm development. It has been shown that in dandelions those three processes are controlled by more than one locus (Vijverberg *et al.*, 2004). As the separate processes would be a disadvantage to an otherwise sexually reproducing individual, the start of asexual reproduction in dandelions must have involved more or less simultaneous mutations at several loci, which is likely to be very rare indeed.

Considering such evolutionary constraints to evolving asexuality, the real paradox of sex is the coexistence of sexuals and asexuals within a single species or species complex. Such coexistence has been observed for many taxa where asexuality is present (Bierzychudek, 1987). Of course, it might be possible that the observed coexistence is simply a transient state in the way to fixation of asexuality. In that case, it would not be necessary to invoke any mutational or ecological theory of sex. However, considering the fitness benefit that asexuality is thought to provide, even if the cost of sex is lower than two-fold, fixation should be extremely rapid. This rapid fixation makes it dubious whether all observed cases of coexistence are such transient states.

Though the Red Queen and mutational models for the maintenance of sex have received the most support, they provide very little space for coexistence between sexuals and asexuals. The two models mostly result in fixation of either sexuals or asexuals. Under the Tangled Bank, however, coexistence is easily achieved, especially when only a few different clones are present. In that case, the combined niches of the clones then take up only a part of the total niche space, leaving enough room for the sexuals (Figure 2). When modelling this mechanism, Case and Taper (1986) have shown that existence of sexuals and asexuals is most likely 1) when clones have limited plasticity for the ecological interactions, 2) when there is severe resource exploitation, 3) when there is niche differentiation between the sexuals and the asexuals 4) when sexual individuals have a wider niche than asexual individuals. The working of the Tangled Bank heavily relies on the presence of “Frozen Niche Variation”, the idea that every clone has its own

separate niche space. Tests of the Tangled Bank through testing niche differentiation and difference in niche width between sexuals and asexuals should therefore include tests of the Frozen Niche Variation hypothesis as well.

Clones and diversity

Clonal diversity is a key element in studies regarding the maintenance of sex, as it can have important consequences for both the mutational and ecological models for sex. On one hand, the accumulation of deleterious mutations (Muller's Ratchet) may be speeded up under high clonal diversity (Pound *et al.*, 2004). The Ratchet works independently for every clone and as clone sizes are in general smaller when clonal diversity is high (given a certain total population size) the fixation of deleterious mutations should be higher when diversity is high. On the other hand, Red Queen and Tangled Bank mechanisms stop working when clonal diversity is high as these models depend on sex to create diversity that is assumed not to be present in the asexuals. For the Tangled Bank, this problem

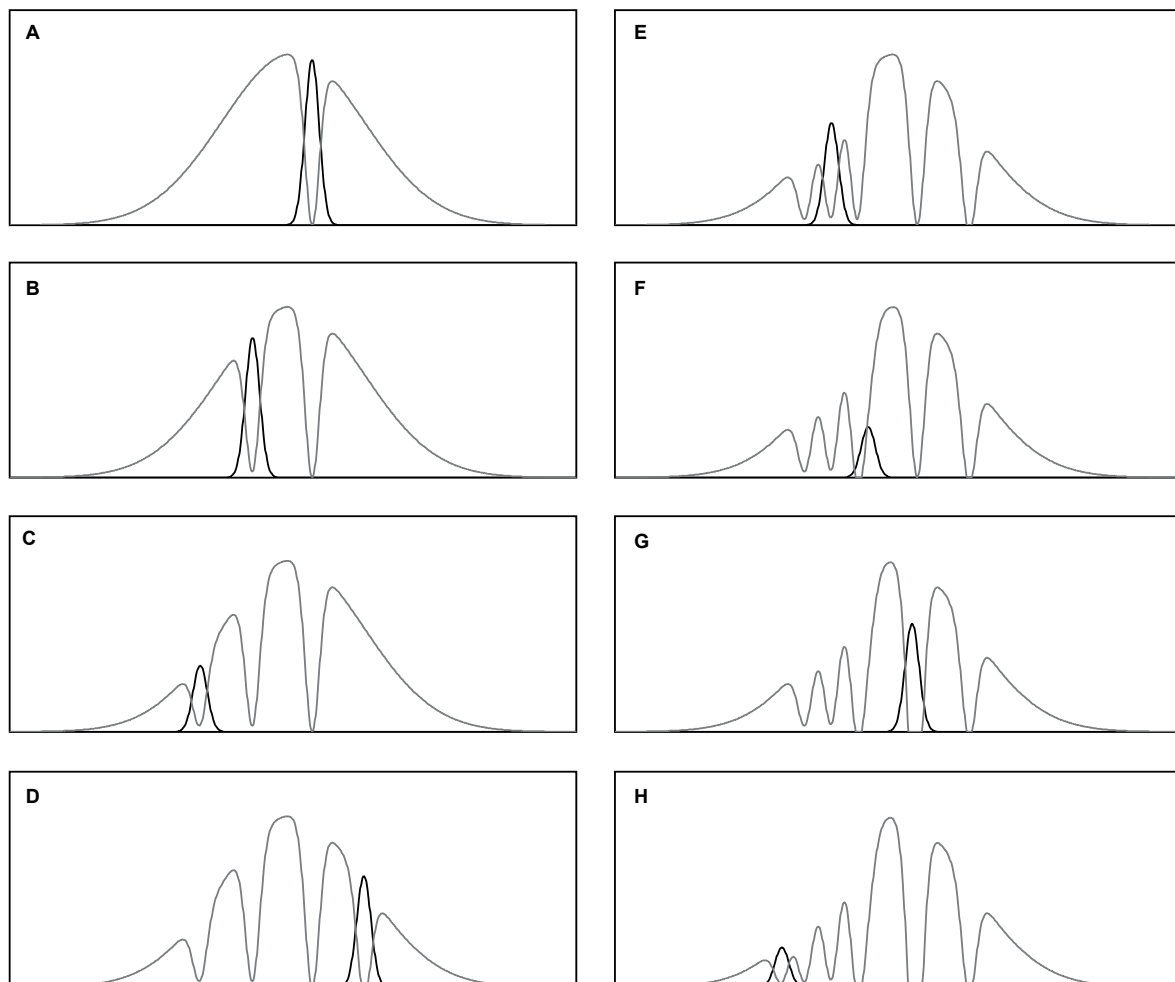


Figure 2: A simplified representation of the Tangled Bank Model. When a single clonal lineage invades a population of sexuals (A), the niche space taken up by the asexuals (black curve) represents only a part of the total niche, leaving enough niche space for the sexuals (grey curve). As more clonal lineages invade the population (B-H), increasingly less niche space is left for the sexuals. In figures B-H, the niche curves of previously invaded clonal lineages are left out, for clearer representation.

can be solved by adding extra dimensions to the niche space (Weeks, 1993), though it is doubtful whether this is a viable approach for populations containing hundreds or even thousands of different asexuals clones (e.g. Van der Hulst *et al.*, 2000).

Initially, little attention was given to the idea of clonal diversity; until the rise of molecular methods in population genetics, genetic variation was thought to be low in all species, but especially so in asexuals. Even after the introduction of allozyme techniques in the beginning of the 1970's, when the overall genetic variation in sexual species was found to be surprisingly high, people still thought that asexually reproducing organisms would be an exception to this. However, when Ellstrand and Roose (1987) reviewed the literature on the genotypic diversity in clonal plant species, they found it to be surprisingly high. Since then, even higher diversity has been found after the introduction of highly variable PCR-based genetic markers.

There are two major explanations for the high level of diversity that is usually found in asexual species (Robinson *et al.*, 2002): 1) Niche differentiation between the different clonal lineages (the above-mentioned Frozen Niche Variation hypothesis (Vrijenhoek, 1979; Vrijenhoek, 1989) and 2) continuous creation of new lineages through hybridisations with sexual relatives. Evidence has been found for both these mechanisms, often in the same species (e.g. in the freshwater snail *Potamopyrgus antipodarum* (Jokela *et al.*, 2003)). In such cases, it is questionable whether the niche differentiation actively maintains any of the clonal diversity, or whether all diversity is due to the hybridisations with the sexual relatives. The ability of the Frozen Niche Variation hypothesis to maintain variation has however been shown in *Penthaleus major*, an earth mite without any known sexual relatives (Robinson *et al.*, 2002).

If the clonal diversity arises from hybridisations with sexual relatives, these hybridisations have important consequences for the mutational models for the maintenance of sex; they help to purge deleterious alleles from the population of asexuals. Even though the purging is slower through this process than through full sexuality, it may be more than enough for the asexuals to win the competition with the sexuals. In fact, theoretical studies have shown that a little bit of sex can be enough to purge deleterious mutations and can be more advantageous than having sex every generation (Green, Noakes, 1995; Hurst, Peck, 1996).

Although (or maybe because) clonal diversity is an important concept in studies of asexual organisms, there is a plethora of different indices used to describe it, most of which have an estimation bias. Furthermore, different authors use different concepts of what they consider a "clone". This makes it difficult to compare diversity between studies.

Polyploidy

There is an important link between asexuality and polyploidy: almost all asexual plants and a large number of asexual animals are polyploid (Stebbins, 1980). It is not exactly known where this relationship stems from, though it has often been suggested that the

polyploid asexuals are allopolyploids that originate from hybridisation between species. The reasoning behind this is that allopolyploid offspring, being hybridogeneous by definition, are thought to be able to maintain themselves on evolutionary timescales only if they somehow manage to reproduce asexually. However, not all asexual polyploids are allopolyploids and therefore the result of hybridisation; in these cases the link between asexuality and polyploidy must have a different basis.

Apart from asexuality issues, polyploidy is of significant evolutionary importance in itself as a large percentage of plant species is polyploid or of polyploid origin (Soltis *et al.*, 2004); only a small part of these polyploids reproduce asexually. Like the coexistence of sexuals and asexuals, also the coexistence of different ploidy levels within one (sexual) species is an evolutionary puzzle. Nevertheless, in a large number of species, different ploidy levels have been found to coexist within populations. The reason why long-term coexistence of two ploidy levels (cytotypes) is thought to be impossible is a mechanism that has been dubbed the “Minority Cytotype Exclusion Principle” (Levin, 1975). According to this mechanism, individuals suffer from fertilisations by partners from another ploidy level, leading e.g. to inviable or infertile offspring. The ploidy level with the lowest frequency in the population, the minority cytotype, will suffer most from such unsuccessful fertilisations. This will decrease the minority cytotype’s frequency in the population, leading to even more unsuccessful fertilisations. This process may however be avoided if there is flowering time displacement or some other form of ecological differentiation between the cytotypes (Fowler, Levin, 1984; Felber, 1991; Van Dijk *et al.*, 1991).

In complexes with asexual polyploids, the Minority Cytotype Exclusion Principle works somewhat differently: only the sexuals will suffer from unsuccessful fertilisations as the asexuals obviously do not need fertilisation at all. Therefore, the frequency of sexuals in a population will decrease whenever there are asexuals present. This cost of hybridisation is one of the costs of sex mentioned above, but it is of course only present when the asexuals retain some male reproductive output.

Polyploidy has important complications for studies where asexuals and closely related sexuals are compared: if the asexuals are polyploid and the sexuals diploid, it is hard to find out whether any observed difference between them is due to asexuality per se, to polyploidy per se or perhaps due to some form of interaction between them. It is known that polyploidy itself can have drastic effects on the life-history and ecology of a species, e.g. due to differences in (cell) size, differences in heterozygosity etc (Ramsey, Schemske, 2002). Therefore, coexistence of sexuals and asexuals may be possible simply due to the fact that they are ecologically different because of the ploidy difference.

Some remaining problems

As the ubiquity of sexual reproduction can very well be explained by constraints to the evolution of asexuality, one of the main problems to solve is why sexuals and asexuals are able to coexist stably in so many taxa. In my opinion, the Tangled Bank hypothesis

is a good candidate for explaining such coexistence. Next to testing hypotheses for coexistence directly, it is important to address other issues that have an influence on their workings. The realised cost of sex in a species determines how strong the mechanism should be to allow coexistence, and in hermaphrodites an estimate of this cost depends among others on the amount of resources invested in male and female reproduction, the benefits of reproductive assurance and the rate of gene flow between the two reproductive types. This rate itself determines the clonal diversity, however there is no consensus on what constitutes a clone and on how to measure clonal diversity. The rate of gene flow also determines whether a genetically based ecological difference can evolve between the sexuals and asexuals. The influence of polyploidy is important to investigate as it may allow coexistence of sexuals and asexuals more directly. This thesis studies some of these topics in dandelions.

Dandelions

Sexual and asexual dandelions

Dandelions (genus *Taraxacum*) are a good system to study questions around the maintenance of sex for several reasons: There are both sexual and asexual dandelions, dandelions are very common and thus accessible as a study object and the geographic distribution of the two reproductive types is relatively well known for some of the sections in which the genus is divided. Furthermore, sexuals and asexuals have been extensively crossed in experiments, which makes that there is already a lot of information about the genetic system underlying the asexuality in dandelions (Van Dijk *et al.*, 1999; Van Dijk, Bakx-Schotman, 2004; Vijverberg *et al.*, 2004). The sexually reproducing dandelions are diploid and are in general self-incompatible and therefore obligate outbreeders. Asexually reproducing dandelions are polyploid and reproduce through apomixis, with autonomous endosperm development. Unlike some other apomictic plants such as blackberries (Kollmann *et al.*, 2000), asexual dandelions therefore do not need pollination to trigger endosperm development.

The two different modes of reproduction present within the genus *Taraxacum* complicates the taxonomy of the genus to a very large extend. From historical times, taxonomists have described many species and species groups, see for instance Von Handel-Mazetti (1907), however, it proved to be difficult to establish an undisputed taxonomy. This is largely due to the large number of so-called “microspecies” that has been described, on the basis of the assumed reproductive isolation between apomictic lineages. Many of these microspecies consist of only a single clonal lineage and in fact all should, according to the axiom of reproductive isolation. Already about 3000 of such microspecies have been described so far, which nevertheless covers only a small part of the total clonal diversity in the genus. The genus *Taraxacum* is divided into sections rather than species, with sections as loose collections of dandelion taxa sharing morphological

traits. Genetic analysis revealed that most of these sections are not monophyletic groups; a large part of the chloroplast-DNA variation was shared between sections (Wittzell, 1999; Kirschner *et al.*, 2003). Phylogenetic analysis of the chloroplast variation is difficult due to a large amount of homoplasies, resulting in a largely unresolved phylogenetic tree (Mes *et al.*, 2000; Kirschner *et al.*, 2003). The special taxonomic status of the sections is further exemplified by the fact that a number of sections include both sexual and asexual dandelions. Kirschner *et al.* (2003) suggested that, apart from polyploidisation events, also hybridisations between distantly related lineages might have contributed to the complex pattern of morphology and genetic markers. Despite these taxonomic difficulties, the subdivision into sections is the most often used way to describe the morphological variation present in the genus *Taraxacum*.

Section Ruderalia

The focus of this thesis is on dandelions of the most widespread section: *Taraxacum* sect. *Ruderalia* Kirschner, H. Øllg. & Štěpánek. This section covers the common, weedy, dandelions and is one of the sections in which both sexuals and asexuals are present. Whereas sexual *Ruderalia* are diploid, asexuals are mostly triploid though tetraploid asexuals can be found in very low frequencies (<1%). Originally confined to Europe, dandelions of sect. *Ruderalia* have spread together with humans colonising Asia and America and nowadays have a more or less cosmopolitan distribution. Remarkably, only the asexuals have managed to spread globally, and the sexuals are still confined to their supposed original distribution areas. Apart from the human-induced difference in the distribution of sexuals and asexuals, there is a clear pattern of geographical parthenogenesis in dandelions (Figure 3). The (original) distribution area of the asexuals covers most of Europe, but the sexuals are only found in the South, where they can co-occur with the asexuals in mixed populations (Den Nijs, Sterk, 1980; Den Nijs *et al.*, 1990).

Comparable patterns of geographical parthenogenesis, with the asexuals in the North and sexuals in the South, have been found in a large number of sexual/asexual complexes in both plants and animals (Bierzychudek, 1987). One explanation for these patterns stems from the colonisation of the North after the last ice age; asexuals are thought to be faster in colonising empty habitats and sexuals would therefore be lagging behind. Other explanations for geographical parthenogenesis are based on the Red Queen and Tangled Bank: As ecological interactions are thought to be less complex in the North, parasite pressure (Red Queen) or niche variation (Tangled Bank) would be lower and asexuals would have more opportunity to outcompete the sexuals. Despite extensive testing of the Red Queen and Tangled Bank hypotheses themselves, their role in causing geographical parthenogenesis has hardly been tested, neither in dandelions nor in other species (but see Meirmans, 2005).

A noteworthy aspect of the distribution area of the sexual dandelions of section *Ruderalia* is that it is split into two main parts: one area in France, Switzerland and Southern Germany (though low amounts of sexuals can be found up to The Netherlands) and a

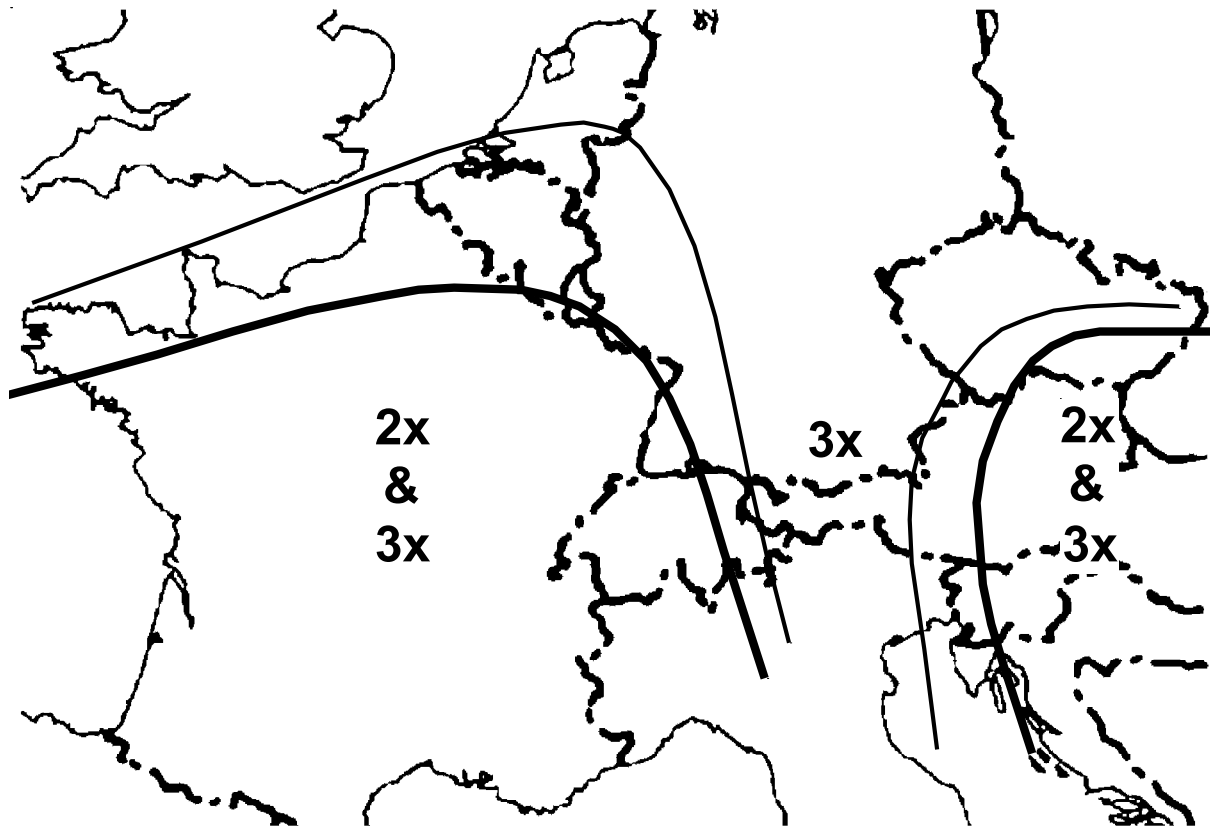


Figure 3: Distribution of sexual diploid (2x) and asexual triploid (3x) dandelions over Europe. The thin lines indicate the border of the distribution area of the sexuals, the thick lines indicate where sexuals start to become frequent.

second area in Eastern Austria, Czech republic and the Balkans (Figure 3). Remarkably, no sexuals have been found in between these two areas (Den Nijs, Sterk, 1980). This disjunct distribution area might be explained by postglacial recolonisation from two glacial refugia, one on the Iberian Peninsula and one on the Balkans (Den Nijs, Sterk, 1980; Den Nijs *et al.*, 1990). A problem with this explanation is that, given the high dispersal rate of dandelions, it would be surprising that the sexuals did not manage to invade the small part in between the two distribution areas.

Inheritance of apomixis

Like the sexuals, most asexual dandelions of sect. *Ruderalia* are hermaphrodites, which means that they have anthers and produce pollen. Due to the triploidy of the asexuals, however, male meiosis is unbalanced and the produced pollen is very irregular in size and largely infertile. Nevertheless, it is possible to use this pollen to fertilise ovules of sexual individuals. The offspring of such crosses is usually a mixture of diploid, triploid and tetraploid individuals (Verduijn *et al.* (2004b) provide a list of crossing studies and their results). Diploids are usually most abundant in the offspring, though a large part of the diploid offspring is the result of selfing by the motherplant. This selfing can take place in the otherwise self-incompatible sexuals as pollination with pollen from asexuals breaks

down the incompatibility system in the sexuals (Morita *et al.*, 1990a). Luckily, diploids that are the result of selfing can easily be detected using molecular markers. Another interesting result from crosses between sexuals and asexuals is the high percentage of tetraploid offspring among the hybrids. Tetraploids are very rare in natural populations where they usually take up less than one percent of the populations.

Although most of the triploids and tetraploids that are produced by crosses between asexual fathers and sexual mothers are apomicts, also non-apomictic polyploids are produced. Using a large number of crosses, Van Dijk and co-workers (among others: Van Dijk *et al.*, 1999; Van Dijk, Bakx-Schotman, 2004; Vijverberg *et al.*, 2004) showed that diplospory, the formation of unreduced megaspores, is regulated by a specific dominant allele at a single locus. Diplospory is one of the main elements of apomixis, together with autonomous embryo and endosperm development. An important characteristic of the diplospory allele is that it has a lethal effect on the developing haploid pollentube; only diploid and triploid pollen grains are able to transfer the allele (Vijverberg *et al.*, 2004). This immediately explains why asexuality is never found in diploid dandelions. The reason why the diplospory is lethal in haploid setting is not known, but it is probably due to the accumulation of recessive deleterious mutations closely linked to the diplospory allele. As the recombination rate around the allele is very low (Vijverberg *et al.*, 2004), Muller's Ratchet acts on this part of the chromosome.

Gene flow

In dandelions, the pollen production by the asexuals and the inheritance of apomixis provide the possibility of gene flow from sexuals to asexuals and vice versa. The rate at which this gene flow takes place in natural populations is not known, but it is important to estimate since this rate has major consequences for the realised cost of sex in dandelions, the clonal diversity, the purging of deleterious alleles and the possibility of ecological differentiation between the sexuals and asexuals. That some gene flow is indeed taking place in natural dandelion populations has been suggested by Menken *et al.* (1995), who showed that the sexuals and asexuals share most of the allozymes variation. They also found that the differentiation between sexuals from different populations was stronger than the differentiation between sexuals and asexuals within the same population. The latter results were however based on *significance* of tests of differentiation, which does not necessarily say much about the *amount* of differentiation. Unbiased statistics that estimate the amount of differentiation between ploidy levels (Hardy, Vekemans, 2002) were however not available at that time.

Gene flow can also be estimated from field experiments in mixed sexual/asexual populations through the inspection of the progeny of diploid sexuals for triploid and tetraploid offspring. Verduijn *et al.* (2004b) showed that only a small portion of the seeds that are produced by sexual dandelions are the product of hybridisations between the ploidy levels. Verduijn *et al.* suggested that the rare tetraploids may play an important role in the gene flow between sexuals and asexuals: In contrast to the triploids, the

tetraploids produce regularly sized pollen grains, all of which are diploid. In controlled crosses, pollen from tetraploids was found to have a higher success in fertilising sexual eggs than pollen from triploids (Verduijn *et al.*, 2004b).

Although insightful, results from field experiments have to be treated with some caution when inferring the amount of gene flow between the sexuals and asexuals: The hybridogenous offspring may suffer from outbreeding depression or otherwise be unable to establish themselves. That outbreeding depression actually takes place in dandelions was found by De Kovel and De Jong (2000), who compared a number of reproductive characters in newly synthesized clones and established clones from a natural population. They found that the new clones had lower seed set and lower germination rates than the established ones.

Clonal diversity

Gene flow between sexuals and asexuals results in a continuous creation of new clonal lineages and thus to a high clonal diversity. In accordance to the suggested gene flow between sexual and asexual dandelions, dandelions indeed show a high amount of clonal diversity. Using AFLP's, Van der Hulst *et al.* (2003), found 33 different genotypes in a sample of 68 dandelions from a single population from Viborg, Denmark. This asexual population was at least 600 kilometres removed from the nearest known sexual population. Van der Hulst *et al.* also found that the distribution of the AFLP-markers over the individuals was not compatible with strict clonal evolution, but rather with recombination.

The extremely high clonal diversity in the Danish dandelions raises the question whether constant creation of new clones in the mixed sexual/asexual populations in Southern Europe is enough to maintain this diversity. Theoretically, ecological differentiation between clones can maintain diversity, under the Frozen Niche Variation hypothesis. There is little evidence whether there is indeed such differentiation between dandelion clones. The sole study in this direction (Solbrig, Simpson, 1974; Solbrig, Simpson, 1977), suggested that the two genotypes under study differed in their investment in reproduction and in their competitive ability. The first clone produced more seeds but was the worst competitor, the second one produced less seeds but was a better competitor. This indicates some form of frozen niche variation, but it does not allow inference whether this differentiation makes the coexistence possible of the more than thirty clones within the one population studied by Van der Hulst *et al.* (2003).

This thesis

Sex in dandelions

The many costs of sex make the coexistence of sexual and asexual dandelions in mixed populations quite paradoxical. For this thesis, I have looked at the genetic and ecological interactions between the sexual and asexual dandelions, but also at the interactions between the different asexual clones, as these interactions provide essential information on our understanding of the coexistence of sexual and asexual reproduction. Looking at these interactions also helps to reveal which of the potential costs of sex are realised in dandelions.

Genetic interactions

I looked at the (population) genetic interactions as I think it is essential for our understanding of asexuality to know the dynamics of the population of asexuals: the rate at which new lineages are created and the clonal diversity that is present in asexual dandelions. As discussed above, these processes have major consequences on the working of both mutational and ecological models for the maintenance of sex. The amount of gene flow between sexuals and asexuals is therefore one of the most important elements in sexual/asexual complexes. Chapters 3, 4 & 5 discuss, among others, gene flow between sexual and asexual dandelions by looking at the distribution of genetic variation within and between the sexuals and asexuals. Chapter 4 & 5 also discuss the origin and maintenance of the high clonal diversity in a population of dandelions. Chapter 6 describes a method to assign individuals to clonal lineages and methods to analyse clonal diversity. This chapter also describes two computer programs that implement these methods.

Ecological interactions

I looked at the ecological interactions as I think that ecological explanations for the maintenance of sex as presented by the Tangled Bank are currently undervalued compared to the mutational mechanisms. Especially the possibility of niche differentiation between the types, perhaps in combination with a difference in niche width, is a powerful mechanism to explain the coexistence of sexuals and asexuals. Nevertheless, the Tangled Bank has received much less attention in the literature than the Red Queen and the mutational models. Chapters 2 & 3 discuss ecological differentiation between sexual and asexual dandelions on two different geographical scales: Chapter 2 mainly handles ecological differentiation between populations on a regional scale, while Chapter 3 handles differentiation on a within population scale. Chapter 4 also looks at ecological differentiation, but focuses on the difference in the reaction of sexuals and asexuals, and different clones within the asexuals, on an ecological change in time. Niche differentiation is only able to explain coexistence between sexuals and asexuals if there is Frozen Niche

Variation in the asexual clones. Chapter 5 therefore uses genetic markers to distinguish between clones and looks simultaneously at niche differentiation between sexuals and asexuals and niche differentiation between clones.

Male sterility

The cost of males is often seen as the most important cost of sex. Therefore, it is surprising that most asexual dandelions still produce pollen, as they may be better off by reallocating the available resources to, for example, the production of more seeds. In Chapter 7, the cost of males is analysed for dandelions by looking at pollen producing and male sterile asexuals and comparing their fitness and their relatedness to the sexuals.

**Anthropogenic disturbance and habitat
differentiation between sexual diploid and apomictic
triploid *Taraxacum* sect. *Ruderalia***

2



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1999, Folia Geobotanica, 34, 451-469

Abstract

Co-occurrence of sexual diploid and apomictic triploid *Taraxacum* section *Ruderalia* has been reported frequently. Many suggestions have been put forward with respect to the existence of an ecological differentiation between the cytotypes. In a study of 116 *Ruderalia* populations in the area around Neuchâtel (Switzerland) such a habitat differentiation has been proven. Large differences in the diploid/triploid ratio have been found between different field types. In a Canonical Correspondence Analysis significant vegetation differences were found between fields with mainly diploid *Ruderalia* and fields with mainly triploid *Ruderalia*. Two environmental indicators were significantly positively correlated with ploidy levels, altitude with diploids, and the percentage of therophytes in a vegetation with triploids. The percentage of therophytes is thought to be an indicator for the amount of disturbance due to human activities. At lower altitudes, the diploid/triploid ratio is largely determined by the amount of disturbance. Highly disturbed fields contain mainly triploids, relatively stable fields contain mainly diploids. At higher altitude there are mainly diploids. The presence of triploid *Taraxacum* sect. *Ruderalia* in the region around Neuchâtel appears to be the result of the high amount of human activities of the last few hundred years. Whether the triploid presence is due to immigration or to local formation from diploids is yet unknown.

Introduction

Ecological differentiation has already been documented for many of cytotypes within polyploid complexes, for example *Anthoxanthum* (Felber-Girard *et al.*, 1996), *Antennaria* (Bayer *et al.*, 1991), *Draba* (Brochmann, Elven, 1992), *Tripleurospermum* (Kay, 1961) and many others. A very tight relation exists between polyploidy and asexual reproduction. Most asexually reproducing plant species are polyploid, but only a small fraction of the polyploid plant species are asexual (Lewis, 1980; Stebbins, 1980). Both asexual reproduction and polyploidy can have great effects on the competitive ability and choice of ecological niche of the species in question. Because of the relationship between ploidy level and asexuality, it is very difficult to distinguish the effects of the two phenomena. Ecogeographical distribution studies in sexual/apomictic plant complexes showed patterns that suggest apomictic taxa have wider areas of distribution that extend into more harsh ecological conditions (Bierzychudek, 1987).

The taxonomically very complex genus *Taraxacum* Weber ex F.H. Wigg, with some 50 sections and 3000 agamospecies described (Kirschner, Štěpánek, 1997), is well known for its widespread apomictic reproduction. The largest and most widespread section is section *Ruderalia* Kirchner, H. Øllg. & Štěpánek, which comprises more than 2000 known agamospecies. Less than 10% of the *Ruderalia* species are diploid ($2n=2x=16$); they are obligate sexuals and are normally highly self-incompatible. All other *Ruderalia* species are apomictically reproducing triploids ($2n=3x=24$). Diploid and triploid *Ruderalia* are found to co-occur in mixed populations, with the diploid/triploid ratio differing per population (Den Nijs, Sterk, 1980; Den Nijs, Sterk, 1984; Den Nijs, Van der Hulst, 1988; Den Nijs *et al.*, 1990). Long-term coexistence of diploid and triploid individuals is claimed to require ecological differentiation between the cytotypes (Levin, 1975).

Figure 1 shows the distribution of diploid and triploid *Ruderalia* in Europe. This map is a consequence of extensive cytogeographical work by e.g. Fürnkranz (1966), Valentine and Richards (1967), Richards (1970), Den Nijs and Sterk (1980; Den Nijs, Sterk, 1984), Den Nijs and Van der Hulst (1988), Roetman *et al.* (1988) and Den Nijs *et al.* (1990), the map itself is redrawn from Den Nijs (1997). The diploids seem to be confined to two separate areas in Central Europe, in which they coexist with triploids. This disjunct pattern is generally interpreted as resulting from post-glacial colonisation from two glacial refugia (Mogie, Ford, 1988). The triploids are said to have spread much faster because of their greater colonising ability. As is apparent from Figure 1, the triploid *Ruderalia* have a larger range than the diploid *Ruderalia*, and their distribution extends further north than that of the diploids.

It has recorded previously before that triploids prefer more disturbed habitats than diploids. Elzinga *et al.* (1987) found that in Limburg, The Netherlands, diploid *Ruderalia* are mostly confined to unfertilised nature reserves, but they were also found in intensively managed grasslands. Fürnkranz (1966) noted that triploids are more confined

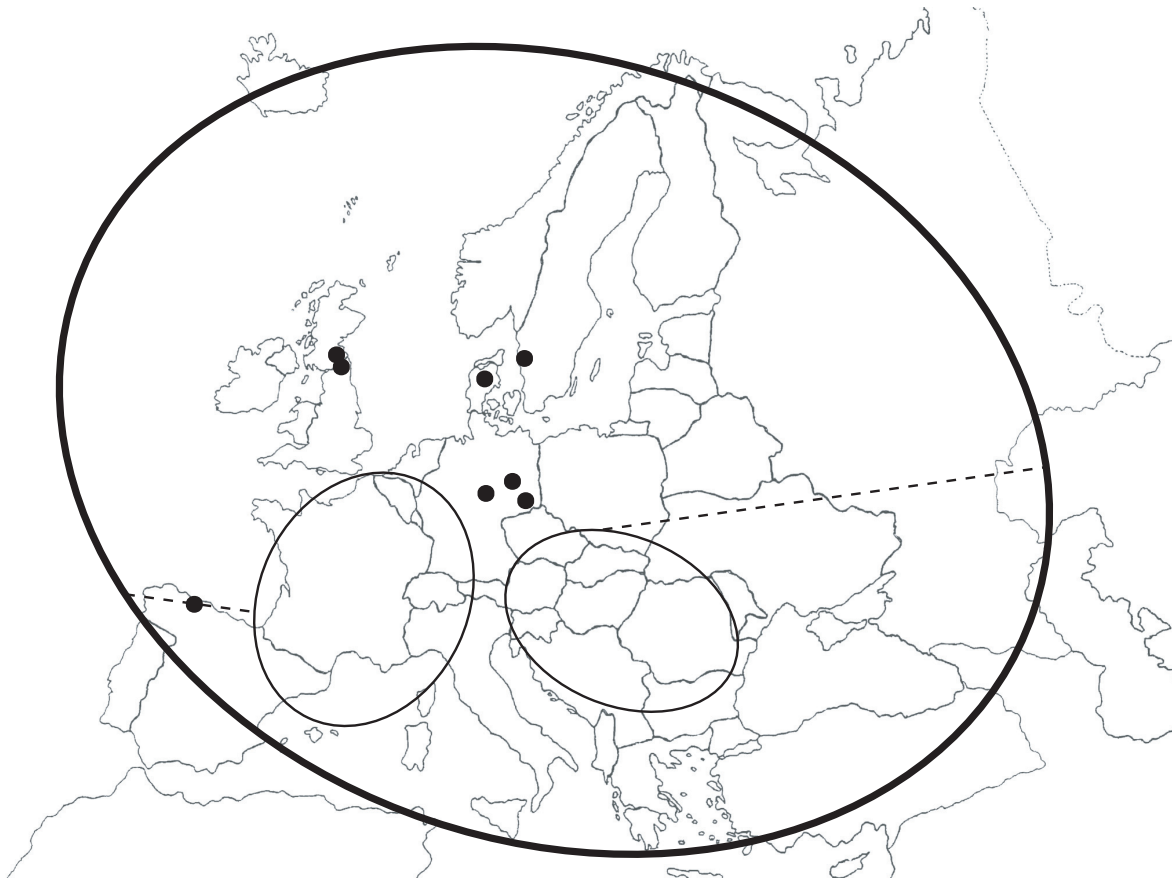


Figure 1: Distribution of diploid *Ruderalia* in Central and Western Europe. The thicker line shows the supposed original distribution of section *Ruderalia* in Central and Western Europe. Diploid *Ruderalia* occur within the thinner solid lines and south of the dashed lines they are assumed to occur. The black dots are isolated locations where diploids have been found. After Den Nijs (1997).

to disturbed fields than diploids, but after revisiting his sample-fields, Den Nijs and Sterk (1980) found no support for this. Incidentally, Den Nijs and Sterk (1984) found large differences in the percentage of diploids between two adjacent fields with different management intensities. From several studies it appears that, especially in the northern margins of their distribution area diploids have a preference for slightly xeric conditions (see e.g. Den Nijs, Sterk, 1980; Den Nijs, Sterk, 1984; Roetman *et al.*, 1988). On the border of the diploid area in Czechoslovakia, the diploids also seem to prefer dry, sunny places (Den Nijs *et al.*, 1990).

On the other hand, in the cytogeographical investigations, no correlation was found between the occurrence of a certain *Ruderalia* cytotype and the altitude (Den Nijs, Sterk, 1980; Den Nijs, Sterk, 1984; Jenniskens *et al.*, 1985). Den Nijs and Sterk (1984) even reported the presence of *Ruderalia* diploids at up to 2000 meters in the Western Alps. Calame (unpublished data) has investigated transects along altitudinal clines in the Alps and in the Jura, and found only two fields containing triploids, both at the lower

end (~530 meters above sea-level) of a transect near Neuchâtel in the Jura. All other fields were purely diploid. It thus appears that diploids are not ecologically restricted to the relatively mild climates of lower altitudes. These results contradict Morita's (1976) findings for a *Taraxacum* sect. *Hondoense*, in Japan. Here, diploids were found mostly in the southern part of the investigated region, while the triploids were common all over the region. Within the diploid distribution area, the diploids grew mostly in man-made habitats below 200 meters. Triploids were common up to 1800 meters altitude.

In conclusion, the general geographical patterns Bierzychudek (1987) found for apomicts and their sexually reproducing relatives seem to be present in *Taraxacum* sect. *Ruderalia* too. The apomictic triploids have a much larger and more northerly reaching range than the sexuals. However, with respect to the concrete differences in ecological preferences between the cytotypes, detailed data are remarkably scarce, and unequivocal. On the one hand, diploids appear to prefer relatively warm and undisturbed environments. On the other hand, some data show the occurrence of diploids at rather high elevations in the southern part of Europe (Den Nijs, Sterk, 1984; Calame, unpublished data), other data show a diploid preference for low altitudes in the southern part of Japan (Morita, 1976).

Aim of this study

The aim of this research was to study possible ecological differences between diploids and triploids within a selected area, by analyzing: the regional cytotype distribution of representatives of *Taraxacum* sect. *Ruderalia*, the relation of the distribution with ecological parameters, such as altitude, land use and amount of disturbance, and some Ellenberg based ecological indicator values, the vegetation relevés of the sample sites, the within-population spatial distribution of the cytotypes using transect sampling.

Materials & Methods

Sampling sites

During the spring and summer of 1997, the ratio of diploid and triploid *Ruderalia* was established for a total of 116 populations from around Neuchâtel, Switzerland (Figure 2). The elevation of this area ranged from 430 m.a.s.l. to 1155 m.a.s.l.. The town of Neuchâtel is situated between 430 and 600 m.a.s.l.. The sampled populations grew in a wide range of field types: roadsides, parks, pastures, hay meadows and vineyards. For 93 of the sampling sites vegetation relevés were made using the cover scale (see Table 1) proposed by Gauch (1977). These are the sample sites used for calculations and the production of graphs. Only for the map (Figure 2) all 116 fields were used. Nomenclature is according to the Flora Helvetica (Lauber, Wagner, 1996). For a list of the sampling sites and a concise description, see Appendix 1.

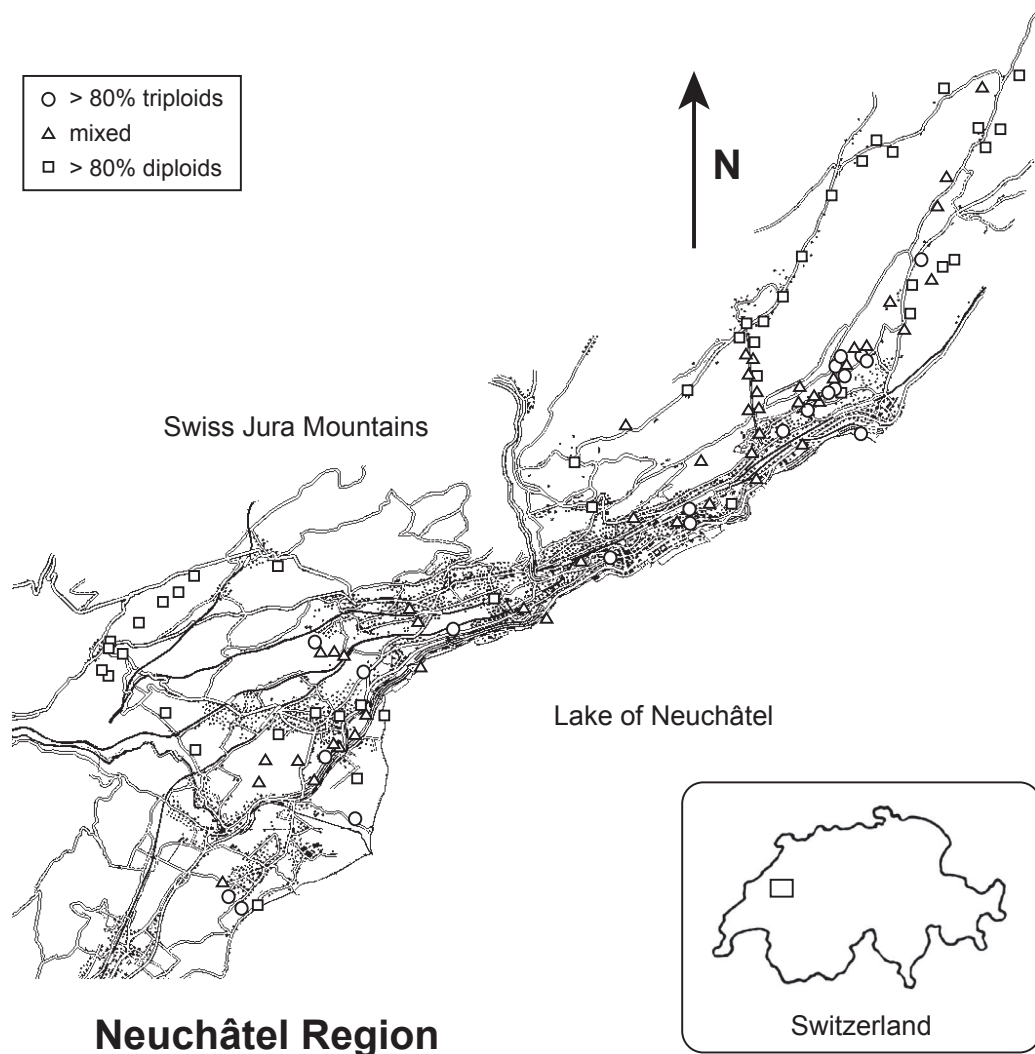


Figure 2: The distribution of the sampling sites around Neuchâtel, and an indication of the cytotype ratio of the *Ruderalia* populations. Squares indicate populations with more than 80% diploids, circles indicate populations with more than 80% triploids, triangles indicate populations with intermediate cytotype ratios. The altitudinal trend is from 430 meters at the lakeside to approximately 1000 meters at the edge of the map.

Cytological data

In order to establish the ploidy level, leaves of *Taraxacum* sect. *Ruderalia* specimens were collected in the fields. One leaf per individual plant was sampled selecting plants from set intervals while crossing the field along imaginary lines. Flowering plants as well as vegetative plants were sampled. Circa 30 plants per field were sampled, or when there were less than 30 plants, the maximum number possible. No identification to the microspecies level was performed. The ploidy of the sampled plants was established by the use of a Becton-Dickinson Facscan flow-cytometer. A flow-cytometer measures the quantity of DNA in the nuclei of cells, using the fluorescence properties of a stain (Propidium Iodide) which binds to DNA in a quantitatively proportional way (see e.g. De Laat *et al.*, 1987; Brown, 1991). For cytometry, a slightly modified version of the

Table 1: The cover-scale used for most of the vegetation-relevés (Gauch, 1977)

Value	% Cover:
0	absent
1	0-0.53
2	0.5-1
3	1-2
4	2-4
5	4-8
6	8-16
7	16-32
8	32-64
9	64-100

protocol of Galbraith *et al.* (1983) was followed. Throughout this article, the percentage of diploids will be used as a descriptor for the ratio of diploid and triploid *Ruderalia*. This is not a perfect indication of the cytotype ratio, due to the occurrence of tetraploid *Ruderalia*, but because of the very low frequency of these tetraploids (less than 1% of the total), this is considered not to be a problem.

Ecological data

In interviews with the farmers and/or managers of the fields, information was gathered concerning the number of cuts per year and also the current and former use of the field. The altitude of the fields was assessed using the National Map of Switzerland, scale 1:25.000 (Anonymus, 1987). The amount of shadow on a field was estimated using three classes: low (1), medium (2) and high (3). Some additional data were derived by converting the vegetation relevés into ecological indicator values for humidity (F), acidity (R), nutrients (N) and temperature (T) according to Landolt (1977). The Landolt indicator values are comparable to indicator values of Ellenberg *et al.* (1992), but are adapted especially for Switzerland. The conversion of the species indicator values into values for the relevés, was made according to the technique of ‘Weighted Averages’ (Curtis, McIntosh, 1950; Curtis, McIntosh, 1951). This approach was also taken to calculate the weighted percentage of the different Raunkiaer-life-forms within the relevés, according to Ellenberg *et al.* (1992). The few species that can show two different life-forms were counted twice. Weighted percentages were only calculated for the four most abundant life-forms: hemicryptophytes (hemi), therophytes (thero), geophytes (geo) and chamaephytes (cham). Hereafter, the term ‘environmental indicators’ will be used for the whole set of variables, those measured, those deduced from the interviews and those calculated from the Landolt indicator values and Raunkiaer-life-forms.

Statistical analysis.

To see whether the occurrence of any plant-species were related to the occurrence of a certain *Ruderalia* cytotype, a weighted average cytotype ratio (H_i) was calculated for the plant species that were found in ten relevés or more, using the following formula:

$$H_i = \sum_{j=1}^n A_{ij} R_j / \sum_{j=1}^n A_{ij}$$

A_{ij} is the abundance of species i in population j and R_j is the ratio of diploids and triploids within population j . Here the percentage of diploids is used as a descriptor for R_j , so H_i actually stands for the weighted average percentage of diploids of all the fields species i is growing in. A high value of H_i indicates that a plant species co-occurs mainly with diploid *Ruderalia*, and a low value of H_i indicates that a plant species co-occurs mainly with triploid *Ruderalia*. An intermediate value of H_i can indicate either that the species is living in fields with both *Ruderalia* cytotypes equally present, or that the abundance of the species is not related to the cytotype ratio.

The environmental indicators were compared to each other and to the percentage of diploids in a Spearman Rank Correlation test. For this test, and all other univariate statistics, the statistical computer program Systat, version 5.2.1 for the Macintosh, was used. A sequential Bonferroni correction (Rice, 1989) was performed when necessary.

The computer-program CANOCO (Ter Braak, 1992) was used for ordination of the vegetational data. A Detrended Correspondence Analysis (DCA, Hill, 1979) was performed to check for the main tendencies within the data. The first two ordination axes were compared to the environmental indicators and the cytotype ratio, using Spearman rank correlation tests.

We performed a Canonical Correspondence Analysis (CCA, Ter Braak, 1992) with the percentage of diploids as the only variable. A Monte-Carlo permutation test was applied to test whether there are significant differences in vegetation between fields with mainly diploid *Ruderalia* and fields with mainly triploid *Ruderalia*. The Monte Carlo test was set to 9999 unrestricted permutations, giving a maximum significance of $p=0.0001$.

Transect analysis

In a mixed field (76% diploids) at 600 m. elevation, 4 transects of 30 meters each (VA, VB, VC and VD) have been randomly set. Transect ends were kept at 4 meters distance minimally from the edges of the field. All *Taraxacum* individuals growing within 10 cm right of the transects were collected ($n_{\text{tot}} = 314$). The position of each of them was coded as distance from the start ($x = 0$ to 3000 cm) and distance on the right of the transect ($y = 0$ to 10 cm). The transect field was uniform in slope and exposition, vegetation pattern was homogeneous.

A Mantel Correlogram (Legendre, Vaudor, 1991) was used to analyze the spatial distribution of the cytotypes within the transects and to detect an eventual difference from random distribution. The correlation between cytotypes for different classes of distances was tested. The number of classes of distance (40, 40, 20 and 30 classes for VA, VB, VC and VD respectively) was chosen in order to have at least 30 pairs of *Taraxacum* plants per class. A sequential Bonferroni correction (Rice, 1989) was performed according to the number of classes.

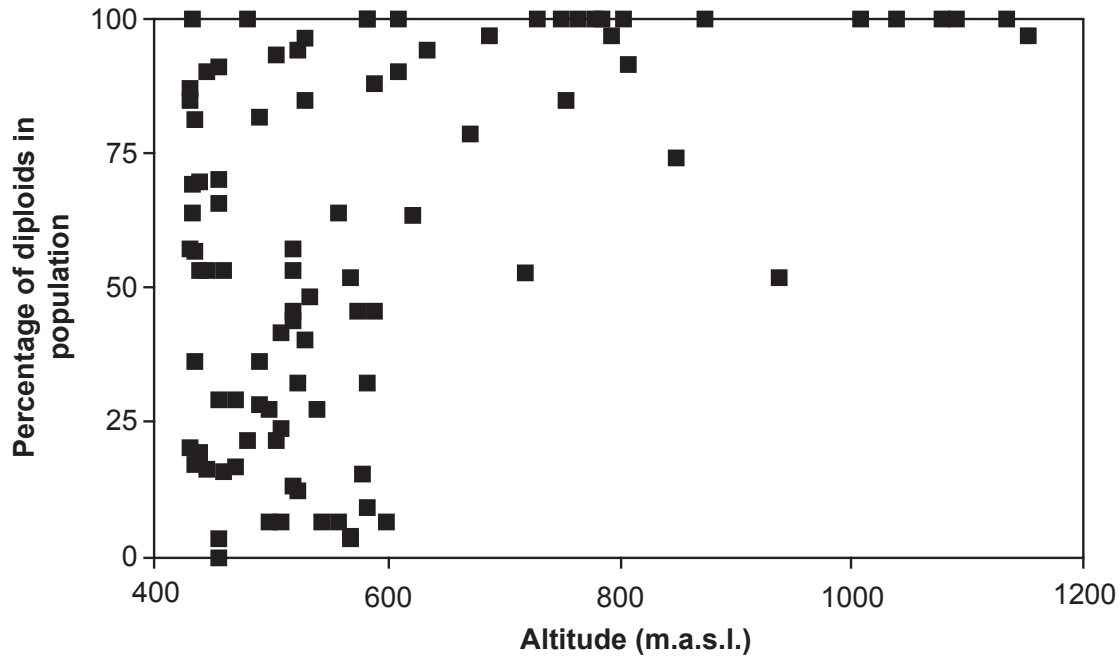


Figure 3: The altitude of the sampling sites versus the percentage of diploid *Ruderalia* at the sites.

Results

Cytometer data

In the area around Neuchâtel, diploids are more abundant than triploids. From a total of the more than 3600 plants investigated, 63% were diploid, 36% were triploid and less than 1% were tetraploid. The majority of the 116 sampling fields consisted of mixed populations, one was fully triploid, and 26 were fully diploid. The cytometer results and the environmental indicators for all the fields are given in Appendix 1. Figure 2 shows the distribution of the fields around Neuchâtel, and an indication of the percentages of the ploidy levels found. The fields with mainly diploid *Ruderalia* were mostly situated outside the city, the triploid *Ruderalia* seemed to be confined to the city and to the vineyards. The altitude of the sample fields ranged from 434 to 1155 meters above sea-level. The lowest fields sampled were on the shore of the Lake of Neuchâtel, the highest population sampled was on a roadside verge, in an agricultural village not far from Neuchâtel.

The map (see Figure 2) already suggests a relation between altitude and percentage of diploids. This relation of the cytotype ratio with altitude is not linear. Figure 3 shows the percentage of diploids as a function of the altitude. Above 600 meters there are mainly fields with a high percentage of diploids. Under 600 meters there seems to be no clear tendency. The border of the urban conurbation of Neuchâtel is at approximately 600 meters. Above 600 meters there are mainly forests, hay meadows, and some small villages, but all these can also be found below 600 meters.

Table 2: The species that occur in 10 or more relevés, sorted on their H_i -value. H_i is the weighted average percentage of diploid Ruderalia of all the relevés the species occurs in. The abbreviations for the Raunkiaer life forms (from Ellenberg, 1992) are: *c* is a herbaceous chamaephyte, *g* is a geophyte, *h* is a hemicryptophyte, *n* is a nanophanerophyte, *p* is a phanerophyte, *t* is a therophyte and *z* is a woody chamaephyte. Combinations indicate that the species can show both life-forms.

Species	# of relevés	H_i	Life-form	Humidity	Temperature
<i>Alchemilla vulgaris</i>	14	0.86	h	4	2
<i>Phleum pratense</i>	17	0.85	h	3	3
<i>Holcus lanatus</i>	11	0.85	h	3	3
<i>Tragopogon pratensis</i>	10	0.81	h	2	3
<i>Ranunculus acris</i>	23	0.79	h	3	3
<i>Glechoma hederacea</i>	20	0.76	g,h	3	4
<i>Rumex obtusifolius</i>	29	0.75	h	3	3
<i>Fragaria vesca</i>	10	0.74	h	3	3
<i>Rumex acetosa</i>	10	0.73	h	3	3
<i>Heracleum sphondylium</i>	17	0.72	h	3	3
<i>Leontodon hispidus</i>	15	0.72	h	3	3
<i>Prunella vulgaris</i>	18	0.72	h	3	3
<i>Leucanthemum vulgare</i>	10	0.72	h	3	4
<i>Trifolium pratense</i>	60	0.71	h	3	3
<i>Lotus corniculatus</i>	15	0.68	h	2	3
<i>Dactylis glomerata</i>	50	0.67	h	3	4
<i>Capsella bursa-pastoris</i>	14	0.66	t	2	3
<i>Arrhenatherum elatius</i>	32	0.65	h	3	4
<i>Anthriscus sylvestris</i>	14	0.65	h	3	3
<i>Salvia pratensis</i>	16	0.65	h	2	4
<i>Medicago sativa</i>	18	0.63	h	2	4
<i>Plantago lanceolata</i>	51	0.63	h	2	3
<i>Ranunculus repens</i>	22	0.63	h	4	3
<i>Poa pratensis</i>	20	0.62	h,g	3	3
<i>Plantago major</i>	41	0.62	h	3	3
<i>Urtica dioica</i>	20	0.62	h	3	3
<i>Plantago media</i>	22	0.61	h	2	3
<i>Daucus carota</i>	35	0.61	h	2	4
<i>Poa trivialis</i>	22	0.61	h,c	3	2
<i>Trifolium repens</i>	77	0.60	c,h	3	3
<i>Galium mollugo</i>	21	0.59	h	3	4
<i>Potentilla reptans</i>	13	0.57	h	3	3
<i>Achillea millefolium</i>	39	0.57	h	2	3
<i>Cerastium fontanum</i>	23	0.57	c	3	2
<i>Bellis perennis</i>	40	0.55	h	3	3
<i>Poa annua</i>	37	0.54	t,h	3	3
<i>Lolium perenne</i>	67	0.54	h	3	3
<i>Bromus erectus</i>	10	0.54	h	2	4
<i>Silene vulgaris</i>	18	0.52	h,c	2	3
<i>Medicago lupulina</i>	33	0.52	t,h	2	4
<i>Hedera helix</i>	11	0.51	z,p	3	4
<i>Geranium molle</i>	26	0.51	t	2	4
<i>Hieracium laevigatum</i>	14	0.50	h	2	4
<i>Erigeron annuus</i>	15	0.48	h,t	2	4
<i>Lapsana communis</i>	11	0.44	h,t	3	4
<i>Trifolium dubium</i>	10	0.44	t	3	4
<i>Polygonum aviculare</i>	18	0.44	t	3	3
<i>Geranium pusillum</i>	15	0.42	t	2	4
<i>Bromus sterilis</i>	14	0.41	t	2	4
<i>Convolvulus arvensis</i>	27	0.32	g,h	2	4
<i>Veronica arvensis</i>	14	0.32	t	3	4
Average	23.46	0.604		2.686	3.333

Table 3 Spearman rank correlation coefficients for the cytotype ratio and the altitude with each other and the other environmental indicators; also the correlations of the cytotype ratio with the same variables, using only the sample fields below 600 meters (*: $p=0.05$, **: $p=0.01$, ***: $p=0.001$, after a sequential Bonferroni correction).

Variable	Normal n=96		Below 600m. n=67	Source of variable
	% diploids	altitude	% diploids	
Altitude	0.41**	X	-0.14	Field
Number of cuts	-0.06	-0.11	0.03	
Shadow	-0.12	-0.04	-0.15	
Humidity	0.29	0.25	0.12	Calculated from
Acidity	-0.20	-0.14	-0.11	vegetation data
Nutrients	-0.03	-0.04	-0.07	using indicator
Temperature	-0.41**	-0.45***	-0.19	values
Therophytes	-0.45***	-0.30*	-0.40*	Calculated from
Geophytes	-0.08	-0.06	-0.03	vegetation data
Hemicryptophytes	0.39**	0.33*	0.31*	using life forms
Chamaephytes	-0.10	0.02	-0.12	

Relative cytotype presence may differ strongly between fields, even on a very small geographical scale. Fields numbers 201 and 202, a vineyard and an orchard separated by a small road, have very different cytotype ratios. The orchard population contains 88% diploids and the vineyard only 18%. A similar difference is found between fields 209 and 210, a maintained and an unmaintained part of the same vineyard, and also between fields 166 and 178, a roadside and the adjacent hay meadow (see Appendix 1).

Vegetation relevés

The total number of species found in the relevés is 219, most of them occurred only a few times. Vegetation relevés of six representative sampling sites are given in Appendix 2. For the 52 species that occurred 10 times or more, the weighted average cytotype ratio (H_i) was calculated (Table 2). The greater part of the species have a H_i -value close to the average, but there are also some species with a remarkably high or low H_i -value. From these extreme H_i -values it is concluded that some species are relatively strongly associated with diploid *Ruderalia*, and others with triploids. There are significant correlations between the H_i -values and the indicator values for humidity and temperature ($p=0.001$). Species with a high H_i -value, associated with diploid *Ruderalia*, have higher humidity indicator values and lower temperature values than species with a low H_i -value. Therophytes have a significantly lower H_i -value than non-therophytes ($p=0.001$, independent t-test). Hemicryptophytes have a significantly higher H_i -value than non-

hemicryptophytes ($p=0.001$, independent t-test). Most of the species associated with triploid *Ruderalia*, like *Lapsana communis*, *Polygonum aviculare* and *Convolvulus arvensis* are important weeds of vineyards. Not all the fields with a high percentage of triploid *Ruderalia* are vineyards, but the vegetation of these other fields is often very similar to that of the vineyards. Species with a high H_i -value, like *Phleum pratense*, *Holcus lanatus* and *Tragopogon pratensis* grow mainly in rather undisturbed hay meadows.

Correlation among environmental indicators

Table 3 shows the Spearman correlation coefficients after a sequential Bonferroni correction (Rice, 1989) of the percentages of diploids and the environmental indicators. The cytotype ratio is significantly correlated with the following indicators: altitude (+), temperature (-), the percentage of therophytes (-) and the percentage of hemicryptophytes (+). Some of these indicators are also correlated with the altitude. Thus, to see whether the correlation of these indicators is mainly due to their correlations with the altitude, or whether there is a correlation with these indicators separately from the altitude, the effect of the altitude should be removed from the indicators. Figure 3 does not show a clear linear relation between percentage of diploids and altitude. Below 600 m there is no relation at all. So, to test the correlation between the percentage of diploids and the

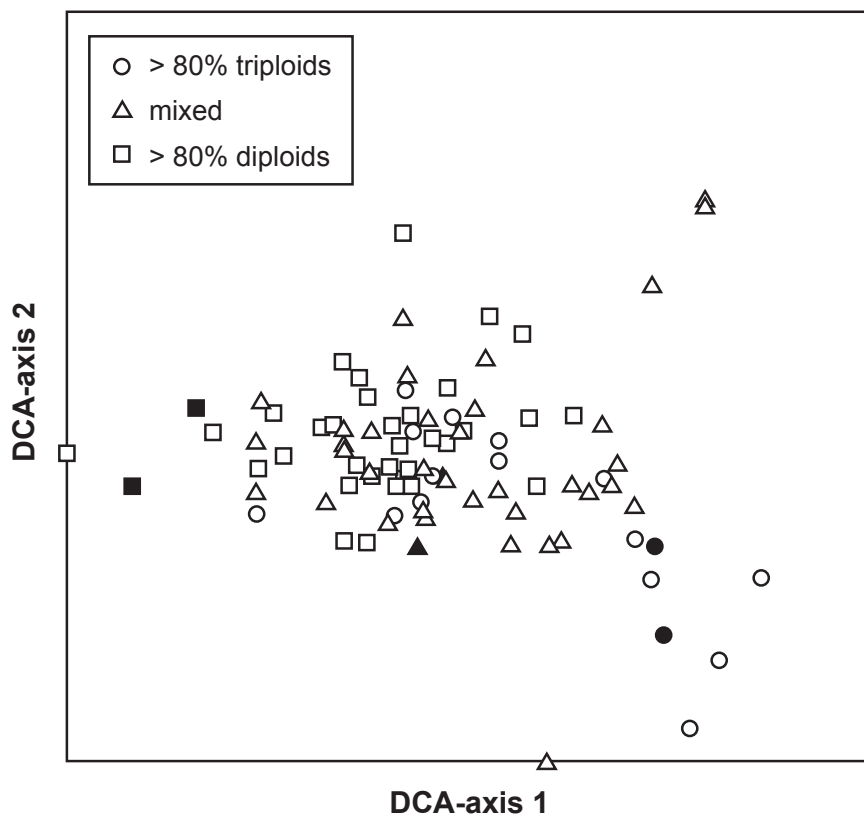


Figure 4: Ordination biplot, of a Detrended Correspondence Analysis (Hill, 1979) of the vegetation data. Squares indicate populations with more than 80% diploids, circles indicate populations with more than 80% triploids, triangles indicate populations with intermediate cytotype ratios. Vegetation relevés of the sites indicated by dark symbols are given in Appendix 2.

environmental indicators without the influence of altitude, another test was performed, using only the sample fields below 600 m (see also Table 2). It emerged that the correlations between the percentage of diploids and temperature disappeared, however, correlations with percentages therophytes and hemicryptophytes remained significant.

Vegetation and the environmental indicators

The first DCA-axis (Figure 4) shows an ordination from fields with a low percentage of therophytes to fields with a high percentage of therophytes (correlation $p=0.001$); the first axis is also significantly correlated with the percentage of diploids ($p=0.001$). The fields with less than 20% diploids are mostly concentrated on the right side of the graph, the fields with more than 80% diploids are on the left side of the graph. This implies that fields dominated by diploids have different vegetations than “triploid” fields.

Vegetation differences and cytotype ratio

According to the DCA results and the distribution of the H_i -values, there appears to be a relationship between the vegetation and the *Ruderalia* cytotype ratio. There are probably some biotic or abiotic variable(s) that cause them to change congruently. To further validate this relation, a CCA was performed. In this CCA the vegetation data was related to the cytotype ratios to obtain the canonical ordination axis. Because this was the only variable used for the CCA, only the first axis was valid. To see whether there are significant vegetation differences between diploid dominated and triploid dominated fields, the built-in Monte Carlo test of CANOCO was used. The differentiation was significant at $p=0.0001$. Another CCA was performed using only the diploid and triploid fields below 600 meters, where there is no relation between ploidy level and altitude (Figure 3). Also in this second test, a significant difference was found ($p=0.034$). CANOCO has the option to use one or more variables as so called “covariables”; it will remove the effects of these variables from the ordination. If following the ordination, the Monte Carlo test is performed, and still shows a significant difference in vegetation, the covariables played no role in causing the difference. Using the altitude as covariable for an ordination still showed a significant difference in vegetation when running the Monte Carlo test ($p=0.009$).

Cytotype distribution within a mixed field

The data from the transect analysis for cytotype distribution in the mixed field are shown in Table 4. For each transect and each distance class the number of pairs compared and their t-values are listed. The higher distance classes containing less than 30 pairs of individuals have been removed. The cytotypes along transect VA are totally randomly distributed. Only at a few distance classes significant negative correlations were detected.

Table 4: Mantel test of random distribution of cytotypes for different distance classes. Upper limits of each class are given in centimeters, significance of t -value * for $p(t) < 0.05$ after sequential Bonferroni.

VA			VB			VC		
Upper limit of class	Number of pairs	t-value	Upper limit of class	Number of pairs	t-value	Upper limit of class	Number of pairs	t-value
61	438	2.26	64	337	2.39	138	210	1.91
121	413	-1.35	128	267	-3.14*	275	95	-1.50
182	318	1.23	192	202	-0.78	413	160	0.52
243	277	-1.80	256	208	1.87	551	92	1.30
303	249	0.35	320	177	0.50	689	145	1.47
364	248	2.99	384	151	-0.04	826	120	0.93
424	254	1.14	448	150	-2.78	964	86	0.42
485	235	1.18	512	210	1.07	1102	53	-2.16
546	284	0.76	576	207	0.83	1239	62	-0.55
606	241	0.65	640	157	-1.25	1377	106	0.06
667	210	1.78	703	156	0.21	1515	37	-2.82*
728	151	1.47	767	134	-0.67	1652	72	-0.09
788	112	-1.45	831	87	-1.47	1790	50	-0.99
849	97	-0.34	895	73	0.94	1928	50	-1.19
909	91	-0.10	959	55	0.29			
970	81	1.29	1023	59	0.23			
1031	63	-0.44	1087	61	-0.02			
1091	76	0.56	1151	96	-0.48	VD		
1152	74	-0.49	1215	143	2.28	55	167	1.30
1213	97	-1.50	1279	137	1.80	109	107	-0.43
1273	76	-0.76	1343	114	-3.34*	164	74	0.59
1334	76	-0.69	1407	132	1.24	219	78	-1.20
1394	89	-1.99	1471	146	2.02	273	79	1.27
1455	97	-1.02	1535	88	-0.01	328	80	1.74
1516	98	-0.93	1599	77	0.28	382	58	0.57
1576	90	-0.70	1663	77	-1.06	437	97	-0.20
1637	71	-1.13	1727	121	2.27	492	79	-1.67
1698	76	-1.69	1791	129	2.08	546	69	-1.70
1758	65	-1.70	1855	120	-2.28	601	63	2.00
1819	45	-1.42	1919	119	-0.68	656	65	1.60
1879	54	-1.57	1982	99	-1.56	710	111	-0.03
1940	58	-1.06	2046	65	-1.28	765	54	-1.22
2001	73	0.18	2110	41	1.72	820	67	-3.32*
2061	52	0.73	2174	36	-2.50	874	42	-0.60
2122	56	-1.09	2238	35	-1.30	929	61	0.33
						983	62	2.04

Discussion

As has been suggested before (Den Nijs, Sterk, 1984; Jenniskens *et al.*, 1985), there is habitat differentiation between diploid and triploid *Taraxacum* sect. *Ruderalia*. Correlations have been found between the cytotype ratio and some of the ecological indicators. There are also significant vegetation differences between fields with mainly diploid *Ruderalia* and with mainly triploids.

Disturbance versus Altitude

There appears to exist a correlation between the percentage of diploids and the altitude. Most fields above 600 meters contain mainly diploid *Ruderalia*; in the 13 sampling fields situated above 1000 meters, only 3 triploid individuals were found (see Appendix 1). Of all the species for which a H_i -value was calculated, *Alchemilla vulgaris* had the highest value (Table 2). In the region around Neuchâtel, *A. vulgaris* is only found at relatively high altitude. In comparable fields at lower altitudes, *A. vulgaris* is either very rare or absent. There is also a correlation between the altitude and the percentage of therophytes in a field (Table 3). Vegetations with a high percentage of therophytes grow at lower altitudes. In general, increasing altitude has certain physiological consequences for therophytes, that may cause this correlation, but in our study it is questionable whether the small altitudinal cline (430-1155 m) has such a strong effect on the amount of therophytes. In the study area the city of Neuchâtel, the vineyards, arable lands, pastures, hay meadows and roadsides are situated at lower elevations. At higher elevations there are mainly pastures, hay meadows, roadsides and some scattered agricultural villages. Apparently, relatively stable habitats are more common on the higher elevations, and the low percentages of therophytes at higher elevations may be explained by the amount of human disturbance, being covariate with altitude. This suggests that the altitude/cytotype ratio correlation may also be due to such covariation. If only the fields below 600 m are considered, the relation between altitude and cytotype ratio disappears. The correlation between cytotype ratio and the weighted percentage of therophytes of the vegetation of the field (Table 3) remains significant. Triploid dominated fields have more therophytes than diploid dominated fields. Among the 10 species with the lowest H_i -values, there are 7 therophytes. In non-natural communities, therophytes are typical for early successional vegetations. They are good colonisers, but in the absence of man-made disturbance, the short-lived species are often quickly outcompeted by perennial species.

Considering the series of CCA's, it appears that there are significant vegetation differences between diploid and triploid dominated fields. The Monte Carlo tests reveal significant differences both when the CCA is performed without covariables, when only fields below 600 m are considered and when altitude is set as covariable. Thus altitude, though correlated with the cytotype ratio, is not the determining factor for vegetation differences between the diploid and the triploid fields. Differences in the agricultural use

of the fields, which are partly determined by altitude, are a more likely source for these vegetation differences.

Both climatic and environmental dynamics have been suggested as determinants of the presence and abundance of triploids relative to diploids in *Taraxacum* sect. *Ruderalia* (Elzinga *et al.*, 1987; Den Nijs, Van der Hulst, 1988; Roetman *et al.*, 1988; Den Nijs *et al.*, 1990). Site disturbance has been proposed to be disadvantageous for diploids by a.o. Fürnkranz (1966) and Den Nijs & Sterk (1984). Because disturbance is a term that is hard to define, and even harder to quantify, these authors found no straightforward proof of this. The results of our study, however, give very strong indications for such a preference. The cytogeography of *Ruderalia* in the area around Neuchâtel (Figure 2), shows that the triploids are merely confined to the conurbation of Neuchâtel and the neighboring vineyards. Outside this region, triploids become increasingly rare and many purely diploid populations are found. Inside the conurbation, human pressure is high, most habitats are small and only available for a short time. Outside the conurbation, habitats are larger and, according to the owner/farmers, most of the sites have been managed at a low intensity for decades. Indeed, most vineyards are also very old, but they are ploughed every few years. Furthermore, in some vineyards herbicides are applied. Thus, the level of external environmental dynamics in the latter type of habitat is much larger, the sites are anthropogenically kept in pioneer phase of vegetation development.

A random distribution of both cytotypes in a mixed field would describe a true sympatric situation, in other words, without differentiation of micro-niches. The transect data did show a random distribution. The very few significant distance classes in the transects don't show any coherent pattern. Moreover, the significant cases are flanked by classes with very different t-values. We must conclude that this study field did not show variation in disturbance in such a degree that ploidy levels respond.

Conclusions

This study of the Neuchâtel area allows for the following generalities:

- 1) At low altitudes the cytotype ratio is largely determined by the amount of (man-made) disturbance. Highly disturbed fields contain mainly triploids, relatively undisturbed fields contain mainly diploids.

- 2) At higher altitudes there are mainly diploids. At some highly disturbed places, triploids can be found, but only in low numbers.

We hypothesize that in western Switzerland diploid *Ruderalia* used to occur in all available habitats, irrespective of altitude, but that human influence on the landscape lead to a shift. Whether the found triploids are newly originating from diploid stock (a mechanism suggested by Menken *et al.* (1995) and Den Nijs (1997)) or are in fact immigrants is as yet an unsolved question.

At the northern border of the distribution area of the diploids, they mainly occur in dry, sunny places (Elzinga *et al.* 1987, Roetman *et al.* 1988, Den Nijs *et al.* 1990). From

these studies however, the diploids' low tolerance for disturbance is not as apparent as in the Neuchâtel area. Elzinga *et al.* (1987) and Roetman *et al.* (1988) found that in The Netherlands, diploids prefer undisturbed grasslands with a southern projection. Contrarily, Den Nijs *et al.* (1990) found that in Czechoslovakia diploids are mainly found in highly disturbed village lawns, while the triploids seem to be common everywhere. At the northern border of the distribution area of the diploids microclimate appears to be more crucial than disturbance in determining the presence of diploid *Ruderalia*.

The overall distribution area of the diploid *Ruderalia*, compared to that of the triploids, suggests that diploids have a smaller tolerance for low temperatures than triploids. However, well in the centre of the diploid area, the diploids may be able to grow at the full range of altitudes, including the higher, colder ones. The preference for lower altitudes found for *Taraxacum* sectio *Hondoense* in the center of its distribution area by Morita (1976) contradicts this pattern. Without a detailed comparative eco-physiological study, this difference remains unexplained.

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*Appendix 1: List of sampling sites and rough data collected. Vegetation relevés (data not shown) were only made for the first group of fields (upper part). No environmental indicators are available for the fields in the second group. The totals and averages are calculated for both groups combined. Vegetation relevés of sites marked with a * are given in Appendix 2. F, R, N and T are the Landolt indicator values for humidity, acidity, nutrients and temperature, respectively. Thero, Geo, Hemi and Cham are the weighted percentages of therophytes, geophytes, hemicryptophytes and chamaephytes in the vegetation.*

Site	Type	n	% dip.	% trip.	% tet.	Altitude	# cuts	Shadow	F	R	N	T	Thero	Geo	Hemi	Cham
2	vineyard	33	46	49	6	520	4	m	2.7	3.0	3.5	3.4	0	11	100	14
5	sportsfield	39	69	31		434	4	l	2.6	3.1	3.6	3.3	31	10	70	10
9	hay meadow	33	100	0		585	3	l	2.5	3.2	3.1	3.3	6	8	91	3
10	roadside verge	30	63	33	3	624	3	m	2.5	3.3	3.1	3.3	13	3	87	7
12	park	32	97	3		690	6	h	2.9	3.1	3.5	3.2	13	7	82	10
14	roadside verge	31	74	26		850	3	h	3.0	3.1	3.7	3.2	21	4	78	12
15	lawn	38	100	0		1010	4	m	2.9	3.1	3.2	3.0	0	9	97	8
16	roadside verge	32	100	0		1092	3	m	2.8	3.0	4.0	3.0	34	14	64	9
29	hay meadow	34	100	0		1040	3	m	3.1	3.0	3.7	3.2	0	12	88	7
30	pasture	31	52	48		940	3	l	2.9	3.0	3.6	3.1	1	5	95	13
42	hay meadow	37	78	22		672	4	l	2.5	3.3	3.1	3.5	12	6	87	9
47	lawn	41	88	12		590	6	l	2.7	3.2	3.3	3.1	14	1	85	9
50 *	roadside verge	33	27	73		540	3	l	2.6	3.2	3.5	3.3	12	14	88	21
56	hay meadow	29	97	3		530	3	m	2.8	3.2	3.7	3.2	11	36	74	2
57	park	33	12	82	6	525	6	l	2.7	3.1	3.6	3.3	15	0	79	19
58	vineyard	31	13	81	6	520	4	l	2.4	3.2	3.4	3.7	62	11	30	6
60	lawn	38	100	0		434	4	l	2.6	3.3	3.2	3.3	12	2	88	9
63	broad roadside verge	30	57	43		435	4	l	2.6	3.3	3.5	3.5	13	6	86	4
65	hay meadow	36	69	31		439	3	l	2.6	3.1	3.3	3.4	3	5	95	9
67	roadside verge	35	17	83		435	3	l	2.7	3.2	3.6	3.3	26	22	72	10
69	vineyard	32	53	47		460	4	m	2.5	3.0	3.4	3.4	27	23	68	12
72	hay meadow	32	28	72		490	2	l	2.4	3.4	3.0	3.3	6	0	94	10
73	hay meadow	33	82	18		490	2	m	2.7	3.2	3.6	3.5	4	0	96	14
75	roadside verge	30	100	0		730	3	h 6	2.8	3.1	3.4	3.3	13	33	87	13
77	biological wheat field	31	100	0		875	1	l 3	2.8	3.1	3.7	3.2	43	0	53	14
78	hay meadow	34	97	3		795	2	l	3.0	3.1	3.6	3.1	0	0	100	5
80	hay meadow	33	100	0		805	3	l	2.9	3.1	3.5	3.1	0	0	100	13
81	roadside verge	24	92	8		810	3	l	2.8	3.0	3.7	3.2	38	0	63	5
83 *	vineyard	33	0	100		455	4	h	2.7	3.3	3.7	3.3	0	31	100	31
84	orchard	35	100	0		752	3	m	2.9	3.1	3.5	3.3	0	0	100	9
85	hay meadow	33	85	15		755	3	l	2.9	3.0	3.7	3.2	18	6	82	10
86	hay meadow	33	100	0		780	3	l	2.9	3.0	3.5	3.1	6	13	94	13
87	park	31	90	10		445	6	h	2.7	3.0	3.3	3.3	5	5	92	8
88	orchard	11	36	64		435	5	m	2.7	3.1	3.7	3.5	43	4	39	6
151	hay meadow	33	64	36		560	3	l	2.9	3.1	3.9	3.3	0	0	100	35
152	roadside verge	31	7	94		560	3	m	2.8	3.1	3.7	3.2	30	1	72	7

Appendix 1: Continued

Site	Type	n	% dip.	% trip.	% tet.	Altitude	# cuts	Shadow	F	R	N	T	Thero	Geo	Hemi	Cham
153	lawn	40	15	85		580	5	m	2.6	3.1	3.3	3.3	14	14	86	9
154	lawn in forest	33	46	55		591	6	h	3.0	3.0	3.7	3.0	6	24	94	27
155	garden	31	32	68		523	1	m	2.0	3.8	2.7	4.0	22	33	78	0
156	garden	24	42	58		510	6	m	2.7	3.1	3.7	3.4	30	0	70	14
157	roadside verge	17	24	71	6	510	3	m	2.5	3.2	3.4	3.5	20	5	79	16
159 *	hay meadow	20	85	15		530	3	m	2.5	3.3	2.7	3.4	9	0	77	8
160	hay meadow	30	40	57	3	530	3	h	2.9	3.1	3.7	3.2	7	0	89	13
161	vineyard	32	6	94		510	4	l	2.3	3.1	3.4	3.9	63	7	37	17
162	pasture	22	9	91		585	3	l	2.6	3.2	3.3	3.4	8	0	83	16
163	edge of meadow	34	32	68		585	2	h	2.7	3.1	3.3	3.3	12	1	84	13
164	path	28	4	96		570	3	m	2.9	3.1	3.9	3.1	35	5	65	0
165	path	32	3	97		570	3	m	2.7	3.1	3.4	3.2	15	10	80	14
166	roadside verge	32	6	94		600	3	l	2.7	3.1	3.6	3.5	18	5	83	9
167	roadside verge	40	53	48		720	3	h	2.8	3.2	3.3	3.2	5	8	86	14
176	roadside verge	31	52	48		570	3	l	2.6	3.1	3.3	3.3	6	17	83	9
177	hay meadow	33	46	55		575	3	l	3.0	3.0	3.6	3.0	0	0	100	20
178	pasture	31	100	0		585	3	l	2.9	3.1	3.8	3.2	20	22	84	18
179	path	31	90	10		610	3	m	2.8	3.1	3.4	3.2	6	4	90	15
180	hay meadow	32	100	0		1080	3	l	2.4	3.4	2.8	3.2	7	3	85	8
181	roadside verge	34	97	3		1155	3	h	3.1	3.1	3.7	3.1	17	9	80	17
183	roadside verge, next to wall	32	16	78	6	460	3	m	3.0	3.0	3.4	3.3	39	0	62	0
184	lawn	14	21	79		505	3	l	2.5	3.2	3.3	3.4	44	6	51	18
185	roadside verge	15	53	47		445	3	m	2.4	3.2	3.3	3.7	21	5	79	11
186	roadside verge	32	53	47		439	3	h	2.9	3.1	4.0	3.5	22	0	57	12
187	lawn	20	20	80		430	6	l	2.7	3.1	3.5	3.3	36	0	64	19
188	rocky beach	31	87	13		430	0	l	2.9	3.0	3.8	3.2	38	0	58	4
189	roadside verge	31	84	13	3	435	3	l	2.7	3.0	3.6	3.3	31	11	60	10
192 *	hay meadow	36	100	0		1135	3	l	3.0	3.0	3.7	3.1	0	0	95	9
194	pasture	35	100	0		765	4	l	3.0	3.0	3.7	3.1	4	13	94	16
195	pasture	33	100	0		787	3	l	2.8	3.0	3.3	3.2	6	0	90	4
196	roadside verge	30	93	7		505	3	h	2.8	3.0	3.8	3.4	39	9	53	4
197 *	lawn	29	48	48	3	535	6	l	2.5	3.1	3.3	3.3	5	0	93	16
198	park	30	17	83		470	4	h	2.8	3.2	3.4	3.2	10	0	96	23
199	roadside verge	22	36	64		490	3	m	2.7	3.2	3.6	3.6	41	7	52	10
200	unused field in city	33	21	79		480	0	m	2.8	3.1	3.6	3.3	30	8	69	9
201	vineyard	34	18	82		440	2	m	2.8	3.1	3.8	3.4	31	21	62	14
202	hay meadow	33	85	12	3	430	4	m	2.8	3.1	3.5	3.2	3	6	97	22
203	cut sunflower field	33	6	91	3	500	1	l	2.6	3.0	3.3	3.8	78	0	39	0
204	vineyard	33	27	70	3	500	6	m	2.9	3.1	3.8	3.1	39	16	65	0
205	vineyard	34	3	94	3	455	4	m	2.6	3.2	3.7	3.5	53	11	47	24
206	vineyard	33	6	94		545	4	m	2.8	3.3	3.8	3.4	60	10	41	5

Appendix 1: Continued

Site	Type	n	% dip.	% trip.	% tet.	Altitude	# cuts	Shadow	F	R	N	T	Thero	Geo	Hemi	Cham
207	hay meadow	35	57	43		520	3	l	2.7	3.1	3.6	3.3	2	0	100	8
208	hay meadow	33	91	9		455	6	l	2.9	3.1	3.7	3.2	19	10	77	8
209	vineyard	35	66	26	9	455	4	m	2.7	3.1	3.6	3.6	31	13	57	6
210	*vineyard	31	19	81		440	6	h	2.8	3.2	3.8	3.5	39	14	58	0
211	roadside verge	35	57	43		430	3	h	3.0	3.0	3.7	3.1	0	9	100	13
212	hay meadow	34	94	6		525	4	l	2.9	3.0	3.7	3.1	0	0	100	21
213	hay meadow	29	100	0		610	3	l	2.9	3.0	3.8	3.2	0	0	100	22
214	roadside verge	33	64	36		434	3	l	2.7	3.2	3.2	3.6	26	8	36	0
215	parking place	31	29	71		455	0	l	2.5	3.1	3.5	3.5	69	0	31	4
216	roadside verge	31	29	71		470	3	m	3.0	3.3	3.2	3.4	11	11	74	11
217	pasture	34	94	6		635	3	l	2.8	3.0	3.5	3.1	2	0	98	10
218	lawn	31	16	84		445	4	m	2.6	3.2	3.5	3.5	24	5	68	10
219	park	34	100	0		480	3	m	2.5	3.2	3.1	3.5	4	0	88	3
220	vineyard	32	44	53	3	520	5	m	2.9	3.0	3.8	3.1	33	9	58	21
221	vineyard	31	26	74		520	5	l	2.6	3.1	3.9	3.3	50	9	50	13
222	garden	30	70	23	7	455	3	l	2.8	3.1	3.6	3.5	27	11	62	14
18	pasture	49	96	4		1100										
21	roadside verge	31	100	0		1137										
22	pasture	30	100	0		1120										
23	pasture	30	100	0		1090										
25	hay meadow	32	100	0		1110										
33	cut corn field	50	84	16		900										
37	hay meadow	49	100	0		840										
38	path	50	100	0		830										
39	lawn	49	98	2		815										
48	hay meadow	24	100	0		560										
54	roadside verge	28	71	29		580										
55	hay meadow	33	6	94		550										
150	wet spot in hay meadow	48	31	69		920										
168	park	33	82	18		525										
169	roadside verge	26	54	46		625										
170	railside	30	77	20	0.03	625										
171	railside	5	40	60		740										
172	path	7	71	29		920										
173	path	30	73	27		920										
174	path	10	80	20		730										
175	path	30	33	63	0.03	660										
182	nature reserve	32	100	0		1400										
193	path	25	100	0		1000										

Appendix 2: Vegetation relevés of six representative sampling sites. Plant nomenclature follows Lauber and Wagner (1996). Sites are sorted on the percentage of diploid Ruderalia, species are sorted by their score on the first DCA-axis (data not shown)

Site nr.	83	210	50	197	159	192
Type	vineyard	vineyard	roadside verge	lawn	hay meadow	hay meadow
% Dip.	0	19	27	48	85	100
Species						
<i>Veronica persica</i>			1			
<i>Vitis vinifera</i>	8	8				
<i>Erigeron canadensis</i>		1				
<i>Lamium purpureum</i>		1				
<i>Setaria verticillata</i>		1				
<i>Senecio vulgaris</i>		4				
<i>Rubus caesius</i>		2				
<i>Geranium pusillum</i>		1		3		
<i>Polygonum aviculare</i>		4				
<i>Convolvulus arvensis</i>	5	5	7			
<i>Calystegia sepium</i>		2				
<i>Hordeum murinum</i>		1				
<i>Bromus sterilis</i>			1			
<i>Erigeron annuus</i>						1
<i>Veronica arvensis</i>		2				
<i>Hieracium laevigatum</i>				4		
<i>Urtica dioica</i>		3				
<i>Potentilla reptans</i>		4				
<i>Lapsana communis</i>		2				
<i>Lolium perenne</i>	6	7	8	6		
<i>Plantago major</i>			1		1	3
<i>Geranium molle</i>			1			
<i>Oxalis fontana</i>			1			
<i>Daucus carota</i>		3		3		
<i>Bellis perennis</i>		5	1	7		
<i>Trifolium repens</i>	5		3	7	1	3
<i>Plantago lanceolata</i>			1	8	2	
<i>Bromus hordeaceus</i>		3	1			
<i>Medicago lupulina</i>			1		2	
<i>Rumex obtusifolius</i>						2
<i>Galium mollugo</i>			2			
<i>Vicia sativa</i>					1	
<i>Achillea millefolium</i>		1	4	4		4
<i>Bromus erectus</i>			5		2	
<i>Trifolium pratense</i>			1	4	4	2
<i>Medicago sativa</i>				5		
<i>Dactylis glomerata</i>			2	2	5	8

Appendix 2: Continued

Site nr.	83	210	50	197	159	192
<i>Tragopogon pratensis</i>						1
<i>Cerastium fontanum</i>				1	2	
<i>Myosotis arvensis</i>			1			
<i>Poa trivialis</i>			8			
<i>Heracleum sphondylium</i>			3			3
<i>Ranunculus acris</i>						4
<i>Phleum pratense</i>						7
<i>Silene vulgaris</i>			1	1	3	
<i>Arrhenatherum elatius</i>			3		7	
<i>Anthriscus sylvestris</i>						5
<i>Vicia sepium</i>						1
<i>Festuca ovina</i>					5	
<i>Vicia cracca</i>						2
<i>Lathyrus pratensis</i>						2
<i>Lotus corniculatus</i>					3	
<i>Salvia pratensis</i>				2	3	
<i>Leontodon hispidus</i>					1	
<i>Alchemilla vulgaris</i>						3
<i>Festuca pratensis</i>						7
<i>Veronica chamaedris</i>						3
<i>Leontodon autumnale</i>					3	
<i>Leucanthemum vulgare</i>						4
<i>Cynosurus cristatus</i>						6
<i>Centaurea scabiosa</i>					3	
<i>Anthoxanthum odoratum</i>					4	
<i>Crepis biennis</i>						4
<i>Knautia arvensis</i>					4	
<i>Betula pubescens</i>					6	
<i>Onobrychis vicifolia</i>					4	
<i>Geranium sylvaticum</i>						3
<i>Sanguisorba minor</i>					3	
<i>Briza media</i>					4	
<i>Cerastium arvense</i>						1
<i>Helianthemum nummularium</i>					2	

Spatial ecological and genetic structure of a mixed population of sexual diploid and apomictic triploid dandelions

3



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Abstract

Ecological differentiation is widely seen as an important factor enabling the stable coexistence of closely related plants of different ploidy levels. We studied ecological and genetic differentiation between co-occurring sexual diploid and apomictic triploid *Taraxacum* section *Ruderalia* by analyzing spatial patterns both in the distribution of cytotypes and in the distribution of genetic variation within and between the cytotypes. A significant relationship between ploidy level and elevation was found. This mode of ecological differentiation however was not sufficient to explain the significant spatial structure in the distribution of diploids and triploids within the population. Strong congruence was found between the spatial genetic patterns within the diploids and within the triploids. We argue that this congruence is an indication of gene flow between neighboring plants of different ploidy levels.

Introduction

Although polyploidy is one of the major aspects of plant evolution, the mechanisms by which newly formed polyploids can become established in their parent populations remain unclear. Central to polyploid theory is Levin's (1975) minority cytotype exclusion principle that predicts which two conspecific cytotypes cannot coexist stably. Fitness loss due to unsuccessful hybridizations will occur more frequently in the less than in the more abundant cytotype, and this will ultimately lead to the exclusion of the least abundant cytotype from the population. Newly formed polyploids will often start as a minority cytotype in an existing population so their establishment seems very unlikely, except in small populations where drift is an important factor. Coexistence of two cytotypes can be possible however, when there is increased self-compatibility in the minority cytotype or when there is ecological differentiation between the cytotypes (Fowler, Levin, 1984; Rodriguez, 1996; Felber, Bever, 1997).

In almost all asexually reproducing plant species, asexuality is linked with polyploidy (Stebbins, 1980). In polyploid complexes comprising both asexual polyploid and sexual diploid individuals, the cytotype exclusion principle works differently and will always lead to the exclusion of diploids from mixed populations of any composition. Assuming that the asexual polyploids still produce pollen but do not need pollination to produce seeds, unsuccessful hybridisations will only take place in the sexual diploids, which leads to a selective disadvantage of the diploids. Furthermore, diploids can produce polyploid offspring from pollinations from polyploid fathers, but the polyploids will always only make polyploid offspring. This will cause a decrease in the relative frequency of diploids in the population and will thus lead to more frequent hybridizations, which in turn will decrease the relative frequency of diploids even further (Mogie, Ford, 1988).

Ecological differentiation has been proven for several polyploid complexes, including some complexes with asexually reproducing polyploids (see Petit *et al.*, 1999 for an overview). Most of these studies have focused on ecological differences among diploid, mixed and polyploid populations, but only few have studied within-population differentiation. As most ecological variables are spatially structured (Legendre, Legendre, 1998), ecological differentiation between cytotypes leads to the expectation of patchy distribution of the cytotypes within a population. Only few studies have investigated spatial patterns within populations of coexisting cytotypes (Chapter 2; Keeler, 1992; Husband, Schemske, 2000). Husband and Schemske (2000) and Hardy and Vekemans (2001) found significant patterns in their species, respectively *Chamerion angustifolium* and *Centaurea jacea*. Both Keeler (1987) and Meirmans *et al.* (Chapter 2) found no spatial structure in the distribution of cytotypes of *Andropogon gerardii* and *Taraxacum* sect. *Ruderalia*, respectively.

The polyploid complex *Taraxacum* section *Ruderalia* Kirschner, H. Øllg. & Štěpánek (dandelions, *Asteraceae*) contains both diploid ($2n=2x=16$) and triploid ($2n=3x=24$) individuals, whereas higher ploidy levels are rare. Diploid *Ruderalia* reproduce obligatorily

sexually and are usually self-incompatible while triploid *Ruderalia* reproduce through apomixis but nevertheless produce pollen that is largely sterile. Based on the cytotype exclusion mechanisms and the fact that diploids and triploids are frequently found together (Den Nijs, Sterk, 1980), ecological differentiation is expected in *Taraxacum* sect. *Ruderalia*. Tschermak-Woess (1949) and Fürnkranz (1966) found a difference in flowering time between the ploidy levels: in Central Europe, diploids flower approximately one week earlier than co-occurring triploids. The significance of this difference can however be questioned as there still is a lot of overlap between the respective flowering periods. Elzinga *et al.* (1987) report a preference of diploids for relatively warm south facing slopes in the southern part of The Netherlands. Meirmans *et al.* (Chapter 2) found that the relative frequency of triploids in populations from Neuchâtel, Switzerland, increased with an increase in the amount of disturbance by human activities.

Apomixis, as well as some forms of ecological differentiation (e.g., in flowering time), will lead to reproductive isolation between two cytotypes. Such reproductive isolation has been found in several polyploid complexes (Petit *et al.*, 1997; Van Dijk, Bakx-Schotman, 1997; Gauthier *et al.*, 1998a), but genetic studies show that in *Taraxacum* section *Ruderalia* this is not the case. Menken *et al.* (1995) found large homogeneity in allozyme allele frequencies between diploids and triploids from mixed populations. This intrapopulation homogeneity between the ploidy levels contrasted strongly with the genetic differentiation between different populations. As this analysis was performed by analyzing population level variation, the spatial scales at which the interactions between diploids and triploids take place within a population remain unknown.

As a result of limited seed dispersal and spatially restricted pollination, genetic variation may not be distributed homogeneously over a population (isolation by distance, Wright, 1943). If this holds true for *Taraxacum*, the asexuality of the triploids leads to clear expectations regarding the genetical population structure. If the triploids are strictly asexual and reproductively isolated from the diploids, the spatial genetic patterns of the two cytotypes should be independent. The two spatial genetic patterns are expected to differ as isolation by distance in the diploids builds up as a result of both restricted seed and pollen dispersal while in the triploid it depends on the seed dispersal only. If there is some gene flow between the two cytotypes, the two spatial genetic patterns should show congruency, even though they might still be slightly different.

The aim of this study is to look for ecological and genetic differentiation within a mixed diploid-triploid population of *Taraxacum* sect. *Ruderalia*. Ecological differentiation between the cytotypes is studied by analyzing the spatial distribution of cytotypes within the population and its relation to the elevation pattern within the field. Genetic differentiation within and between the ploidy levels was studied by analyzing the spatial distribution of allozyme variation and comparing the spatial patterns found in diploids and triploids.

Materials & Methods

Sampling

In spring 1986, we studied a *Taraxacum* sect. *Ruderalia* population from Waldmichelbach/Oberschönmattenweg in the Odenwald, Germany. This is the same locality, but not the same sample, as population number T20 from Menken *et al.* (1995). The population was situated in a moderately manured haymeadow, approximately 30 meters wide and 110 meters long. At the time of sampling, the field had been in use as a haymeadow for ca. 25 years. In the middle of the field, a grid was laid out measuring 25 by 90 meters, subdivided in 360 subplots of 2.5 by 2.5 meters. For every subplot, the ratio of diploid and triploid *Taraxacum* individuals was estimated by analyzing 10-15 plants using the pollen-analysis method of Tschermak-Woess (1949). Due to disturbed meiosis, pollen from triploids is irregularly sized, whereas pollen from diploids is regularly sized; simply checking the pollen under a field microscope is a quick method for determining the ploidy level of an individual. The accuracy of the method has been verified with chromosome counts (Den Nijs, Sterk, 1980). The elevation of each subplot was measured in spring 2000, using a level instrument. As the corners of the grid were marked when leaving the field in 1986, it was possible to exactly reconstruct the grid in 2000.

Based on the overall cytotype distribution, four subpopulations (A, B, C and D, see Figure 1) each comprising several adjacent subplots were chosen for further analysis. From these subpopulations a total of 421 plants were randomly picked out, checked for ploidy level, mapped, dug out and transported to the greenhouse in Amsterdam.

Electrophoresis

We performed allozyme analysis on the 361 (86%) of the plants that survived the transfer to Amsterdam. No distinct pattern was detectable in the distribution of the died plants within the field, so we assume that any selection during the transport of the plants

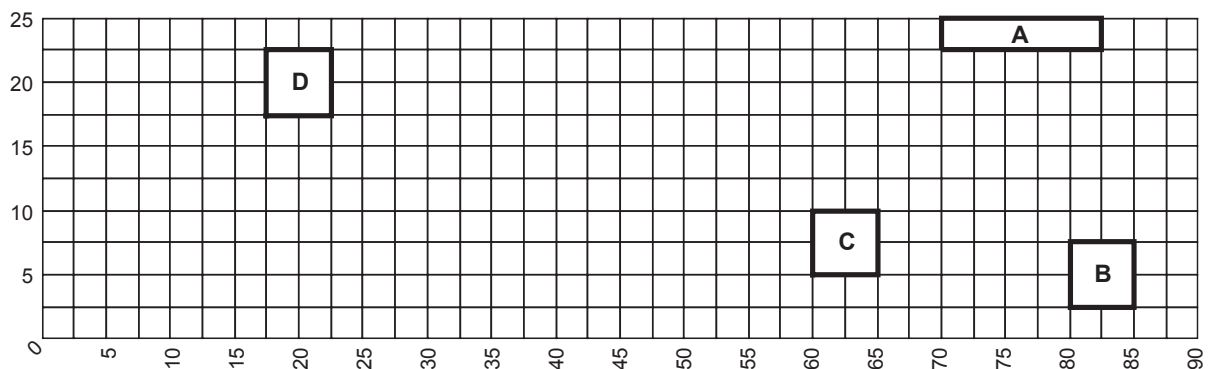


Figure 1: Odenwald study field and the gridsystem of the analysis. Thin lines indicate borders of subplots. Bold lines indicate borders of subpopulations used for genetic analysis.

has had no influence on our analysis. Protein extraction, electrophoresis, and allozyme specifications followed the protocol of Menken *et al.* (1989). Two enzyme systems were used: 6-phosphogluconate dehydrogenase (with two loci: *6Pgdh-1* and *6Pgdh-2*) and malate dehydrogenase (with one locus: *Mdh-1*). All three enzymes are dimeric and in the absence of dosage compensation allow for easy recognition of both kinds of triploid heterozygotes, the loci are located in the nuclear DNA, show Mendelian inheritance, and are unlinked (Menken *et al.*, 1989).

Genetic population structure

Allozyme data from the four subpopulations was analyzed for deviation from Hardy-Weinberg expectations for both diploids and triploids, using the log likelihood G-statistic. For triploids H.W.-expectations were calculated following $(p+q)^3$ for a locus with two alleles (Menken *et al.*, 1995), significance was assessed by permutating alleles between individuals. F_{is} values (Nei, 1987) were calculated for both ploidy levels. The calculated F_{is} values were not used as test statistics for testing H.W.-equilibrium, as in triploids a population with $F_{is}=0$ may be out of equilibrium. Imagine, for example, a population consisting for 25% of genotype aaa and for 75% of genotype AAa. This population would have a F_{is} of zero as the frequency of heterozygotes exactly matches the expectation of $1-2p^3$ ($= 0.75$). The population is nevertheless clearly out of H.W. equilibrium as it lacks two of the four possible genotypes.

Differentiation in allele frequencies between pairs of subpopulations was tested using the G-statistic, for diploids and triploids separately. Significance was determined by randomizing genotypes between pairs of populations. This permutation scheme was chosen as it does not assume random mating within the subpopulations (Goudet *et al.*, 1996). The same method was used to test for differentiation between diploids and triploids from the same subpopulation. When necessary, significance levels were corrected for multiple testing using the sequential Bonferroni procedure (Rice, 1989).

Spatial analysis.

Spatial autocorrelation analysis tests the association of the value of a geographically distributed variable (here, cytotype and allele frequencies) with the value of the same variable at another location (Sokal, Oden, 1978). Geographic relationships between pairs of samples are grouped in a number of distance classes and for every distance class an autocorrelation coefficient is calculated. As autocorrelation analysis involves multiple testing, correction is necessary. Therefore, Hewitt *et al.* (1997) developed the progressive Bonferroni correction, a correction method that is especially meant for correlograms.

For analyzing spatial patterns in the distribution of the cytotype ratio over the 360 subplots we used Moran's *I* (Moran, 1950), with 35 distance classes, where the input variable was the fraction of diploids found in the subplots (the fact that the number of distance classes equals the number of columns on our sampling grid is entirely coincidental). For the analysis of correspondence between the spatial patterns in cytotype

Table 1: (Partial) Mantel tests for association between spatial distance, elevation, and difference in percentage of diploids for the 360 subplots. ** $p < 0.001$ after Bonferroni correction

Matrix A	Matrix B	Corrected for:	Mantel's r
Geogr. distance	% of diploids	-	0.185 **
Geogr. distance	% of diploids	Elevation	0.172 **
Elevation	% of diploids	-	0.123 **
Elevation	% of diploids	Geogr. distance	0.102 **

distribution and elevation, we used a combination of standard Mantel tests (Mantel, 1967) and partial Mantel tests (Smouse *et al.*, 1986). Standard correlation analysis methods are not appropriate in cases where both variables are spatially autocorrelated (Legendre, 1993). This is the case here, where both the cytotype ratio and the elevation of the subplots are spatially dependent. A method to test the relationship between two spatially dependent variables is to perform a partial Mantel test, that calculates the relationship between two variables while correcting for the influence of a third one. We used a series of four Mantel and partial Mantel tests to elucidate the relationships between ploidy, elevation and space (see Table 1).

Analysis of fine-scaled patterns in the distribution of allelic variation was possible as the spatial coordinates of every sampled plant were measured in the field. For this analysis we used the coefficient of relationship, ρ_{ij} , which is independent of ploidy level, following Hardy and Vekemans (2001). Under two-dimensional isolation by distance, the coefficient of relationship between individuals is expected to linearly decrease with the logarithm of the distance between the individuals. The coefficient of relationship between two individuals is defined as $\rho_{ij} = \sigma_{ij} / \sigma^2$, where σ_{ij} is the covariance between the allele frequency of individuals i and j , and σ^2 is the variance of the allele frequency in the cytotype. A multi-locus, multi-allele estimation can be calculated by weighing the contributions of individual alleles by $p_{la}(1-p_{la})$, where p_{la} is the frequency of the a th allele at the l th locus (Loiselle *et al.*, 1995). The coefficient of relationship can also be used to calculate relationships between individuals of different ploidy levels. In that case it is defined as: $\rho_{ij} = \sigma_{ij} / \sigma_d \sigma_t$, where σ_d and σ_t are the standard deviations of the allele frequencies for the diploids and triploids respectively and σ_{ij} is calculated using the cytotype specific allele frequencies.

Spatial autocorrelation analysis was performed by calculating ρ_{ij} for every pair of individuals. The matrix of pairwise geographical distances was divided into 10 distance classes, and for every distance class the average ρ_{ij} was calculated. The upper limits of the 10 distance classes were 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 meters. Significance was assessed by randomizing genotypes over the distance classes. This test was performed for the diploids and the triploids separately and for the interaction between diploids and triploids.

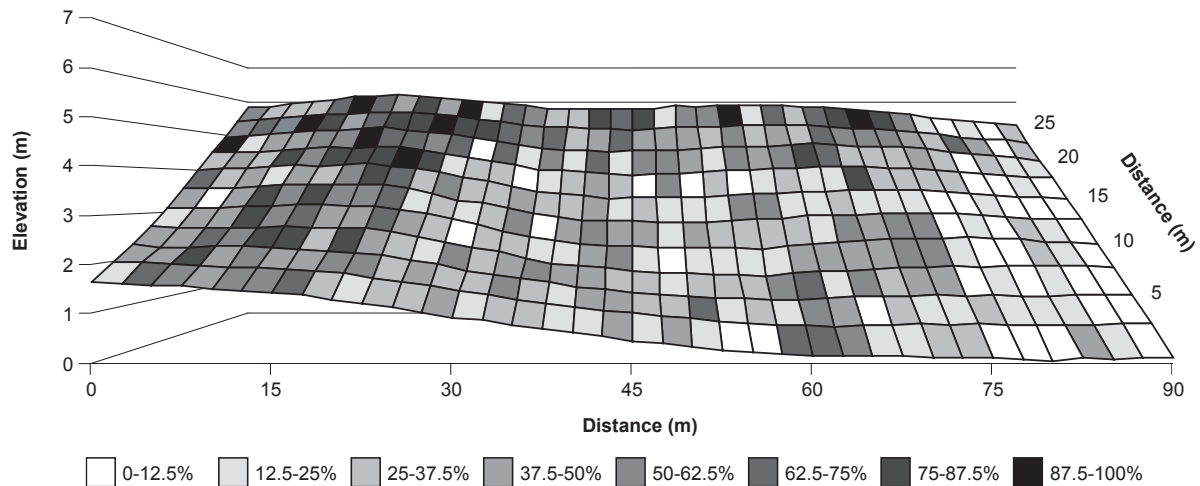


Figure2: *Distribution of cytotypes over the sample field. The greyscale indicates the percentage of diploids found in a subplot after analysis of 10-15 individuals.*

The number of randomizations for all permutation tests mentioned above was 9999. Calculations for the patterns in the distribution of the cytotypes were performed using the software package 'R', version 4.0d3 (Legendre, Vaudor, 1991). Genetic autocorrelation analyses were performed using the program AutocorG (available upon request from ohardy@ulb.ac.be). The G-tests were performed by a Perl script run in MacPerl 5.2.

Results

Distribution of cytotypes

The pollen analysis for the distribution of the cytotypes over the entire field showed that diploids were the minority cytotype: 33.6% of the analyzed plants were diploid, the remaining 66.4% were triploid. In 37 of the 360 subplots no diploids were found; the maximum percentage of diploids in a single subplot was 90%. The cytotypes were not randomly distributed over the field; two areas could be discerned in which the percentage of diploids was higher than elsewhere (Figure 2). The correlogram (Figure 3) shows the significance of this heterogeneous distribution; the majority of the distance classes shows a significant spatial autocorrelation. For distances smaller than 21 meters there is a significant positive autocorrelation, meaning that subplots less than 21 meters apart are more equal in cytotype ratio than would be expected from random distribution. For distances larger than 57 meters there is a significant negative autocorrelation, meaning that subplots more than 57 meters apart are less equal in cytotype ratio than would be expected from random distribution.

There appears to be an association between the cytotype ratio of the subplots and the topography (relief) of the field (Figure 2). This relationship was tested using a series of

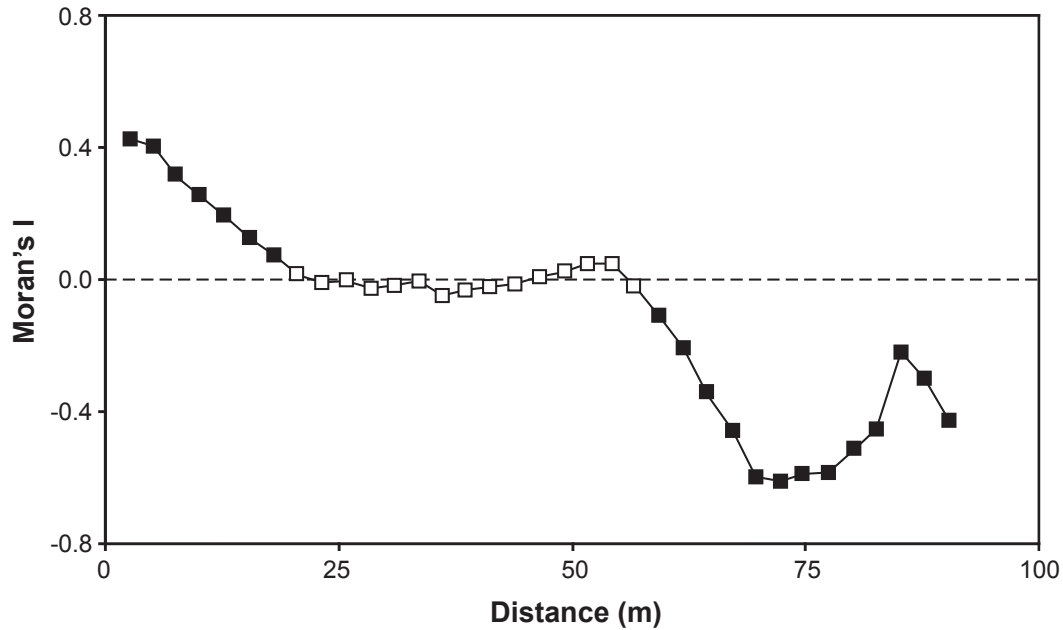


Figure 3: Correlogram for spatial patterns of variation of the percentage of diploids in the 360 subplots. Black symbols indicate significance ($p < 0.05$) after progressive Bonferroni correction.

(partial) Mantel tests (Table 1). The first standard Mantel test showed, like the correlogram, a significant ($p < 0.001$) positive relationship between the geographical distance and the difference in cytotype ratio. The second partial Mantel test tested the same relationship, corrected for the influence of the elevation. This test showed only a slight decrease in the value of r_M compared to the uncorrected test; the corrected relationship between space and cytotype ratio remained significant at the $p < 0.001$ level. Elevation per se therefore explains only a small part of the spatially dependent variation in the cytotype ratio. The third test tested directly for the relationship between elevation and cytotype ratio, and showed a strong relationship between these two variables. The last test tested what part of this relationship was caused by autocorrelation present in both variables, by correcting for geographical distance. Compared to the uncorrected test, there was only a slight difference in r_M , and the test was still strongly significant ($p < 0.001$). This indicates that spatial autocorrelation in the data explained only a small part of the observed correlation between cytotype ratio and elevation.

Genetic analysis

A total of eleven alleles was found, four for *6Pgdh-1*, three for *6Pgdh-2* and four for *Mdh-1*. Most alleles were shared by the two cytotypes; out of eleven alleles, three were cytotype specific, but these had an overall frequency lower than 2% (see Table 2).

In the triploids, 46 different three-locus genotypes ('clones') were found, 23 of these were found only once. The most frequent clone was found 41 times: 29 times in subpopulation B (where it made up 28% of the sampled triploids), seven times in C, five times in A and it was absent from subpopulation D. The next-most frequent clone

Table 3: Inbreeding coefficients and Hardy Weinberg equilibrium. Shown are F_{is} values calculated separately for the diploids and triploids. F_{is} values were not tested for deviation from 0, as in triploids an F_{is} of 0 does not necessarily imply random mating. Instead, deviation from H.W. expectations was tested using a G-statistic. * $p < 0.05$ after sequential Bonferroni correction

Locus	A	B	C	D	
<i>6Pgdh1</i>	-0.15	-0.10	0.18	-0.13	diploids
	0.16	-0.06	0.02*	-0.08	triploids
<i>6Pgdh2</i>	-0.10	-0.10	-0.19	-0.14	diploids
	0.06	-0.06	-0.08	0.14	triploids
<i>Mdh1</i>	0.09	-0.25	-0.18	-0.06	diploids
	0.17	-0.20*	-0.02	-0.03	triploids

was found 34 times; 23 times in A (=40% of the sampled triploids), five times in B, four times in C, and two times in D. Most other clones that were found more than once were also unevenly distributed over the subpopulations.

Most inbreeding coefficients in both the diploids and the triploids were slightly negative (Table 3). In the diploids, none of the tests revealed significant deviations from Hardy Weinberg expectations; in the triploids only two out of twelve tests showed a significant deviation. In one of these two cases (locus *6Pgdh1* in subpopulation C) the F_{is} value was remarkably close to zero, illustrating the uselessness of F_{is} as a statistic for testing for random mating in a polyploid population.

The pairwise tests for subpopulation differentiation (Table 4) showed significant differentiation between most subpopulations. Only subpopulations C and D were not significantly differentiated, both in the diploids and in the triploids. Allele frequencies in the diploids and triploids from the same subpopulations were significantly different in subpopulations A and B, but not in C and D.

Table 4: P-values from pairwise tests of multilocus subpopulation differentiation. Values above diagonal are from tests between diploids from different subpopulations, values below diagonal are from tests between triploids from different subpopulations. The values on the diagonal (gray) represent tests between diploids and triploids from the same subpopulations. * $p < 0.05$ after sequential Bonferroni correction

Subpop	A	B	C	D	
A	0.0003*	0.0001*	0.0001*	0.0001*	triploids
B	0.0001*	0.0083*	0.0002*	0.0002*	
C	0.0001*	0.0035*	0.699	0.040	
D	0.0001*	0.0008*	0.518	0.024	
	diploids				

Spatial genetic analysis

The correlograms calculated separately for diploids and triploids show some similarity (Figures 4a and b) although they do differ in significance at certain distance classes. Both correlograms show positive autocorrelation for distance classes up to 16 meters, and negative for distance classes at 32 and 128 meters. This means that individuals less than 16 meters apart are genetically more similar to each other than would be expected from a random distribution. Individuals which grow more than 16 meters apart are genetically less similar to each other than would be expected from a random distribution. For both ploidy levels, the correlograms are globally significant ($p < 0.001$).

The similarity in the spatial genetic patterns of the two ploidy levels which is apparent from Figures 4a and b does not necessarily imply that there is genetic exchange between the diploids and triploids. In case of independence of the isolation by distance structures, there would be no local genetic relationships between diploids and triploids, but simply equal patch sizes. To test this, a third correlogram was computed, including only distances between diploids and triploids, leaving out diploid-diploid and triploid-triploid comparisons. The structure in this third correlogram (Figure 4c, global test: $p < 0.001$) is practically the same as the structure of the correlograms calculated separately for the two ploidy levels. This shows that diploids and triploids growing in each others direct vicinity are more related to each other than diploids and triploids growing further apart.

Discussion

Distribution of cytotypes

Based on the minority cytotype exclusion principle (Levin, 1975; Fowler, Levin, 1984; Felber, Bever, 1997), ecological differentiation between coexisting cytotypes is expected. This may lead to a patchy distribution of the cytotypes within a population as a result of ecological heterogeneity. Of the studies known to us where within-population cytotype distribution was analyzed (Chapter 2; Keeler, 1992; Husband, Schemske, 2000) two were able to detect significant spatial structuring. Husband and Schemske (2000) reported that in the population of *Chamerion angustifolium* they studied, different patches of plants had different ratios of diploids and tetraploids, but they made no further inference on the causes of this patchy distribution. Hardy and Vekemans (2001) used autocorrelation statistics to show spatial segregation of diploid and tetraploid *Centaurea jacea*, but they did not test for any ecological mechanisms to explain the segregation. Meirmans *et al.* (Chapter 2) found no spatial pattern in the distribution of cytotypes in an analysis of four transects through a single population of *Taraxacum* sect. *Ruderalia* in Northwest Switzerland. As this population was chosen because of an apparent ecological homogeneity, the absence of spatial patterns in cytotype distribution

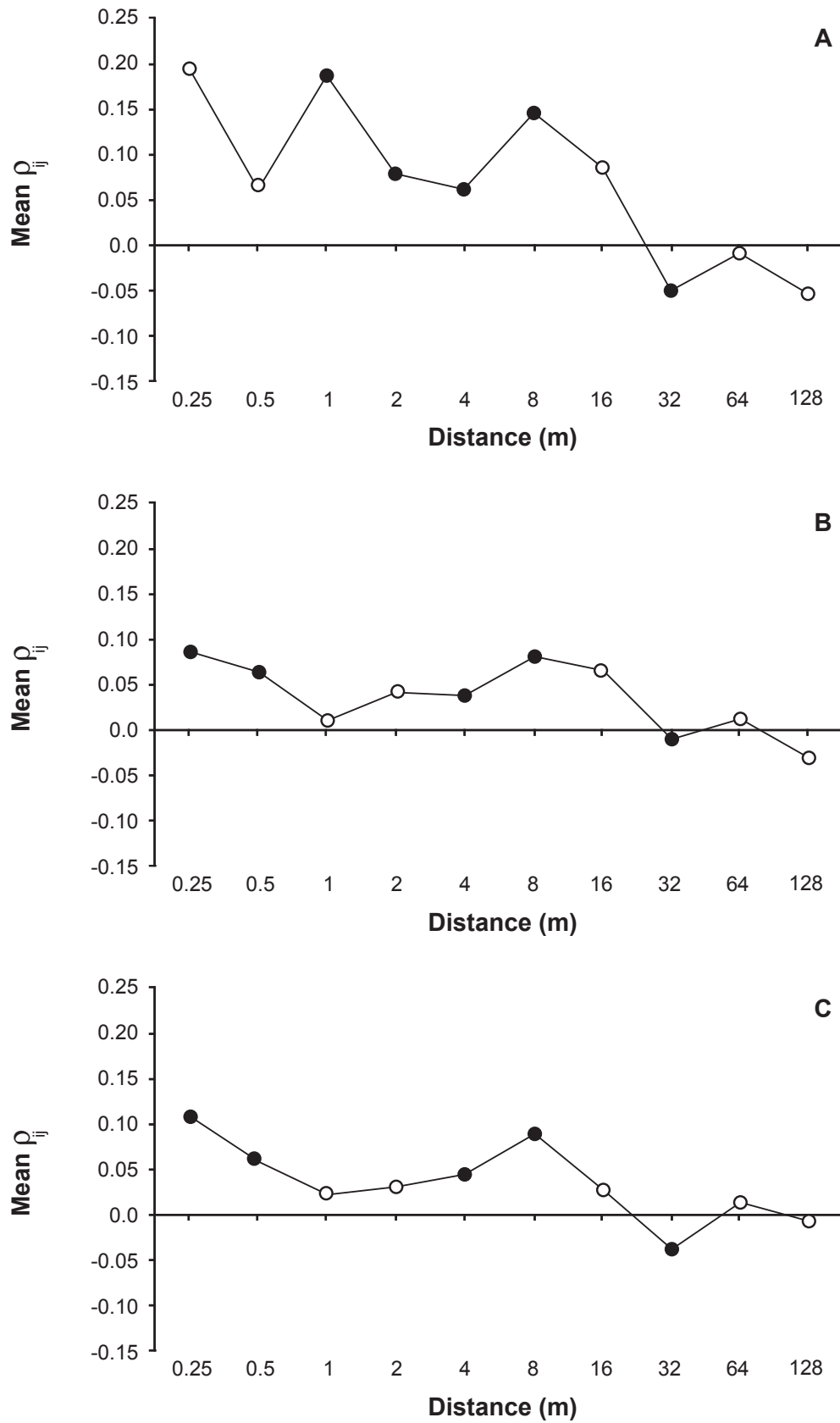


Figure 4: Coefficient of relationship ρ_{ij} (dark line) as a function of distance in diploids (a), in triploids (b) and between diploids and triploids (c). Black symbols indicate significance ($p < 0.05$) after progressive Bonferroni correction.

may not be surprising. The mechanisms enabling the coexistence of the cytotypes in that population, however, remain unknown. In Keeler's (1992) study of *Andropogon gerardii*, the lack of spatial structure may be the result of a lack of statistical power rather than of the absence of any spatial patterns within the populations. We re-analyzed her data using Moran's *I* statistic (results not shown) and found that significant autocorrelation patterns were present in two of the four populations she presented as being representative. So there probably is some ecological differentiation between the two cytotypes of *A. gerardii*.

In the present study, the spatial autocorrelation test showed that there is a highly significant patchy distribution of the two *Taraxacum* cytotypes in the Odenwald population studied. This patchy distribution may explain the difference between the overall cytotype ratio we found (34% diploids) and the ratio Menken *et al.* (1995) found (60% diploids) as they sampled only from a small part of the field (pers. comm.). The series of (partial) Mantel tests also showed that there is ecological differentiation between the diploids and triploids: the correlation between cytotype ratio and elevation was highly significant, and remained so when correcting for the influence of spatial autocorrelation in both variables. This relation between cytotype and elevation nevertheless only explained a small part of the spatial autocorrelation in the distribution of the cytotypes, as is shown by the small decrease of the Mantel correlation coefficient when correcting for elevation. This means that the observed heterogeneity in the distribution of the cytotypes is predominantly caused by other factors than elevation. These may be either ecological variables not measured in our study, or demographic factors. Different parts of the field may have different colonization histories, the results of which may still be visible in the present day distribution of the cytotypes. Another possibility is that the observed distribution is the result of a kind of ecological 'isolation by distance' process. Due to limited seed dispersal, patches of cytotypes may develop, comparable to the patches of alleles or haplotypes that are the result of genetic isolation by distance processes (Sokal *et al.*, 1989).

The Mantel approach does not provide a direct indication of the exact nature of the correlation between cytotype ratio and elevation; it does not tell which ploidy level prefers higher elevations and which prefers the lower elevations, but simply states that plots from the same elevation have comparable cytotype ratios. A look at the distribution map (Figure 2) however tells that the diploids are more frequent at the higher elevations, and the triploids at the lower.

The precise ecological effect of the elevation on the two cytotypes is unclear. Possibly, elevation influences the drainage patterns of the field. Water flowing down from the woods above the field may be flowing predominantly through the little "trough" in the middle causing small-scale differences in moisture and texture of the soil and differences in temperature regime. On a regional scale in Neuchâtel, Switzerland, Meirmans *et al.* (Chapter 2) found a relation between the presence of triploids and the amount of disturbance due to human activities. Although, according to the owner, the Odenwald field under study here has experienced a low intensity management for at least the last 15

years before our sample, the field has occasionally been grazed by cattle. These can display preferences for certain parts of the field and thus create differences in the amount of disturbance. Besides this, certain parts of the field may be more vulnerable to trampling (e.g. because they are wetter due to the local drainage patterns described above), and ecological differences within the field may increase due to the presence of grazers.

As a result of cytotype exclusion principles (Levin, 1975; Felber, Bever, 1997) a stable coexistence of two ploidy levels is not expected unless there is some form of ecological differentiation between them. It can be questioned however, whether the difference in elevation we found between the diploids and triploids, provides a strong enough ecological differentiation to promote long-term coexistence, as the elevation explains only a small part of the variation in ploidy level.

Distribution of genetic variation

As a result of hybrid inviability, coexisting cytotypes are expected to show some form of reproductive isolation. Genetic studies have proven that reproductive isolation is present in polyploid complexes with sympatric cytotypes such as *Arrhenatherum elatius* (Petit *et al.*, 1997) and *Plantago media* (Van Dijk, Bakx-Schotman, 1997), but absent in others such as the *Lotus alpinus/corniculatus* complex (Gauthier *et al.*, 1998a; Gauthier *et al.*, 1998b). In *Taraxacum* sect. *Ruderalia* reproductive isolation is expected between the cytotypes because of the apomictic mode of reproduction of the triploids. Nevertheless, Menken *et al.* (1995) showed that there was high homogeneity in allele frequencies between diploids and triploids from mixed populations. The Odenwald population in this study was one of the populations previously sampled by Menken *et al.* (1995); they found no significant genetic differentiation between the diploids and triploids. In our study, however, we found significant differences in allele frequencies between the diploids and triploids in two of the four subpopulations (A and B). This difference between our results and those of Menken *et al.* (1995) may be a result of the difference in power of the tests, as they sampled a total of 44 plants and tested single-locus, we sampled on average about 90 plants per subpopulation and used a multi-locus statistic. Subpopulations C and D, in which we found no differentiation between the two cytotypes are also the two subpopulations with the smallest sample sizes, which may also indicate that lack of significant differentiation may be due to a lack of statistical power.

Usually, within-population differentiation is ascribed to isolation by distance mechanisms caused by restricted gene flow (Peakall, Beattie, 1995; Hossaert-McKey *et al.*, 1996; Streiff *et al.*, 1998). If isolation by distance mechanisms are indeed the cause of the spatial genetic patterns found in the studied *Taraxacum* population, the congruence between the patterns of the two ploidy levels would imply gene flow between the sexual diploids and the apomictic triploids. Hardy and Vekemans (2001) found no such congruence in the spatial distribution of allozyme variation within mixed populations of diploid and tetraploid *Centaurea jacea* and concluded that at this spatial scale, the two cytotypes were reproductively isolated.

The most probable mechanism for gene flow between the cytotypes of *Taraxacum* sect. *Ruderalia* is hybridization between triploid fathers and diploid mothers (Morita *et al.*, 1990b). Triploids produce seeds by means of apomixis for which no pollen input is required, but they also produce pollen. Male meiosis in triploids is highly disturbed and as a result of this, pollen of irregular ploidy is produced, most of which is aneuploid and sterile. Crossing experiments between triploid fathers and diploid mothers resulted in very low seedset. Calame (2000) found 28.5% seedset in handpollinated crossings and 10.6% seedset in diploid plants which were transplanted into a fully triploid field. Tas *et al.* (1999) found a seedset of 22% after manual cross-pollinations. Triploid pollen is known to induce breakdown of the self-incompatibility system of diploids (Morita *et al.*, 1990a), so possibly a large part of the seedset of these crossing experiments is the result of selfing. Tas *et al.* (1999) screened the offspring from their crosses for allozyme variation and found that 88% resulted from selfing and in most of the remaining 12% real hybrids seedset was reduced. Despite these results, hybridization may very well occur in the wild. Calame (2000) found that among the offspring of diploid plants transplanted into a triploid field, 8.4% was triploid and 2.2% was tetraploid. The high incidence of tetraploids in experimental hybridizations (sometimes higher than 50%, R. van der Hulst, pers. comm.) contrasts strongly with the low incidence in wild populations (Chapter 2). The lack of tetraploids in the field, the breakdown of the self-incompatibility and the reduced fertility of the hybrids make that the order of magnitude of the effective hybridization rate in wild populations is still unknown, even though hybrids are readily obtainable from crosses and transplantation experiments.

There are no theoretical models that relate the amount of congruence in within-population spatial genetic patterns to the level of hybridization between two ploidy levels. The only theoretical study that included hybridization between ploidy levels, modeled isolation by distance between populations rather than within a population (Hardy, Vekemans, 2001). Hardy and Vekemans found that the correlation between the isolation by distance patterns of diploids and tetraploids indeed increases with decreasing hybridization barrier. In general, the spatial structure within the diploids was much stronger (higher $F_{st}/(1-F_{st})$) than within the tetraploids, as a result of the higher effective population size for the tetraploids at an equal number of individuals. This effect was also visible in our study population (Figure 4). In Hardy and Vekemans' model, the $F_{st}/(1-F_{st})$ value for the smallest distance classes calculated between ploidy levels, became intermediate between the values for the diploids and the tetraploids at high (10%) levels of hybridization. In our study the correlogram calculated between cytotypes was indeed an intermediate between the two within cytotype correlograms (Figure 4). Assuming that the results for the between-populations model of Hardy and Vekemans is also applicable to within population trends, this might indicate a hybridization rate which is remarkably high considering the results of experimental crossings (Tas, Van Dijk, 1999; Calame, 2000).

Sokal *et al.* (1989) modeled the effects of selection on spatial genetic patterns and tested this using autocorrelation statistics. They found that spatial selection gradients yielded clear clinal correlograms. Other types of spatially restricted selection, such as patches, also resulted in significant autocorrelations although the correlogram patterns were less clear. Selection therefore could provide an alternative explanation for the spatial genetic patterns we observed in *Taraxacum*. If the same selection would act on the diploids as well as on the triploids, it would also explain the congruence between the cytotypes. However, the multi-locus correlograms we presented are much clearer and more significant than each of the three single-locus correlograms (not shown) which suggests that there is redundancy between spatial patterns present in the loci. This redundancy would suggest that the selection works tandemly on different, unlinked and presumably neutral allozyme loci. We therefore conclude that the observed congruence between the spatial genetic patterns of the two ploidy levels is not the result of selection, but the result of gene flow between the diploid sexuals and the triploid apomicts.

High levels of gene flow between sexual and apomictic *Taraxacum* would result in the constant creation of new polyploid lineages, and would explain the high number of different triploid genotypes usually found in population samples of *Taraxacum* (Van der Hulst *et al.*, 2000). Van der Hulst *et al.* (2000) found a high number of genotypes in samples from three purely triploid populations. They showed that the distribution of AFLP-markers over the genotypes was more compatible with a sexual than with a clonal population structure. The diploid-triploid cycle and the constant creation of new clonal lineages may therefore not only have an influence on the population structure of mixed populations, but also on the structure of purely triploid populations, even though these populations may be hundreds of kilometers away from the diploid distribution area.

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Genetic and ecological changes in a population of sexual and asexual dandelions after a 14-year interval

4



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Abstract

Many studies have looked at populations of closely related sexually and asexually reproducing taxa. Nevertheless, there are only a few long-term studies of such populations, which is surprising as changes in mixed sexual/asexual populations may provide essential information on the mechanisms that maintain sexual reproduction. We studied a population of sexual and asexual dandelions (*Taraxacum* sect. *Ruderalia*) during a 14-year period of decreasing anthropogenic disturbance. From previous analyses of the correlation between disturbance and the frequencies of sexuals and asexuals, we predicted the frequency of sexuals to increase at the cost of the asexuals. Indeed, the frequency of sexuals doubled, from approx. 30 to 60 per cent. Moreover, the change was accompanied by a drastic reduction in population size. We also looked at the distribution of genetic variation and found a slight increase in the genetic differentiation between the sexuals and the asexuals after the 14-year period. During this period, there also were significant changes in the frequencies of clonal lineages, and an overall increase in clonal diversity, perhaps caused by the ecological changes in the field, and different selection pressures on different clones. A high rate of gene flow between the sexual and asexual dandelions was found, and the maximum likelihood estimates for gene flow suggested that this gene flow was asymmetric: there was more gene flow from the sexuals to the asexuals than the other way around. We conclude that even in a relatively short period of 14 years time the composition of the dandelion population had radically changed, including plant density, the ratio of sexuals and asexuals in the population, the clonal diversity and frequencies of clonal lineages.

Introduction

The maintenance of sex is one of the major unresolved riddles of evolutionary biology as there are large costs connected to sexual reproduction as compared to asexual reproduction, yet most eukaryotes reproduce sexually (Williams, 1975; Maynard Smith, 1978; Hamilton, 1980; Meirmans, 2005). A large part of the research on the maintenance of sex has focused on the analysis of populations where sexually reproducing species coexist with closely related asexuals. Most of these studies however present only a “snapshot” image of the interactions between sexuals and asexuals, and it is important to establish how informative such short-term studies are. So far, there are only a few longer term studies, such as the study done by Lively & Jokela (2002).

There are two main reasons why mixed sexual-asexual populations might not be stable over longer time periods. The first is that the interactions between the sexuals and asexuals may be very dynamic by nature: the simplest form of such a dynamic interaction is when there is a large cost to sexual reproduction (Maynard Smith, 1978), in which case sexuals should be rapidly replaced following an introduction (or new origin) of asexuals. Secondly, environmental changes may differentially affect the two types. This could be the case when coexistence of sexuals and asexuals is maintained through some mechanism, such as one of the ecological models for sex. These models typically work because sex generates polymorphic offspring that is better able to cope with a complex biotic or abiotic environment (Jaenike, 1978; Hamilton, 1980; Bell, 1982). Any independent change in the environment could then also result in a change of the frequencies of sexuals and asexuals in the population.

The ratio of sexuals and asexuals is not the only variable of interest when looking at the temporal dynamics of mixed sexual/asexual populations; it might be equally important to investigate changes in clonal diversity and frequencies of clones and genetic variation in sexuals. These variables play important roles in all theories for the maintenance of sex and are all likely to be affected by changes in ecological conditions. For example, clone frequencies are liable to change when asexual clones have restricted (‘frozen’) ecological niches (Vrijenhoek, 1979; Vrijenhoek, 1989). In that case, when the environment changes, the present clones will be replaced by others that are better adapted to the new ecological conditions. Changing ecological conditions may also lead to an increase or decrease in clonal diversity. Finally, ecological change may lead to a change in populations size and a concurrent change in the amount of genetic variation, which, in turn, can have important implications for the coexistence of sexuals and asexuals (Otto, Barton, 2001).

Common dandelions (*Taraxacum* sect. *Ruderalia*), provide a good system to study the maintenance of sexual reproduction as sexual and asexual dandelions are found sympatrically. The sexuals are diploid and, due to their self-incompatibility system, fully outcrossing. Asexuals are triploid and reproduce seeds through apomixis. It is not known exactly what mechanisms maintain sexual reproduction in dandelions, but ecological

differentiation between sexuals and asexuals has been found (Chapters 2 & 4); asexual dandelions were found in disturbed habitats that required constant recolonisation, whereas sexual dandelions were found in more stable habitats, where competition is more important. Because of this ecological differentiation, we generally expect that over time, when succession takes place and interspecific competition gets more intense, asexual dandelions be replaced by sexuals.

It is known that there is a high clonal diversity in dandelions (Van der Hulst *et al.*, 2000; Rogstad *et al.*, 2002; Van der Hulst *et al.*, 2003), which most probably originates from occasional gene flow between the sexuals and asexuals. Such gene flow is possible because of the production of pollen by the asexuals. Though this pollen is largely sterile, it can be used to successfully pollinate sexual plants; the offspring of such crosses is usually a mixture of diploid, triploid and tetraploid individuals (Verduijn *et al.*, 2004b). The rate of such gene flow, and therefore the rate of formation of new clonal lineages, is dependent on the relative frequencies of sexual and asexual dandelions and whether new lineages are able to establish. Therefore, the genetic relationships between the sexuals and asexuals are likely to be affected by changes in the environment.

Research goals:

The aim of this research is to look at the changes in a mixed sexual/asexual dandelion population after a 14-year period of reduced anthropogenic disturbance. We specifically ask the following questions: Has there been a change in the ratio of sexual and asexual dandelions over the 14-year period? Has there been a change in overall population density? How is the genetic variation distributed over the sexual and asexual dandelions, and has this distribution changed over the 14-year period? What is the rate of gene flow between sexual and asexual dandelions, and is the gene flow symmetric? Has there been a change in clonal diversity and a change in the frequencies of individual clones? We answer these questions by comparing samples from 1986 and 2000 for the frequency of sexuals and asexuals, population density and frequencies of allozyme alleles, microsatellite alleles and chloroplast haplotypes.

Materials & Methods

Studied population

In 1986 and 2000, we studied a population of dandelions from Oberschönmattenwag in the Odenwald, Germany (see Chapter 3 for more details about the sample of 1986). The field containing the population was situated on a Southwest-facing slope and measured approximately 100 by 30 meters. The study site within the field was 90 by 25 meters, subdivided in 360 subplots of 2.5 by 2.5 meters. Over the 14 years between the two censuses, the valley in which the field was situated has known a decrease in agricultural

management intensity and has been transformed from an agriculture area with pastures and arable fields into a nature-reserve with haymaking activities only. In 1986, the sampling field was mainly used as a hay meadow, regularly fertilised and grazed late in the season, after the last mowing. In 2000, the field was not grazed anymore but still mowed twice a year, with no fertilisation.

Ratio of sexuals and asexuals in the population

In order to estimate the percentage of sexuals in the population, in both years a large number (> 4000 in 1986; > 300 in 2000) of flowering individuals was checked for their mode of reproduction, sampled evenly from the whole field. The variation in pollen grain sizes was used as an indicator of the ploidy level of the flowering plants and thus for their mode of reproduction. While pollen grains of diploid sexual plants are equal in size, pollen grains of triploid asexuals are highly variable, due to disturbed male meiosis. Therefore, the two reproductive types can be distinguished by looking at the pollen of flowering individuals through a field microscope (Tschermaek-Woess, 1949).

The density of the dandelion population was measured by counting the number of flowering individuals in subplots of 2.5 by 2.5 meters. In 1986, the number of flowering individuals was counted in nine of such subplots. These nine subplots were not evenly distributed over the field, but situated close together. However, as these subplots were not picked out because of their density, we take them to be representative for the population density of the whole field. In 2000, the number of flowering individuals was counted in 90 subplots, which were distributed evenly over the field.

Genetic analysis

To analyse genetic variation in sexual and asexual dandelions, we used allozyme, microsatellite and chloroplast markers. Allozyme markers were available for both the sample from 1986 (data taken from Chapter 3, see there for information on the sampling method) and the sample from 2000, and were used to compare genetic variation between the years, as well as between the two reproductive types. We also used the allozyme data to distinguish clones and compare clone frequencies and clonal diversity between the two years. The microsatellite and chloroplast data were only available for the sample from 2000, and were used for comparing genetic variation between the two types in this year only. The microsatellite data were also used to obtain a maximum-likelihood estimate for the rate of gene flow between sexual and asexual dandelions.

In 2000, sampling of plants for genetic analyses took place immediately after the analysis of the ratio of sexuals and asexuals in the population. For the genetic analyses four plants were sampled from each of 90 adjacent subplots at the southeastern half of the field (different from the set of 90 subplots mentioned above that were spread evenly over the whole study field). The plants were dug out and taken to the greenhouse, where the ploidy of all surviving plants (353 out of 360) was established by flow cytometry, using a Partec Ploidy Analyser, following the protocol of Tas and Van Dijk (1999);

the asexual reproduction of the triploids was confirmed in the greenhouse, where only triploid individuals showed spontaneous seed production.

DNA-extraction, microsatellite amplification conditions, chloroplast PCR-RFLP conditions and allozyme analyses were all performed using the protocols described in Van der Hulst *et al.* (2003). Five highly polymorphic microsatellite loci were used for genotyping the plants: msta53, msta64, msta72, msta78 and msta85 (Falque *et al.*, 1998). Microsatellite amplification products were visualised on a Li-Cor GeneReader 4200 sequencer and scored manually twice. The results of the two scoring rounds were compared and inconsistencies scored a third time, individuals that remained doubtful were amplified and run again. In the cases where triploid individuals had only two bands for a locus, band intensities were used to score which allele was present once and which was present twice. This was possible in most of the cases, in the few cases that remained ambiguous after all scoring rounds, the third allele was scored as “missing data”. Chloroplast variation was analysed using both restriction site and length-variation of three regions of the chloroplast genome and amplified using universal primers: intron trnL (Taberlet *et al.*, 1991) cut with Apal1 and Bste2, trnL-trnF (Taberlet *et al.*, 1991) and psbA-trnH (Dumolin-Lapeque *et al.*, 1997) cut with Dra1 and Ssp1. All three regions showed length variation, but the length variation at the psbA-trnH intergenic spacer was hard to score on agarose gels, and also known to contain a lot of homoplasies (Mes *et al.*, 2000), so this variation has been discarded for cp-haplotyping. Allozyme analysis was performed using two enzyme systems, yielding three polymorphic loci: *6Pgdh-1*, *6Pgdh-2* and *Mdh-1*.

Statistics:

We studied the distribution of genetic variation over the years (allozymes) and over the two reproductive types (allozymes, microsatellites and chloroplasts) using the Rho-statistic (Ronfort *et al.*, 1998). Rho is an F_{st} -like statistic that is useful for analyses involving different ploidy levels (we also report F_{st} values to enable comparison with previous studies). Both Rho and F_{st} statistics assume that the used genetic markers follow the infinite allele mutation model. However, microsatellites are often thought to follow a stepwise mutation model; Recently, Hardy *et al.* (2003) developed a method to test whether allele length variation is informative for the tested population differentiation. If this is not the case, (i.e. the test is not significant) the infinite allele model is deemed the most appropriate. As this test was not significant for our data, we used the Rho/ F_{st} approach to test for differentiation for the microsatellite data.

To estimate the rate of gene flow between sexual and asexual dandelions, a maximum likelihood approach with a Monte Carlo Markov Chain was used (Beerli, Felsenstein, 1999). For the Monte Carlo Markov Chain, we made ten independent runs, each run consisting of 20 short chains of length 1000 and 3 long chains of length 10.000, with increments of 20 and a burn-in of 10.000. Of every run, only the results from the last

long chain were used; these results were combined over the ten runs. Like in the ρ/F_{st} analyses, an infinite allele model was assumed for analysing the microsatellite data. For the rest of the settings of the Monte Carlo Markov Chain, we used the program's defaults. As computation of maximum likelihood estimates of gene flow is slow, we used a subsample of the complete dataset by randomly choosing 120 alleles from both sexuals and asexuals.

The maximum likelihood method (Beerli, Felsenstein, 1999) jointly estimates the population parameters Θ and M . The parameter Θ is, for diploids, $4N_e\mu$, where N_e is the effective population size in individuals and μ is the mutation rate. However, in our study we also have triploids, so that the estimates of Θ are expected to be biased: a sample of triploid asexual individuals would contain 1.5 times as many gene copies than an equally sized sample of diploids. Because of this difference in ploidy, it is more appropriate to let N_e express the number of gene copies rather than the number of individuals; in that case, Θ becomes an estimate for $2N_e\mu$. The migration parameter M indicates m/μ , the migration rate (m) between a pair of populations scaled by the mutation rate. The combination of Θ and M gives the gene flow; in our case $2N_e m$. M is calculated for both directions of gene flow between a pair of populations; this allowed us to see whether the gene flow from the sexuals to the asexuals was different from the gene flow from the asexuals to the sexuals.

A special tetraploid individual was found that was for three-quarters genetically identical to a frequent triploid clone. A population assignment test was performed to see whether the remaining quarter stems from the population of sexuals or from the population of asexuals.

We looked for changes between 1986 and 2000 in clonal diversity and in clone frequencies by distinguishing clones based on the allozyme data. Clonal diversity was analysed through Shannon's diversity index with correction for sampling bias (Chao, Shen, 2003), a bootstrap test was used to test for a difference in clonal diversity between the two samples from 1986 and 2000 (Chapter 6). The amount of differentiation in clone frequencies between the two years was tested using the Rho-statistic.

Used software

Most statistical analyses were done using Spagedi (Hardy, Vekemans, 2002), with some exceptions: assignment of individuals to clonal lineages and analysis of clonal diversity was performed using the programs GenoType and GenoDive (Chapter 6), population assignment was performed using Whichrun, version 4.1 (Banks, Eichert, 2000), and the Monte Carlo Markov Chain was performed using Migrate, version 1.7.3 (Beerli, 2004). All tests were done by randomisation with 999 permutations.

Results

Difference in the ratio of sexuals and asexuals

The dandelion population in the Odenwald field experienced a shift in the relative abundances of the sexuals and asexuals between the two censuses. In 1986 the sexuals were in the minority: 33.5% of the dandelions were sexual diploids. In 2000, the relative abundance of the sexuals had increased to 55.9%, shown by the pollen analysis performed on flowering individuals from the whole field. Among the plants that were sampled for genetic analysis in 2000, the sexuals were also in the majority. Flow cytometry revealed that out of the 353 surviving plants, 235 (66.6%) were diploid, 116 (32.9%) were triploid and 2 (0.6%) were tetraploid. We believe that the difference between the two analyses (55.9% vs 66.6% sexuals) was the result of a non-homogeneous distribution of the sexuals and asexuals; the plants sampled for the genetic analysis came exclusively from the south-eastern half of the field. Therefore, the data resulting from the pollen analysis probably presents a more accurate estimate for the percentage of sexuals in the population.

The shift in the ratio between the sexuals and asexuals was accompanied by a drastic decrease in population size. In 1986, an average of 185.1 (± 49.6 sd.) flowering dandelions were found per 2.5 x 2.5 meter subplot, in 2000 only 3.7 (± 2.6 sd.) flowering plants were found. Although sampling in 2000 took place in a later stage of the flowering season than in 1986, we believe that this difference is not simply a result of a difference in the percentage of plants that were actually flowering when performing the field work. In 2000, when sampling the plants for the genetic analysis, we also recorded whether the plants were in flower; of the sampled dandelions, 15.4% were flowering. This percentage gives an estimated maximum number of $3.7 / 0.154 = 23.8$ dandelions per subplot, both flowering and vegetative. This is only 13% of the number of flowering dandelions found in 1986, so the population has shrunk with at least 87% in size during the 14-year period. Note that this is a conservative estimate as the number of vegetative plants next to the 185 flowering ones from 1986 is unknown.

The annual decrease in population size was much higher for the asexuals than for the sexuals. We can see this by combining the decrease in overall population density and the shift in the ratio of sexuals vs. asexuals into separate estimates of population decline for the sexuals and asexuals. In 2000, the sexuals made up 55.9% of the overall dandelion population, however the size of this population was only 13% of that of the population of 1986. So relative to the total population of 1986, the frequency of sexuals in 2000 was only $13\% * 55.9\% = 7.2\%$. The population of sexuals has therefore declined from 33.5% to 7.2%, which constitutes a decline of 79%. Likewise, the relative frequency of asexuals becomes $13\% * 44.1\% = 5.7\%$. Compared to their frequency of 66.5% in 1986 the population of asexuals has declined with 91%. So the 87% decline in overall dandelion population size is actually a combination of a 79% decline for the sexuals and a 91%

Table 1: Genetic differentiation between sexuals and asexuals. A: differentiation between samples from 1986 and 2000, based on allozymes. Above diagonal: F_{st} , below diagonal: Rho . B: differentiation between sexuals and asexuals based on microsatellites, allozymes and chloroplast RFLP's, from the 2000 sample. Significant values, after Bonferroni correction, are marked in bold.

A

All loci		1986		2000	
		Sexuals	Asexuals	Sexuals	Asexuals
1986	Sexuals		0.003	-0.002	0.016
	Asexuals	0.008		0.002	0.003
2000	Sexuals	-0.004	0.005		0.012
	Asexuals	0.039	0.010	0.027	

B

2000 Sexuals vs. Asexuals	F_{st}	Rho
Microsatellites	0.046	0.098
Allozymes	0.012	0.027
Chloroplasts	0.077	0.077

decline for the asexuals. Assuming a constant exponential decline in population size, this amounts to an annual decline of 10% and 16% for sexuals and asexuals, respectively.

Genetic variation

Allozyme allele frequencies hardly changed in the 14 years in between the two censuses. This lack of change is apparent from the very low Rho -values for this differentiation (Table 1): -0.004 for the sexuals and -0.001 for the asexuals, both not significantly different from zero. The differentiation between the sexuals and asexuals changed slightly from $Rho = 0.008$ in 1986, to $Rho = 0.027$ in 2000, significant in 2000 ($p=0.001$), but not in 1986. Microsatellite diversity was high, with a total of 91 alleles for the 5 loci. There was significant differentiation between the sexuals and the asexuals, based on the microsatellite allele frequencies from 2000, though the differentiation itself was not very strong, judging from the Rho -value: 0.098. Using PCR-RFLP's, seven different chloroplast haplotypes were found, of which only two had an overall frequency higher than two percent. The most frequent haplotype was present in 81 percent of the sexual individuals and in 64 percent of the asexual individuals. There was a significant difference in haplotype frequencies between the sexuals and the asexuals ($Rho = 0.077$, $p = 0.001$), though also here the amount of differentiation was low.

The maximum likelihood approach revealed a high rate of gene flow between sexuals and asexuals. The asexual dandelions indeed had a larger Θ than the sexual dandelions (Table 2), and this difference was larger than the expected 1.5 fold, based on the difference in ploidy level. The estimate of M was slightly different for the two directions: 20.07 from

Table 2: Maximum likelihood estimation of the rate of gene flow between sexual and asexual dandelions. N_e expresses the number of gene copies rather than the number of individuals.

Type	$\Theta = 2N_e\mu$	Direction of gene flow	$M = m/\mu$	$2N_em$
Sexuals	1.28	From Asexuals to Sexuals:	22.85	29.24
Asexuals	3.05	From Sexuals to Asexuals:	20.07	61.30

sexuals to asexuals, and 22.85 from asexuals to sexuals. Nevertheless, this difference in migration rate had only little influence on the overall asymmetry of gene flow as that was mainly influenced by the difference in Θ between the sexuals and asexuals. The estimate for the gene flow from sexuals to asexuals ($2N_em = 61.30$) was more than twice as high as the estimate for gene flow from asexuals to sexuals ($2N_em = 29.24$). This implies that pollinations of sexuals by asexuals effectively give much more asexual triploid than sexual diploid offspring.

The occurrence of gene flow from the sexuals to the asexuals was further corroborated by the analysis of the two tetraploids: One of these tetraploids shared exactly three-quarters of its microsatellite genotype with a multilocus genotype that was found four times among the triploid asexuals. This tetraploid individual had the complete multilocus genotype of the triploid clone, but had one allele extra for every locus. We performed an assignment test using this extra set of alleles as input, which revealed that this set of alleles was 3.5 times more likely to have come from the sexual population than from the asexual population. This indicates that this tetraploid originated from a sexual motherplant that was pollinated by a triploid pollen grain produced by a member of the triploid clone. The other tetraploid that was found did not have such genetic affinity with any of the triploid clones present in the sample.

Clones

In contrast with the lack of change in allele frequencies between 1986 and 2000, there was a slight, but strongly significant change in clone frequencies between the two censuses (Figure 1; $Rho = 0.016$, $p = 0.001$). Based on the allozyme electrophoresis patterns, 47 multilocus genotypes could be distinguished within the asexuals, which we will treat as 47 different clonal lineages. In 1986, 43 clones were found among the 220 sampled asexual plants and in 2000, twenty clones were found among the 116 sampled plants. Out of the 47 clones, twenty-seven were found only in 1986 and four were found only in 2000, but most of those clones were found in very low frequencies. Nevertheless, there were also some drastic changes in the frequencies of some of the more abundant clones (Figure 1). Clonal diversity decreased significantly from 1.38 in 1986 to 1.14 in 2000 (bootstrap test, 999 permutations, $p = 0.001$).

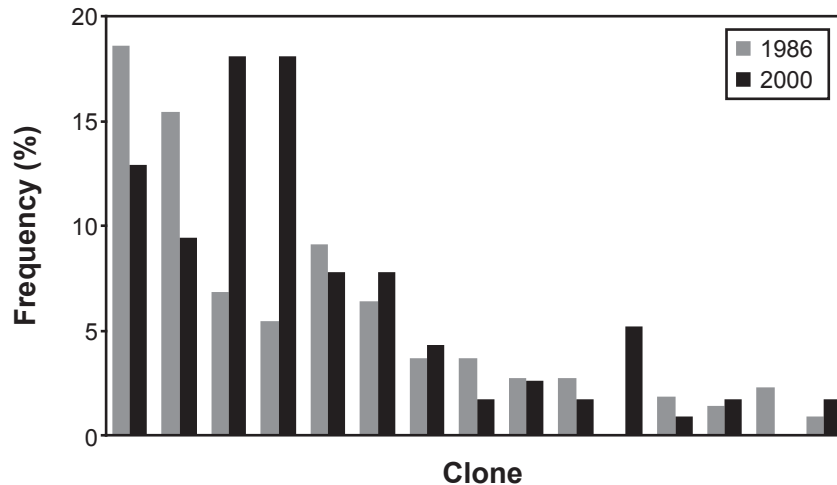


Figure 1: Frequencies of the 15 most abundant clones in the samples from 1986 and 2000.

Discussion

Change in the ratio of sexuals and asexuals

Under the simplest models of sexual versus asexual reproduction, asexuals have a large reproductive advantage over the sexuals (Maynard Smith, 1978), which would lead to a rapid decrease of the frequency of sexuals in a mixed population, until the sexuals will go extinct. This is not what we see in the studied population: the frequency of the sexuals increased after 14 years of decreasing management intensity of the field in which the population was situated. However, this increase in the frequency of sexuals was expected considering the ecological difference between the sexuals and asexual dandelions that was previously found (Chapters 2 & 5). As the less intensive management of the field implies less anthropogenic disturbance, the results also support the finding of Bell (1982) that sex is more common in stable environments.

The preference of the sexual dandelions for stable habitats suggests the following dynamics between the two reproductive types; after disturbance of a patch, asexual dandelions are presumably better at colonising the new empty habitat, but as succession proceeds, competition gets more intense and the sexuals are able to invade and take over. The ratio of sexuals and asexuals in a given patch might only be stable over time when the field is kept in a constant stage of succession, which mostly happens due to human activities or a relatively constant pattern of (natural) grazing. If succession proceeds eventually also the sexual dandelions will disappear from the field; dandelions are a weedy species that need to constantly recolonise new patches. This is also apparent from our study, where the large overall decrease in dandelion population density was most likely the result of the ecological change of the field as a whole, and intensified interspecific competition.

High rate of gene flow between sexuals and asexuals

The low values of the Rho-statistic, calculated for microsatellites, chloroplast haplotypes and allozymes, show that most of the genetic variation was shared between the sexual and asexual dandelions. This confirms earlier results of Menken *et al.* (1995) who studied using allozyme diversity in several populations and found that sexual and asexual dandelions shared all major polymorphisms. The low values of Rho indicate on-going gene flow between the two types, despite the apomictic seed production of the asexuals and the low viability of their pollen. High rates of gene flow were indeed found using the maximum likelihood approach. This high rate of gene flow complements the findings of Verduijn *et al.* (2004b) who looked at the progeny of naturally pollinated sexuals from a mixed population and found that about two percent of their offspring was triploid. Their estimate is however not necessarily indicative for the effective rate of gene flow between the sexual and asexual dandelions as newly produced clones may have difficulties with establishing themselves in the population. Evidence for this was found by De Kovel and De Jong (2000), who showed experimentally that newly created clones had a lower average seed set than established clones from a natural population.

We found a strong asymmetry in gene flow between the two reproductive types; the rate of gene flow from sexuals to asexuals was more than twice as high as the gene flow from asexuals to sexuals. This asymmetry indicates that natural pollinations of sexuals with pollen from asexuals effectively result mostly in asexual diploid offspring, corroborating the results of crossing studies, where a majority of the offspring was found to be polyploid (Tas, Van Dijk, 1999; Van der Hulst *et al.*, 2004). This match between the asymmetry in realised gene flow and the asymmetry in the results of crosses is not what is expected when newly created clones indeed have difficulties with establishing themselves, as suggested by the results of De Kovel and De Jong (2000). In that case, gene flow from sexuals to asexuals should be lower than vice versa. However, the observed pattern of asymmetry can be explained if also the sexuals that result from the crosses have a lower fitness.

Interestingly, a large part of the offspring of artificial crosses is tetraploid (Tas, Van Dijk, 1999; Van der Hulst *et al.*, 2004), indicating that a large percentage of the viable pollen produced by asexuals is triploid. Triploid pollen produced by asexuals explains the genotype of one of the two tetraploids that were found in this study; this tetraploid individual shared three-quarters of its genotype with a common triploid clone. Verduijn *et al.* (2004b) argued that tetraploid asexuals may play an important role in the gene flow between sexuals and asexuals as they produce diploid pollen that is more viable and more successful in pollinating sexuals than the pollen produced by triploid asexuals. If tetraploids indeed play such a big part in the gene flow between sexuals and asexuals, this would also explain the observed asymmetry between sexuals and asexuals, as crosses between asexual tetraploids and sexual diploids, usually give triploid offspring (Verduijn *et al.*, 2004b).

Because of the high rate of gene flow between sexual and asexual dandelions, the two types are not reproductively isolated and thus effectively form a single evolutionary group despite the different modes of reproduction. The high rate of gene flow also has important consequences for the working of models for the maintenance of sexual reproduction in dandelions. The constant creation of new clonal lineages will keep the asexuals free from deleterious mutations (Muller's Ratchet: Muller, 1964) and diverse enough to deal with coevolving antagonists (Red Queen: Jaenike, 1978; Hamilton, 1980).

Changes in clonal diversity

The frequencies of individual clones changed significantly between the two censuses, indicating that the changed ecological conditions have different effects on different clonal lineages. Some clones increased in frequency while others decreased in frequency or possibly became extinct. Even though there was a strong decrease in population density, we can rule out genetic drift as a factor causing the differences in clone frequencies as genetic drift proved to have little effect on the allozyme allele frequencies. Therefore, the change in clone frequencies is most likely the result of differential selection on clones. Such selection is expected to take place as a result of an ecological change if every clone has its own "frozen" ecological niche (Vrijenhoek, 1979). Frozen niche variation has already been shown to exist in several organisms, among which dandelions (Solbrig, Simpson, 1974; Gray, Weeks, 2001; Robinson *et al.*, 2002). Solbrig and Simpson (1974) showed that two dandelion clones differed in their reproductive and competitive abilities. Such differences in competitive abilities could also have caused the change in clone frequencies observed in this study, following the decrease in disturbance. Alternatively, the changes in clone frequencies are not the result of the ecological change, but of interactions with coevolving parasites (Red Queen model for the maintenance of sex: Jaenike, 1978; Hamilton, 1980). Such parasite-mediated turnover in clone frequencies has been shown for the freshwater snail *Potamopyrgus antipodarum* (Jokela *et al.*, 2003). We cannot distinguish whether the change in clone frequencies observed here is the result of selection following the ecological change or of Red Queen mechanisms. However, the Red Queen does not work under high levels of clonal diversity such as observed in dandelions (West *et al.*, 1999).

Next to changes in frequencies of individual clones, we observed a decrease in overall clonal diversity. This decrease either indicates that some of the clones have become dominant in the population or that more clones have become extinct than have managed to invade the population. The decrease in clonal diversity was most likely due to the adverse ecological conditions and the increase in frequency of sexuals: there may not have been enough niche space left available for a large number of clones. A relationship between clonal diversity and adverse ecological conditions has also been found for the asexual earth mite *Penthaeus major*, where clonal diversity decreased when going from the centre of its distribution to the margin, where the environment is less favourable (Robinson *et al.*, 2002).

Conclusions

Our results show that the interactions between sexuals and asexuals, coexisting in mixed populations, are not necessarily stable over the long term, but can change over relatively short time periods, due to ecological changes in their habitat. This means that studying a mixed sexual/asexual population at a single point in time may not reveal the complexity of the sexual/asexual system; however, only a few studies on mixed sexual/asexual populations have looked at longer-term changes in the system. Lively and Jokela (2002) found little change in the ratios of sexual and asexual snails when comparing two censuses that lay more than ten years apart from each other. In contrast to our study organism, the habitat of the snails, New Zealand lakes, may provide a relatively stable habitat.

The natural habitat of dandelions of the section *Ruderalia* consists of temporarily available open patches and intensively managed grassland vegetations. Dandelion populations are only stable when a field is constantly kept in an early stage of succession, which nowadays often happens through human (derived) activities. For instance, intensive grazing by cattle will constantly create a lot of small open patches where vegetation is damaged to such a degree that germination and establishment can take place. Within the dandelions, asexuals, being better colonisers, are found in earlier stages of succession than the sexuals, which are better competitors. The exact causes of the difference in competitive abilities is however unknown. We found that the dynamics of the system during various stages of succession are not restricted to changes in the frequencies of sexual and asexual dandelions in the populations, but also include changes in clone frequencies and clonal diversity

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Competition and colonisation in sexual and asexual dandelions

5



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Abstract

The Tangled Bank hypothesis is one of the few among many theories explaining the maintenance of sex that allow for coexistence between closely related sexual and asexually reproducing species. One of the main assumptions of the Tangled Bank is that all members of a clone have the same “frozen” ecological niche. In this study we look for evidence for the Tangled Bank hypothesis and its assumption by simultaneously looking at ecological differences between sexual and asexual dandelions (*Taraxacum* sect. *Ruderalia*) as well as differences among clones within asexual dandelions. Based on vegetation analyses we found slight ecological differences between the sexuals and asexual dandelions, but no evidence for a different use of resources, as required by the Tangled Bank. Instead, we argue that the difference points to a competition-colonization trade-off, with the sexuals being better competitors and the asexuals better colonizers. Within the asexuals, we found no evidence for frozen niche variation based on the vegetation analysis. However, differences in seed production between the clonal lineages suggest that also clones may differ in colonizing and competitive abilities.

Introduction

Sexual reproduction is the dominant mode of reproduction among higher organisms, despite the fact that sexual reproduction should be highly costly compared to asexual reproduction (Maynard Smith, 1978). Many theories have been proposed to explain the ubiquity of sexual reproduction, but only two of these theories have found widespread support (West *et al.*, 1999): The mutation accumulation hypothesis (Kondrashov, 1988), under which sexual reproduction helps to purge deleterious mutations, and the Red Queen hypothesis (Jaenike, 1978; Hamilton, 1980), under which sexual reproduction provides an advantage for dealing with rapidly coevolving antagonists. Even though the mutation accumulation hypothesis and the Red Queen hypotheses could potentially explain the maintenance of sex, they provide little room for explaining the coexistence of sexuals and asexuals within one species complex (Carrillo *et al.*, 2002); both models lead to fixation of either sexual or asexual reproduction, depending on specific model parameters. Coexistence of sexuals and asexuals has however been observed in a large number of species in which asexuality is present (Bierzychudek, 1987), and any general explanation for sex should take this pattern into account.

Coexistence of sexuals and asexuals is possible under another hypothesis for sex, the Tangled Bank. The Tangled Bank model conceptually connects to more general insights from population ecology, where it is well known that a species with a high growth rate at low densities can coexist with another species if the other species is a better competitor (Hanski, 1983; Nee, May, 1992; Yu *et al.*, 2001). Coexistence of sexuals and asexuals would be possible if a higher reproductive output of the asexuals is compensated by better competitive abilities of the sexuals. Under the Tangled Bank model, these better competitive abilities are thought to be a direct consequence of sexual reproduction itself; sex produces polymorphic offspring through the combined effects of segregation and recombination, and competition between sexuals will be less severe than competition between asexuals (Ghiselin, 1974; Bell, 1982), as sexuals are able to use scarce resources more effectively than asexuals. Theoretical studies in which the Tangled Bank scenario was modelled showed that the main factors that enable coexistence between sexuals and asexuals were: A wider niche of the sexuals, a difference in average niche position between the sexuals and the asexuals (niche differentiation), or a combination of these two factors. Furthermore, coexistence was more likely under severe resource exploitation and when phenotypic plasticity for the environmental variables was low (Case, Taper, 1986; Pound *et al.*, 2002). Case and Taper (1986) also showed that the differences in niche width and in niche position can evolve rapidly, and can therefore be a result of the coexistence of sexuals and asexuals itself rather than being a necessary prerequisite for coexistence.

A main assumption of the above competition models is that all members of a clonal lineage have the same ecological niche, an aspect of asexual reproduction that is generally known as “frozen niche variation” (Vrijenhoek, 1979; Vrijenhoek, 1989). Under the

Tangled Bank model, such frozen niche variation causes competition between asexuals to be generally more intense than between sexuals, and it is exactly this feature of relaxing sib competition among sexuals that results into an advantage for sex. The Frozen Niche Variation hypothesis was originally proposed as a mechanism to maintain variation in clonal lineages per se (Vrijenhoek, 1979), and has mostly been tested as such. The evidence for frozen niche variation in nature is mixed; it has been verified in several species (Jokela *et al.*, 1997; Gray, Weeks, 2001; Robinson *et al.*, 2002), but there is also other evidence that indicates that clones can have an extremely broad ecological niche (Van Doninck *et al.*, 2002), a concept known as a “General Purpose Genotype” (Lynch, 1984). In our view, any test of the Tangled Bank hypothesis should also include a test of the Frozen Niche Variation hypothesis since the latter is a crucial factor for the former, and since frozen niche variation is not a general feature in nature that can be taken for granted.

Dandelions

In common dandelions (*Taraxacum* sect. *Ruderalia*) sexuals are diploid ($2n = 2x = 16$) and are fully outcrossing due to a sporophytic self-incompatibility system. Asexual dandelions are triploid ($2n = 3x = 24$) and produce seeds through apomixis, without any need for pollination. Ecological differentiation between sexual and asexual dandelions has been shown in several studies, even though none of these studies tested the Tangled Bank model directly. Meirmans *et al.* (Chapter 2) found a significant association between the frequency of asexuals and the vegetation in more than a hundred studied dandelion populations in Switzerland: populations with predominantly asexual dandelions were characterized by vegetations that showed indications of a high degree of anthropogenic disturbance, whereas populations dominated by sexuals were associated with a relatively undisturbed habitat. Verduijn *et al.* (2004a) showed that at the Northern border of the distribution area of the sexuals in the Netherlands, sexual dandelions preferred relatively warmer spots compared to the asexuals from the same population. In a dandelion population situated in Germany, there was a clear relationship between the distribution of the two reproductive types and elevation: the sexuals preferred the slightly higher located parts of the habitats (Chapter 3).

One study suggested indirect evidence for the presence of frozen niche variation in asexual dandelions. Solbrig and Simpson (1974), studied and compared two clones, and found that one of them had a much higher production of seeds while the other was competitively superior. They concluded that the first clone was adapted to disturbed habitats necessitating better dispersal capacities, and the second one was adapted to undisturbed habitats necessitating higher competitive abilities. However, such variation might not provide sufficient explanation for the coexistence of as many as forty clones, a normal number found within dandelion populations (Van der Hulst *et al.*, 2003).

Research goals

The aim of this study is to test features of the Tangled Bank model and one of its crucial assumptions; frozen niche variation in clonal lineages. We address the Tangled Bank by comparing the niche width and mean niche position of sexual and asexual dandelions from a mixed population. We do this by testing the association between the mode of reproduction of individual dandelions and the vegetation directly surrounding these individuals. Vegetation analyses can give reliable indications for the quality or type of the habitat, and about the availability of different resources (Schaffers, Sykora, 2000). Furthermore, we test for frozen niche variation among asexual lineages in this population by looking at their ecological niches both in terms of vegetation and reproductive success.

Materials & Methods

Sampling:

A sample of 360 dandelions was taken from a mixed sexual/asexual population from the Odenwald, Germany in the spring of the year 2000. This population, and the sampled set of individuals, has been the subject of several previous studies on the genetics and ecology of dandelions (Chapters 3 & 4). A plot measuring 20 by 25 meters was set out in one half of the field in which the population was situated. This plot was divided in 90 subplots, each measuring 2.5 by 2.5 meters. From every subplot, four dandelions were chosen at random. We measured the coordinates of the chosen dandelions within the plot (regrettably missing one subplot), and made a vegetation analysis of the direct surroundings of the chosen dandelions through scoring the abundance of all higher plant species growing within a circle of 50 cm diameter around the individual dandelions. These individuals were subsequently dug out and transported to the greenhouse.

Out of the 360 dandelions, 353 survived the transportation. We analyzed the ploidy level of the individual dandelions through flow cytometry, using a Partec Ploidy Analyser using the method of Tas and Van Dijk (1999). Ploidy level provides a marker for the mode of reproduction; diploids are sexual and triploids are asexual. We confirmed the mode of reproduction by monitoring all plants for spontaneous seed production during the flowering season of 2002. All occurrences of spontaneous seed set were in individuals that were previously typed as triploids, except for some rare occurrences in diploids. These latter cases were presumably the result of a low level of selfing and were easily recognisable by the large number of thin white inviable seeds. During the flowering season of 2003 we also counted the number of produced seed heads for all asexual plants.

Niche width and mean niche position

The species diversity of the vegetation surrounding a given individual plant is used as a measure of the complexity of the ecological interactions in which this plant is engaged. The combined, or total, species diversity encountered by all individuals in a group is used as a proxy for the niche width of that group. The amount of niche overlap between individuals can then be estimated by comparing the average diversity per individual with the total diversity. In this study, we estimated species diversity by calculating Shannon's diversity index ($H = -\sum p_i \ln(p_i)$, where p_i is the abundance of species i). The average diversity per individual (H_s) is obtained through calculating Shannon's index separately for every individual and averaging over all individuals within a group. The total species diversity of a group (H_t) is obtained by first averaging the species abundances over all individuals and then calculating Shannon's index using these average abundances. The amount of niche overlap between individuals can then be obtained by calculating the fraction of diversity that is distributed among individuals, and subtracting that from one: $1 - (H_t - H_s)/H_t = H_s/H_t$. The partitioning of diversity was done separately for the sexuals and the asexuals, to compare the niche width and amount of niche overlap between the individuals within the two groups. The average diversity per individual was compared between the sexual and the asexual dandelions by means of a t-test.

We looked for niche differentiation between sexuals and asexuals by using a Canonical Correspondence Analysis (Ter Braak, 1986). A CCA ordines vegetation data in relation to ecological variables, a Monte Carlo test can be used to test whether the association between the vegetation and the ecological variables is statistically significant. We used the method in a non-standard way and entered the mode of reproduction of the dandelions as an ecological variable to test for an association with the (log-transformed) vegetation data. To avoid circularity in reasoning, the abundance of dandelions in the vegetation was removed from the dataset before performing the CCA. The dandelion abundance in the vegetation might be important, however, as intraspecific competition is higher when dandelion abundance is high. We used a t-test to see whether sexual and asexual individuals differed in the abundance of (other) dandelions in the surrounding vegetation. We accounted for the possible occurrence of spatial autocorrelation of the different included variables in a series of further statistical tests. Tests in which both variables are spatially autocorrelated may suffer from an inflated type I error (Legendre, 1993). Spatial autocorrelation in the distribution of the two reproductive types in the studied field has previously been proven (Chapter 3), and autocorrelation in the vegetation data is more than likely. We therefore used a spatially explicit setup to test for niche differentiation, and then analysed the relations between mode of reproduction, space and vegetation in a series of (partial) Mantel tests (Smouse *et al.*, 1986). (Partial) Mantel tests check for the correlation between matrices of pairwise resemblance: the resemblance matrix between the vegetation relevees was made using the Steinhaus similarity coefficient (Legendre, Legendre, 1998) on log-transformed species abundance data. The CCA with Monte Carlo test were performed using the program Canoco, v.

3.11 for Macintosh (Ter Braak, 1992), the Mantel tests were performed using R-package version 4.0b (Legendre, Vaudor, 1991).

Frozen niche variation in clones

We distinguished between different clonal lineages in the asexual dandelions using genetic variation occurring at five hypervariable microsatellite loci (using data from Chapter 4). When using highly variable genetic markers for genotyping, individuals belonging to a single clonal lineage can differ because of mutations, PCR-artefacts or scoring errors. We therefore chose a threshold of the number of differences allowed between individuals to belong to the same clonal lineage, based on the frequency distribution of pairwise genetic distances between individuals (Chapter 6).

We tested for frozen niche variation in the clonal lineages by using the differences in vegetation surrounding the clones as measure for their respective niches. We also looked for differences between clones in seed production. Vegetation data were compared using a Mantel test, analogous to the test for niche differentiation between sexuals and asexuals. Frozen niche variation in the vegetation data was also tested by looking at the amount of niche overlap between individuals using Shannon's diversity index, as described above. If there would be frozen niche variation, we expect more niche overlap between asexuals individuals than between sexual individuals. We tested for a difference in the number of seed heads produced by the clones during the flowering season of 2002, using an Anova with clone as a random factor. For the Mantel test and the Anova, we only used clones that were present more than once in the population.

Results

Niche width and mean niche position

Total species diversity (H_t) in the surrounding vegetation of all individuals was equal for the sexual and asexual dandelion populations, indicating that the two reproductive types did not differ in their total niche width (Table 1). However, sexual dandelion individuals were on average found in more diverse neighbourhoods than asexual individuals. The species diversity (H_s) of the vegetation that was growing around sexual individuals was significantly higher than that of the vegetation around asexual individuals ($t = 2.20$, d.f. = 351, $p = 0.028$). The partitioning of the variation revealed there was less niche overlap between the asexual individuals than between the sexual individuals.

There was a slight difference in mean niche position between sexual and asexual dandelions; the Monte Carlo test of the Canonical Correspondence Analysis showed a significant association between vegetation and mode of reproduction ($p=0.01$, see Table 2). However, the association explained only 0.5% of the total variation in the vegetation data, suggesting that the ecological factors causing the variation in the vegetation play

only a small role in causing ecological differentiation between the two dandelion types. Low and high scores for the CCA showed that some plant species were more abundant around sexual dandelions, while other species were more abundant around asexual dandelions (Table 2). The association was most informative for the few plant species that showed a high overall abundance; the association of most of the other, rarer plant species with the reproductive type of the dandelions might also have been due to chance alone. Relatively abundant species associated with sexual dandelions were *Anthoxanthum odoratum*, *Cerastium fontanum* and *Arrhenatherum elatius*, relatively abundant species associated with asexual dandelions were *Dactylus glomerata*, *Rumex acetosa* and *Festuca sp.* There was no difference between the sexual and asexual individuals in the abundance of dandelions in the vegetation surrounding them ($t = -0.44$, d.f. = 351, $p = 0.66$).

The result of the CCA was corroborated by the results from the Mantel tests (Table 3); there was a significant, but low, association between the mode of reproduction of an individual and the surrounding vegetation (Mantel's $r = -0.070$, $p = 0.001$). Other Mantel tests showed that there was spatial autocorrelation present in the data, both in the distribution of the reproductive types over the sampling area (Mantel's $r = 0.055$, $p = 0.001$) and in the vegetation data (Mantel's $r = -0.143$, $p = 0.001$). After correcting for this autocorrelation, the relationship between mode of reproduction and vegetation was still strongly significant (Mantel's $r = -0.063$, $p = 0.001$).

Differences between clonal lineages

The histogram of genetic distances between pairs of asexual individuals, calculated for the microsatellites, showed a clear bimodal distribution (Figure 1a). The first peak was close to zero, representing distances between individuals from the same clonal lineages that may differ to some extent due to mutations or scoring errors. The second peak represented distances between individuals from different clonal lineages, with independent origins. The two peaks were clearly separated, and we took a threshold of seven mutation steps to assign individuals to different clonal lineages.

No association was found between the multilocus genotypes and the surrounding vegetation. Under the frozen niche variation we would expect a histogram of ecological distances between individuals to look more or less the same as the histogram of genetic distances, with distances between clonemates close to zero and with a second peak of distances between individuals from different clones. This is not what we see when we draw a histogram of pairwise differences based on the vegetation data: The distribution is

Table 1: Partitioning of species diversity of vegetation growing around sexual and asexual dandelions, using Shannon's diversity index.

Type	Average diversity per individual (H_s)	Total diversity (H_t)	Niche overlap between individuals (H_s/H_t)
Sexuals	1.76	2.26	0.78
Asexuals	1.69	2.25	0.75

unimodal, and within-genotype distances and between-genotype distances are completely overlapping (Figure 1b). A Mantel test showed that there indeed was no evidence for an association between genotype and vegetation ($r = 0.0064$, $p = 0.351$). However, we found significant variation in the number of seed heads produced by different clonal lineages (Table 4).

Table 2: Species-scores of Canonical Correspondance Analysis (CCA) of the vegetation data using dandelion mode of reproduction as an environmental variable. Negative CCA-scores indicate an association with sexual dandelions; positive CCA-scores indicate an association with asexual dandelions.

Species	CCA-score	Total abundance
<i>Luzula campestris</i>	-0.69	2.7
<i>Lychnis flos-cuculi</i>	-0.69	0.8
<i>Carex otrubae</i>	-0.69	3.2
<i>Anthoxanthum odoratum</i>	-0.16	143.1
<i>Cardamine palustris</i>	-0.15	5.9
<i>Cerastium fontanum</i>	-0.12	65.1
<i>Arrhenatherum elatius</i>	-0.06	206.0
<i>Poa trivialis</i>	-0.06	189.9
<i>Veronica chamaedrys</i>	-0.03	60.6
<i>Lolium perenne</i>	-0.02	145.8
<i>Trifolium pratense</i>	-0.02	331.2
<i>Holcus lanatus</i>	-0.01	197.8
<i>Agrostis stolonifera</i>	-0.01	2.4
<i>Ranunculus acris</i>	0.01	183.3
<i>Poa annua</i>	0.02	0.9
<i>Poa pratensis</i>	0.03	515.5
<i>Plantago lanceolata</i>	0.05	180.0
<i>Veronica serpyllifolia</i>	0.07	30.0
<i>Bromus hordeaceus</i>	0.08	2.2
<i>Alchemilla sp.</i>	0.11	6.1
<i>Festuca sp.</i>	0.11	100.8
<i>Rumex acetosa</i>	0.12	53.2
<i>Dactylis glomerata</i>	0.19	33.3
<i>Cynosurus cristatus</i>	0.33	4.8
<i>Alopecurus pratensis</i>	0.77	6.5
<i>Rumex obtusifolius</i>	0.85	7.0
<i>Bellis perennis</i>	0.91	1.2
<i>Fagus sylvatica</i>	1.44	0.3
<i>Rumex crispus</i>	1.44	1.0
<i>Galium mollugo</i>	1.44	0.3

Table 3: (Partial) Mantel tests for association between ploidy level, vegetation and distance.

Association	Corrected for	Mantel's r	p-value
Ploidy * Vegetation	-	-0.070	0.001
Ploidy * Distance	-	0.055	0.001
Vegetation * Distance	-	-0.143	0.001
Ploidy * Vegetation	Distance	-0.063	0.002

Discussion

Niche width and mean niche position

Under the Tangled Bank hypothesis, coexistence between sexuals and asexuals is possible when the sexuals are able to use resources that are underexploited by the asexuals (Ghiselin, 1974; Case, Taper, 1986). This is the case when the sexuals have a wider overall niche than the asexuals, and/or when the sexuals and asexuals have different mean niche positions. In this study, we set out to measure niches in terms of the vegetation surrounding the dandelions. Vegetation data can provide an indication for the availability of resources in the plot (Schaffers, Sykora, 2000) and can therefore be used to test the Tangled Bank. However, the patterns of vegetation diversity and the association between the dandelion's mode of reproduction and the plant species that we found here did not indicate differences in resource use between the sexuals and asexuals. The use of Ellenberg values (Ellenberg *et al.*, 1992) as indicators for light, moisture and nutrients also did not reveal any differences between sexual and asexual dandelions (results not shown). Furthermore, we did not find a difference in niche width between the sexuals and the asexuals, using the total diversity (H_j) as an estimator for niche width. This means that some of the most crucial features enabling Tangled Bank mechanisms for the coexistence of sexuals and asexuals (Case, Taper, 1986) are entirely lacking.

We did however find a difference in mean niche position as shown by the Canonical Correspondence Analysis and the Mantel tests. Despite being strongly significant, the found difference was weak: most (99.5%) of the variation in the vegetation was independent of the reproductive types of the dandelions. The Canonical Correspondence Analysis revealed the nature of the niche differentiation: those plant species that were associated with sexual dandelions (*Anthoxanthum odoratum* and *Arrhenatherum elatius*), are indicative of relatively stable habitats compared to the species that were associated

Table 4: Anova for differences among clones in number of produced seed heads. Clone was used as a random factor, the number of produced seed heads was ln-transformed prior to analysis.

	SS	df	MS	F	p-value
Hypothesis	10.611	14	0.758	3.079	0.001
Error	14.771	60	0.246		

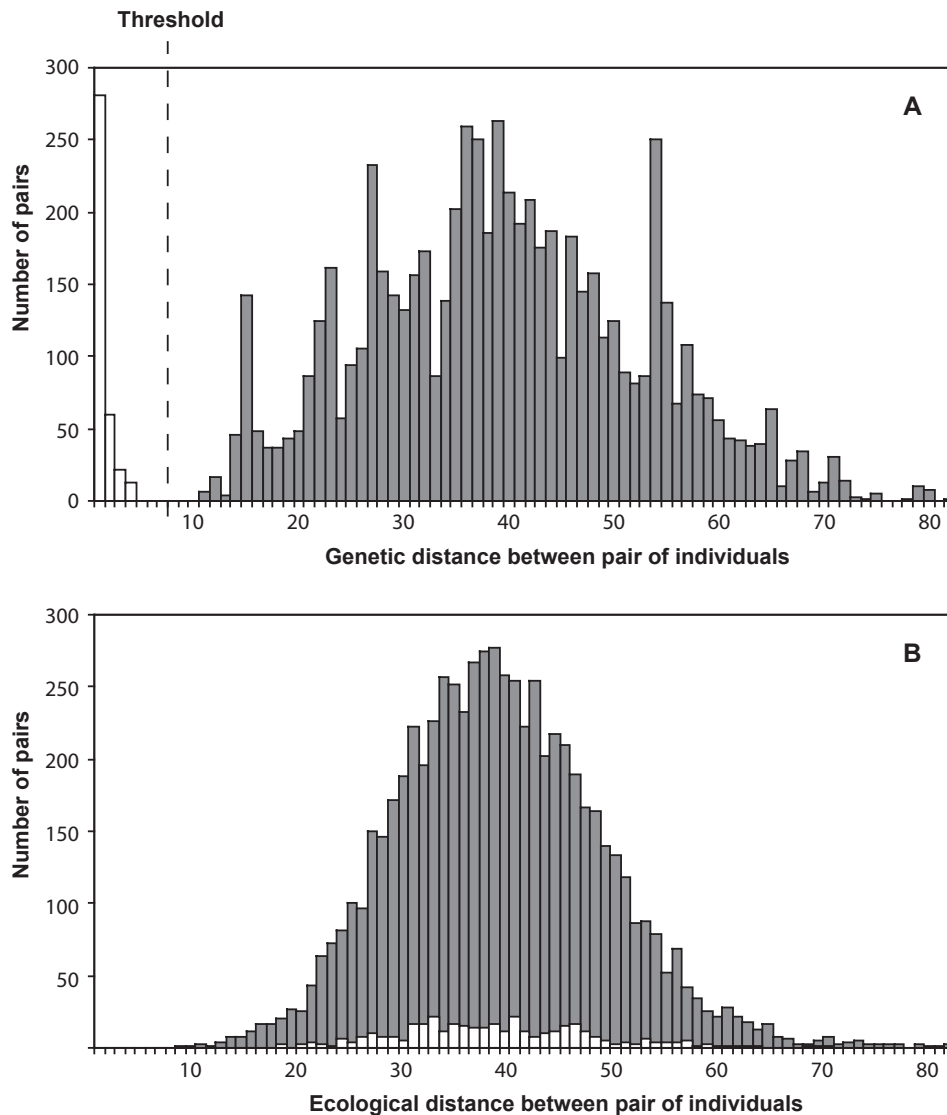


Figure 1: Frequency distributions of pairwise genetic (A) and ecological (B) distances between asexual individuals. The dotted line in figure A is the threshold that has been used to assign individuals to clonal lineages. White bars indicate distances between individuals from the same clonal lineage; grey bars indicate distances between individuals from different clonal lineages.

with asexual dandelions (*Dactylus glomerata* and *Rumex acetosa*). The latter species are indicative of more disturbed habitats, though the difference is not very pronounced, possibly because of the relative homogeneity of the sampled field and the general preference of dandelions for at least some anthropogenic disturbance. The nature of the niche differentiation matches earlier results of Meirmans *et al.* (Chapter 2) who looked at the distribution of sexual and asexual dandelions on a larger geographic scale. By comparing dandelion populations with different ratios of sexuals and asexuals they concluded that the vegetations of populations with a high frequency of asexual dandelions were indicative of a high level of human-induced disturbance. Therefore, the same process, anthropogenic disturbance, that determines differences in the frequency of sexuals and asexuals between populations (Chapter 2), has been found here to determine, albeit to a lesser extent, the distribution of sexuals and asexuals within a population.

Sexuals, when compared to the asexuals, prefer relatively stable habitats, i.e. habitats in a more advanced stage of succession than the habitats preferred by asexuals. Succession can be seen as an increase in (mainly interspecific) competition, with the first species to arrive at an empty patch being good colonisers (e.g. annuals). However, these colonising species will eventually be outcompeted by later-arriving species that invest more in competitive rather than colonising abilities (Hanski, 1983; Nee, May, 1992; Yu *et al.*, 2001). In this light, asexual dandelions can be seen as the better colonisers of the two reproductive types, while sexual dandelions are the better competitors. Note that these competitive abilities do not involve much *intraspecific* competition, which is necessary for the working of the Tangled Bank hypothesis, but rather *interspecific* competition. Intermediate stages of succession often show a higher species diversity than early stages (Connell, 1978; Huston, 1979). In that light, the higher species diversity (as measured by H_s) that was found around sexual individuals can be seen as another indication that there is a difference in competitive abilities between the two reproductive types.

Frozen niche variation

All studies that have modelled Tangled Bank mechanisms for the maintenance of sex assume that all members of a clonal lineage have the same ecological niche, a phenomenon that has been dubbed “frozen niche variation”. Using the vegetation data, we did not find any evidence for frozen niche variation in the asexual dandelions: individuals from the same clonal lineage were not more similar in their habitat than individuals from different clones, and the amount of niche overlap between individuals was larger in the sexuals than in the asexuals. However, we did find frozen niche variation for the reproductive character that was measured in the greenhouse: clonemates were similar to each other in the number of produced seed heads. Apparently some clones consistently produce more seed heads than others, if this is traded-off against another factor such as survival is as yet not known.

The niche overlap between individuals was measured by the fraction of the total diversity that was distributed among individuals; this fraction was higher in the asexuals than in the sexuals. This observation is in contradiction with the expectation under the frozen niche variation hypothesis: under that hypothesis, all members of a clone have the same ecological niche and therefore show considerable niche overlap, which should lower the average amount of niche overlap in the asexual population (Gray, Weeks, 2001). The lack of ecological similarity between clone mates was shown by the unimodal shape of the histogram of ecological distances and confirmed by the Mantel test. Ecological variables measured in the field usually have a large sampling error, but we believe that the absence of frozen niche variation for the vegetation data is not simply the result of a lack of power as comparable studies have successfully found frozen niche variation using field data. For example, using an approach similar to ours, frozen niche variation was shown using the gut-content of *Poeciliopsis*-guppies collected from natural populations (Gray, Weeks, 2001); the amount of niche overlap was found to be greater in asexual

guppies than in sexual guppies. In the freshwater snail *Potamopyrgus antipodarum* some clones were shown to prefer deep water, while others preferred shallow water (Jokela *et al.*, 1997).

The difference between the clones in the number of produced seed heads points to frozen niche variation in a similar way than found by Solbrig and Simpson (1974). They compared two clones in their competitive ability and seed set and found that the better competitor of the two clones produced fewer seeds. In other words, they found a trade-off between reproductive and competitive abilities. If such a trade-off is also present in the dandelions under study here, the factor enabling coexistence between clones is the same as the factor enabling coexistence between sexuals and asexuals: reproductive versus competitive abilities.

Conclusions

Combining the results of the test for niche overlap and the difference in mean niche position, we can conclude that the ecological niches of the sexual and asexual dandelions are largely overlapping. The non-overlapping part does not indicate the working of the Tangled Bank hypothesis, but point at a competition-colonization trade-off that could enable coexistence between the two reproductive types. The Tangled Bank hypothesis for the maintenance of sex focuses on the different utilization of scarce resources; because of their mode of reproduction, sexually reproducing taxa are expected to be able to use these resources more efficiently than asexual relatives (Case, Taper, 1986). This makes the sexuals better at *intraspecific* competition than the asexuals. We found that sexual dandelions are better competitors than asexual dandelions, but not in the sense of the Tangled Bank hypothesis; sexual dandelions were better at *interspecific* competition rather than *intraspecific* competition. If there is a trade-off between reproductive and competitive abilities, this trade-off can be seen as an ecological niche axis, along which taxa can be placed. However, this axis is not a resource axis, in the sense that the resources can be exploited like in the Tangled Bank models. We found that sexual and asexual dandelions, and possibly different clones within the asexuals, occupy slightly different places along this axis. The difference in competitive abilities might therefore contribute to the coexistence of sexuals and different asexual clones (Hanski, 1983; Nee, May, 1992; Yu *et al.*, 2001). The evolutionary origin of this difference is however unknown.

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GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms

6



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Abstract

Investigating diversity in asexual organisms using molecular markers involves the assignment of individuals to clonal lineages and the subsequent analysis of clonal diversity. Assignment is possible using a distance matrix in combination with a user-specified threshold, defined as the maximum distance between two individuals that are considered to belong to the same clonal lineage. Analysis of clonal diversity requires tests for differences in diversity and clonal composition between populations. We developed two programs, GENOTYPE and GENODIVE for such analyses of clonal diversity in asexually reproducing organisms. Additionally, GENOTYPE can be used for detecting genotyping errors in studies of sexual organisms.

Program Note

The study of genetic diversity in asexual organisms is of considerable importance for our understanding of the evolution of sex. A lot of effort has already been put into the analysis of parthenogenetically reproducing plants and animals, as well as clonally (vegetatively) reproducing plants (e.g. Van der Hulst *et al.*, 2003). The common approach when analyzing genetic data of asexuals is to estimate the diversity of different clonal lineages and compare this diversity among populations. Genetic markers are used to genotype all sampled individuals and thus to identify clonemates: individuals with exactly the same multilocus genotype are considered representatives of the same clonal lineage. This approach can also be used in studies that use noninvasive sampling methods, to check whether individual organisms have been sampled twice. Clone frequencies are then used to calculate indices of clonal diversity, which are compared among populations or species in order to test some evolutionary or ecological hypothesis.

This approach, however, has a number of pitfalls. Scoring errors, polymerase chain reaction (PCR) artifacts and (somatic) mutations may cause small differences in the data of individuals from the same clonal lineage, which are then erroneously assigned to different clonal lineages (Duhovnikoff, Dodd, 2003). This causes estimates of clonal diversity to be biased upward. Such estimates also depend on the number of markers scored, as more markers increase the chance of detecting a - real or artificial - difference among clonemates. In addition, some widely used indices of clonal diversity have a large estimation bias, which makes them unsuitable for small or even moderately large sample sizes. Some of these pitfalls can be circumvented by careful analysis of the marker data. We discuss approaches to: (i) distinguish between clonemates and real clones; and (ii) calculate diversity measures based on the assignment of individual samples to clones, and recognize possible bias in the estimation of clonal diversity. These approaches are implemented in two programs, GENOTYPE and GENODIVE, respectively.

Genotype assignment is done by calculating a pairwise distance matrix and then selecting a threshold that defines the maximum distance between two individuals at which they are still assigned to the same clonal lineage (Rogstad *et al.*, 2002). Choosing a threshold higher than zero will cause clonemates with small genetic differences to be assigned to the same clonal lineage. However, choosing a high threshold may cause individuals from different, but genetically related lineages (e.g. siblings in a species with both clonal and sexual reproduction), to be incorrectly assigned to the same clone. Therefore, attempts to reduce an upward bias in clonal diversity through increasing the threshold may at a certain point lead to a downward bias by incorrect assignment of clonemates. Clearly, choosing the appropriate threshold is crucial, although no objective guidelines are available for choosing a suitable threshold. Recently, Duhovnikoff and Dodd (2003) developed a method to calculate an appropriate threshold level, using prior knowledge about the degree of relatedness among and within known clonal lineages; the resulting threshold was subsequently implemented in studies of natural populations.

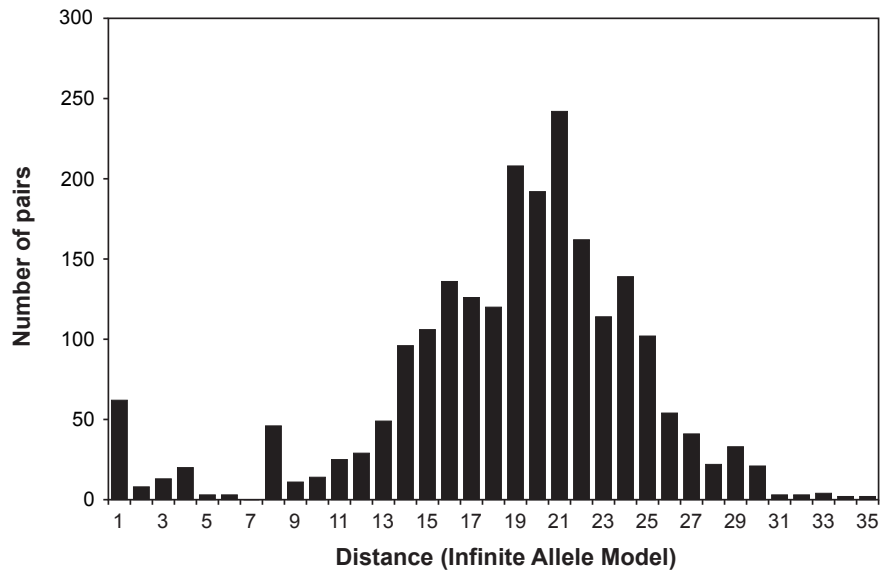


Figure 1: Frequency distribution of pairwise distances, calculated assuming an infinite alleles model, for AFLP-data from a sample of 67 dandelions from Denmark (Van der Hulst *et al.*, 2003).

However, this is not feasible for the analysis of arbitrary population samples without such prior information.

Choosing an appropriate threshold may be helped by drawing a frequency distribution of the values of all pairwise comparisons (Rogstad *et al.*, 2002). Such histograms are often multimodal, with the first peak close to zero, for nearly identical genotypes (see Figure 1). The number and positions of other peaks may depend on the population structure, the specifics of the reproductive system of the species under study, and the age and relatedness of the different clones sampled. For a sexual species that exhibits vegetative growth, for example, the first peak may represent distances between different offshoots (ramets) from the same individual (genet), that differ due to scoring errors or, less likely, somatic mutations. A second peak may represent distances between genets, which, through sib-matings, are closely related (Duhovnikoff, Dodd 2003). Subsequent peaks may be due to population structure, which causes genets from the same population to be more closely related than genets from different populations. The valley between the first and second peak can be considered a good candidate to use as a threshold.

This procedure was implemented in the program GENOTYPE, which assigns individuals to clonal lineages. It can handle data from different kinds of genetic markers, both co-dominant and dominant, such as allozymes, microsatellites, amplified fragment length polymorphisms (AFLPs) and random amplified polymorphic DNA (RAPD). GENOTYPE draws a histogram of clonal differences, and gives for every threshold the number of clones recognized to help the user in choosing a threshold for genotype assignment, which can then be performed by the program. The distance matrix GENOTYPE uses for the histogram can be calculated in three different ways: i) assuming a stepwise mutation model, for use with microsatellite data; ii) assuming an

infinite allele mutation model, intended for use with most other types of markers, or iii) using the Dice-similarity coefficient, for dominant markers. Alternatively, the distance matrix can be imported by the user.

The task performed by GENOTYPE is somewhat similar to that of other programs e.g. API-CALC (Ayres, Overall, 2004), MLGSIM (Stenberg *et al.*, 2003) and GIMLET (Valiere, 2002), although there are important differences. A conceptual difference is that those programs were developed to identify identical genotypes in otherwise sexual populations, whereas GENOTYPE is specifically made for data from species that are known to be asexual. Nonetheless, GENOTYPE can also be useful for duplicate removal and quality control of data from sexual populations. Furthermore, MLGSIM only considers full identity in multilocus genotype (i.e. a threshold of 0). The most important distinction is that GENOTYPE can handle data from polyploids. Many asexual species are polyploid and may even have individuals with different ploidy levels within the same population; GENOTYPE can also handle data from such mixed populations.

As the assignment of individuals to clonal lineages depends on the ploidy levels of the compared individuals, GENOTYPE allows the user to choose between two ways of dealing with ploidy differences: they are either simply ignored or equated to a certain, user-specified, number of mutations or mutation steps. Although there is no theoretical justification for the latter option (there is no biologically meaningful number of mutations that can be seen as equivalent to a change in ploidy level), setting this value very high ensures that individuals with different ploidy levels are never assigned to the same clonal lineage, even if they have a lot of alleles in common. Missing data are treated in the same way as ploidy differences, so either ignored or equated to a specified number of mutations.

Clonal diversity within and among populations is usually analysed using several different diversity indices, the most popular of which are: i) number of genotypes; ii) effective number of genotypes; iii) genotypic diversity, with or without correction for sample size (Nei, 1987); iv) evenness; and v) the Shannon-Wiener index of diversity (for an overview of indices, see: Legendre, Legendre, 1998). Clonal diversity can also be partitioned in within and among population components (using Nei's corrected genotypic diversity index), analogous to calculating Nei's G_{ST} -value (1987) for a single locus with several alleles.

Several additional approaches can be used to further analyse the clonal diversity, for instance comparing different populations, and assessing potential sample size bias in the diversity estimates. These approaches are implemented in the second program, GENODIVE. First, differences in clonal *diversity* between pairs of populations can be tested through bootstrapping (Manly, 1991); resampling, with replacement, genotypes within the populations. Second, differences in clonal *composition* (i.e. whether two populations could be random samples from the same pool of genotypes) can be tested through randomizing genotypes over populations (Manly, 1991). Third, the bias, resulting from small sample sizes, in the estimation of the diversity indices can be

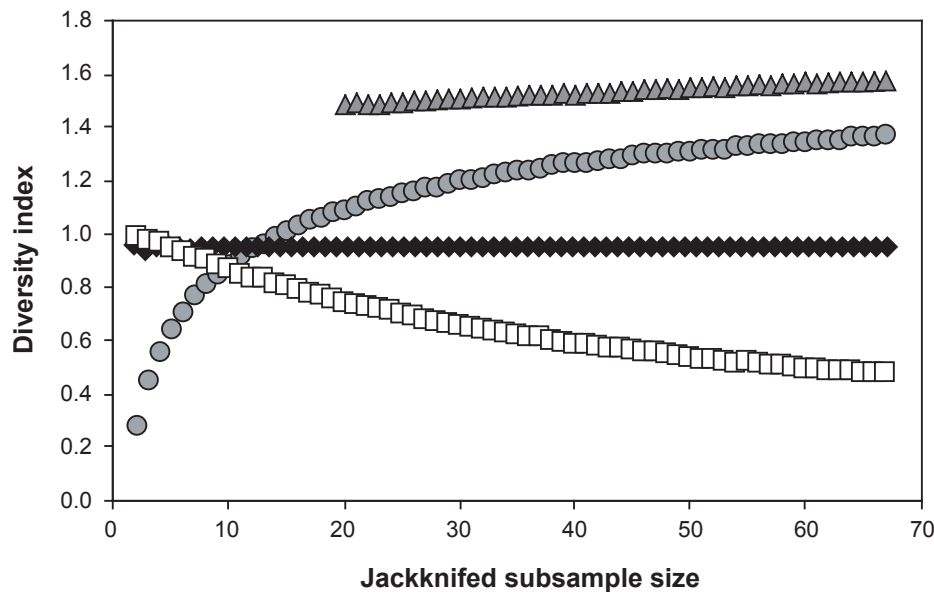


Figure 2: Indices of clonal diversity for increasing subsample size for a sample of 67 dandelions from Denmark (Van der Hulst et al., 2003). For each subsample size the average value of the diversity indices is shown after one thousand jackknifed subsamples. Black diamonds represent Nei's (1987) corrected genotypic diversity, white squares represent the evenness, grey circles represent the Shannon-Wiener diversity index and grey triangles represent the Shannon-Wiener index with bias correction (Chao, Shen, 2003). The latter index could not be calculated for the smaller sample subsample sizes. Nei's genotypic diversity is the only index without bias: the estimate is the same for every subsample size.

assessed through calculating the indices for subsamples of the dataset with different, increasing, sizes for the subsamples (Figure 2). For an unbiased diversity index (such as Nei's genotypic diversity, with correction), the index calculated from such jackknifed datasets should, on average, be the same as the original value for the full dataset. For the biased diversity indices (all others), the average values of the jackknifed estimates either increase or decrease with increasing subsample size, though this trend may level off for moderate to large subsample sizes. Although this is a good argument for not using these indices at all (unless sample size is indeed very high), they may facilitate comparisons with other, already published studies; the jackknifing procedure may then assist in checking whether sampling is sufficient to avoid sampling bias.

The accuracy of the programs has been tested through comparing the results, when possible, with those of other programs e.g. FSTAT (Goudet, 1995) and R-PACKAGE (Legendre, Vaudor, 1991). Options that are not available in any other programs were tested by doing the same calculations by hand and using Microsoft Excel. The calculations on the example files, with 10 000 permutations for the randomization tests, took only a few seconds on an iMac with a 500 MHz G3-processor, with the exception of the jackknifing option of GENODIVE, which still took less than a minute.

GENOTYPE and GENODIVE can be downloaded for free from <http://www.science.uva.nl/~tiendere/software>. Sample input files and a user manual are included. The input files of both programs consist of tab-delimited text-files in specific formats,

though GENOTYPE can also read files in FSTAT format (Goudet, 1995). The programs have no limitations regarding the number of populations, individuals or loci that can be analysed, although the maximum ploidy level of individuals for input in GENOTYPE is hexadecaploid. Both programs are available in versions for Macintosh (OS 8, 9 and X) as well as for Windows (95 and later) computers.

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Male sterility in triploid dandelions: asexual females versus asexual hermaphrodites

7



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submitted to Heredity

Abstract

Male reproductive output, pollen in plants and sperm in animals, has been shown to constitute a substantial cost for many organisms. In parthenogenetic hermaphrodites, selection is therefore expected to reduce the allocation of resources to male reproductive output. However, sustained production of pollen or sperm has been observed in numerous asexual hermaphrodites. We studied the widespread production of pollen by asexual dandelions, *Taraxacum* sect. *Ruderalia*, comparing rare male sterile individuals with pollen producing asexuals. Based on evidence from genetic markers and crosses, we conclude that the male sterility in asexual dandelions is caused by nuclear genes, in contrast to the cytoplasmically inherited male sterility previously found in sexual dandelions. Male sterile lineages did not produce more seeds per flower head, heavier seeds or seeds that were more viable. However, male sterile plants did produce more seed heads and hence seeds than pollen producing ones, indicating that they were able to reallocate resources toward seed production. Considering the difference in seed production, it remains puzzling that not more asexual dandelions are male sterile. It presumably takes time for clonal lineages to accumulate the mutations needed to become male sterile. If so, we would expect that male sterile clonal lineages are genetically more distinct from sexual dandelions than pollen producing lineages. Using the genetic distance at microsatellite loci as a measure for divergence, we found no proof that this is the case. We suggest that frozen niche variation in the asexuals might prevent pollen-producing clones from being replaced by male sterile ones.

Introduction

Male reproductive output, pollen in plants and sperm in animals, has often been found to be costly (Gouyon, Couvet, 1987). As a result of this, in parthenogenetically reproducing hermaphrodites male reproductive output is expected to be eliminated through natural selection (Weinzierl *et al.*, 1998). Nevertheless, numerous asexual hermaphrodites maintain their male function; marine clams (Ó Foighil, Smith, 1995) and flatworms (Weinzierl *et al.*, 1998) produce sperm, blackberries (Kollmann *et al.*, 2000) and dandelions (Richards, 1986) produce pollen. A non-adaptive explanation for the retention of the male function of such asexual hermaphrodites has been given by Maynard Smith (1978), who suggested that the asexual lineages in these species may be too young and may not yet have had the time to accumulate the necessary mutations for male sterility. Alternatively, male function could be retained selectively, if pollen or sperm are used to fertilise sexual relatives and if such matings lead to the production of new clonal lineages or reduce the fitness of sexual competitors. Male function can also be adaptive in pseudogamous species, where parthenogens require sperm or pollen to trigger embryo development (Weinzierl *et al.*, 1998).

The production of pollen is widespread among asexually reproducing forms of common dandelions, *Taraxacum* sect. *Ruderalia* (Richards, 1986). This pollen production has been seen as a pure waste of resources (Maynard Smith, 1978; Van Dijk, 2003); asexual dandelions do not require pollination to trigger endosperm development and they are predominantly triploid and therefore, as a result of unbalanced meiosis, most of the produced pollen is inviable. Sexual dandelions are diploid and, due to a sporophytic self-incompatibility system, outcrossing. There is a clear pattern of geographical parthenogenesis in the distribution of the sexuals and asexuals; the asexuals have a much larger and more Northern distribution than the sexuals, which are confined to Southern and Central Europe, where the two types are often found together in mixed populations (Den Nijs, Sterk, 1980; Den Nijs, Sterk, 1984).

Even though the great majority of dandelions produce pollen, male sterility has been found in low frequencies among asexual but also among sexual dandelions. Among the asexuals, male sterility is present all over Europe but usually only in low frequencies. From observations of a large number of dandelion populations, we estimate the frequency of male sterility to be between one and five percent (P.G. Meirmans, pers. obs.). This observation however does not match that of Richards (1986) who noted that about 20% of the microspecies described for Britain were male sterile. The difference between these estimates is probably that the lower estimate is for dandelions from section *Ruderalia*, while Richards looked at multiple sections. The microspecies studied by Richards are only a subset of the total dandelion diversity, and as useful morphological characters are rare in dandelions, microspecies description may also have been biased towards those that show male sterility, as this is an easily recognisable character.

In sexual dandelions, male sterility was found in two populations from France by Van der Hulst *et al.* (2004), who proved through crosses that the trait is cytoplasmically inherited, and that pollen production can be restored by nuclear modifying genes. The results from the crosses were corroborated by linkage between male sterility and a rare chloroplast haplotype. This haplotype was present in all male sterile sexuals from two populations, and further in only one male fertile individual, in which pollen production was probably restored by (a) nuclear modifier(s). Van der Hulst *et al.* (2004) also found that male sterile asexuals could be generated through crosses between a male sterile sexual mother and asexual pollen donors. If there really were resource reallocation due to male sterility, these new male sterile asexuals would have an immediate fitness benefit over pollen producing asexuals. As pollinations of sexuals by asexuals are also taking place in natural populations (Chapter 3; Sterk, 1987a; Menken *et al.*, 1995), this could entail a faster route to male sterile asexuals than the accumulation of the necessary mutations within asexual lineages. However, even though the populations in which the male sterile sexuals were found were mixed sexual/asexual populations, no male sterile asexuals and no asexuals containing the rare chloroplast haplotype were found in the populations containing male sterile sexuals (Van der Hulst *et al.*, 2004).

Pollination of sexuals by asexuals is possible because not all pollen that is produced by the triploid asexuals is inviable. Crosses between sexuals and asexuals generally result in diploid, triploid and tetraploid offspring, though the latter are very rare in natural populations (Verduijn *et al.*, 2004). Such hybridisations are assumed to be relatively common in natural populations as most variation at allozyme and microsatellite loci is shared between the sexuals and the asexuals (Chapter 3; Menken *et al.*, 1995). If so, the fitness benefit of male sterility in asexuals due to resource reallocation towards the female function could be balanced by the fitness benefit through the pollination of the sexuals. Any new clonal lineage that is created through this process will most likely be male fertile. Therefore, the presence of male sterility in dandelions, as well as other asexual hermaphrodites, is not only determined by the rate of mutation to male sterility, but also by the fitness trade-off between resource reallocation and rate of gene flow with the sexuals (Weinzierl *et al.*, 1998). There is however hardly any information on the importance of these different processes in any asexual hermaphrodite.

Research goals

The aim of this research is to answer the following questions regarding the evolution of male sterility in asexual dandelions: 1) Is there a genetic basis for male sterility in asexuals? 2) If so, is it cytoplasmically inherited and linked to the same chloroplast haplotype as in the male sterile sexuals (Van der Hulst *et al.*, 2004)? 3) Does male sterility provide individual plants with a fitness benefit due to resource reallocation? 4) Are pollen producing clones more related to the sexuals than the male sterile ones, which is expected considering that most gene flow between sexuals and asexuals is thought to occur through pollen from asexuals?

We answer these questions by using chloroplast haplotyping and microsatellite genotyping, crosses between (partially) male sterile asexuals and sexuals, measuring reproductive characters, and by estimating the amount of genetic differentiation by comparing microsatellite data of asexuals and sexuals.

Materials & Methods

Population sampling

In 2001, three dandelion populations with coexisting sexual diploids and asexual triploids were sampled from Brno, Kyov and Znojmo (Czech Republic) as a part of a phylogeographic study. These three population samples were chosen for the present study from more than thirty available samples in our greenhouse because of the high frequency of male sterility among the asexuals in these populations. Sampling was performed by J.C.M Den Nijs and J. Kirschner (who confirmed that all sampled plants belonged to *Taraxacum* section *Ruderalia*). The dandelions were dug out, leaves were removed and the roots were transferred to the greenhouse in Amsterdam, where the plants were regrown. We scored the ploidy level and the mode of reproduction of the plants using four methods: 1) Size variation in the pollen grains (only possible with male fertile plants): triploid plants have a disturbed meiosis and produce pollen of different sizes, whereas diploids produce regularly sized pollen. 2) Spontaneous seed production: insect pollinations are very rare in our greenhouse so nearly all capitula that develop seeds are from asexual plants. 3) Number of alleles at four microsatellite loci. 4) Flow cytometry, using a Partec Ploidy Analyser, according to the instructions of the manufacturer. The latter method was used to score the ploidy level of the male sterile plants and the offspring from the crosses.

Scoring of male sterility

During the flowering seasons of 2002 and 2003, all capitula of all individuals of the three Czech populations were scored for pollen production. This was done by looking at the open capitula with a 10x magnifying glass to see whether the anther tube and stamen of the open florets contained pollen. Capitula were scored in three categories: mf, ms and pms, indicating male fertile, male sterile and partially male sterile, respectively. Capitula were assigned to the latter category when they contained both male sterile and male fertile florets.

Genotyping

DNA-extraction, microsatellite-amplification and chloroplast PCR-RFLP were all performed using the protocols described in Van der Hulst *et al.* (2003). Four microsatellite loci were used for genotyping the plants: msta64, msta72, msta78 and msta85 (Falque *et al.*, 1998). Microsatellite amplification products were run on polyacrylamide on a

Li-Cor GeneReader 4200 sequencer. The banding patterns were scored by hand twice; inconsistencies were scored a third time. Whenever triploid individuals only had two bands at a locus, the relative dosages of the two bands were used to score which allele was present once and which was present twice. Dosage effects were clear in most of these cases; in the few cases that were ambiguous, the third allele was scored as “missing data”. Three regions of the chloroplast genome, known to contain length and restriction-site variation in dandelions (Van der Hulst *et al.*, 2003), were amplified using universal primers: intron trnL (Taberlet *et al.*, 1991) cut with Bst2, trnL-trnF (Taberlet *et al.*, 1991) and psbA-trnH (Dumolin-Lapeque *et al.*, 1997) cut with Dra1 and Ssp1. All chloroplast variation was scored from agarose gels, except the length variation at the psbA-trnH intergenic spacer, for which labeled primers were used and which was run on the Li-Cor.

Crosses

We performed crosses to assess whether the inheritance of the male sterility trait in the asexuals is nuclear or cytoplasmic, even though it is rather difficult to do crosses with male sterile apomicts. When a partially male sterile capitulum was discovered on an individual that was otherwise fully male sterile, this capitulum was immediately used to pollinate a sexual individual. In most of these crosses, the sexuals failed to set seed, but a small number of viable seeds was produced in three of the trials, involving three father plants from three different clonal lineages, and three mothers. All seeds produced from the crosses were put in the climate chamber for germination under the same conditions as described below. It is known that pollination of a diploid sexual motherplant with pollen from a triploid asexual can result in breakdown of the self-incompatibility system in the sexual (Morita *et al.*, 1990a); it is therefore important to check for selfing of the mother plants. We did this through genotyping the parents and all the offspring that survived to maturity at three microsatellite loci: msta53, msta72 and msta78 (Falque *et al.*, 1998). All plants of the progeny that came out as hybrids were subsequently checked for male sterility when they flowered.

Reallocation of resources

To test whether male sterility provides plants with more resources for the production of more and/or better seeds, we measured a number of reproductive traits. In 2002 and 2003, all seed heads were collected from the asexual plants, recording the pollen phenotype of the capitulum when it flowered. We also counted, weighed and germinated seeds produced by 22 male sterile and 29 male fertile plants during the flowering season of 2002. For this, depending on the number of produced seed heads, either one or two heads were used per plant, and if a plant had produced more than two seed heads, two heads were chosen at random. Of every seed head, we counted the total number of seeds as well the number of inviable seeds; these are easily recognisable because they are thin and white, in contrast to the normal bigger, brown seeds. Sixty seeds, including the

pappus, were weighed per seed head. To control for the possibility that, unconsciously, the bigger seeds were picked first, we performed the weighting in two sets of thirty seeds each, allowing us to check whether the first thirty seeds were heavier than the second batch. These same sixty seeds were used for the germination experiments that were performed in two rounds, each round using one of the two sets of thirty seeds. Whether the first or second batch of thirty seeds was used for the first or second germination round was determined at random, by flipping a coin. For the germination experiments, the thirty seeds were placed in a titerplate containing wet filterpaper and all titerplates were placed in a climate chamber with a 18°/14°C temperature and a 16/8 h. dark/light regime. The titerplates were checked daily for four weeks; newly germinated seedlings were counted and removed. No differences were found between the first and second set of thirty seeds that were picked per seed head, nor were there any differences between the first and second round of germination, so the results were combined.

ANOVAs

Seed and germination characteristics were analysed using ANOVAs. Number of seed heads, and percentage of inviable seeds were log-transformed, and the percentage of seeds that germinated was arcsine-transformed prior to analysis. The number of seed heads was analysed with male sterility (sterile vs. fertile), population and year as fixed factors and genotypes nested within sterility by population as random factor. The number of seeds within seed heads, percentage of sterile seeds, seed weight and germination rate were analysed with sex type and populations as fixed factors, and individuals nested within genotypes nested within sterility by population as random factors. ANOVAs were performed using SPSS version 11.

Clone assignment

Multilocus microsatellite genotypes were used to assign asexual individuals to clonal lineages. If such assignment is done based on full identity in multilocus genotype there may be an error as, due mutations or PCR-artifacts, individuals from the same clonal lineage may have slightly different microsatellite profiles. Therefore, to assign individuals to clones we used a threshold: the maximum pairwise genetic distance allowed between individuals to belong to the same clonal lineage (Chapter 6; Douhovnikoff, Dodd, 2003). This was done using the frequency distribution of pairwise distances between individuals. For asexual organisms such a frequency distribution is often bimodal; the peak close to zero represents comparisons between clonemates, the second peak represents comparisons between individuals belonging to different clones. The “valley” between the two peaks is then a suitable candidate for the threshold that is used for assigning individuals to clonal lineages. This assignment was done using the program GenoType (Chapter 6). As a distance measure, we used the number of single basepair length mutations that is needed to transform one genotype into the other. Association between the clonal lineages (hereafter called “clones”) and male sterility, and between the

chloroplast haplotypes and male sterility was done using the log likelihood G-statistic, the significance of which was assessed through 999 random permutations of the data, using the program GenoDive (Chapter 6). This program was also used to test for differences between the populations in the frequencies of clones and cp-haplotypes.

Genetic differentiation

Gene flow between sexual and asexual dandelions is thought to occur mostly due to pollinations of sexuals by pollen from asexuals. Therefore, pollen producing asexuals are expected to be genetically more similar to the sexuals than male sterile asexuals. We tested this hypothesis using the Rho-statistic (Ronfort *et al.*, 1998), an F_{st} -like estimator for the amount of population divergence, based on allele frequencies, that is suited for comparisons involving different ploidy levels. Per population, we estimated the Rho-statistic between the sexuals, the male sterile and male fertile asexuals. For an overall estimate, we averaged the three within population estimates. To test the hypothesis of higher divergence of the male sterile asexuals we took a bootstrapping approach: We calculated a test statistic by subtracting the averaged Rho-estimate of the divergence between the sexuals and the male sterile asexuals from the estimate of the divergence between the sexuals and the male fertile asexuals. We then created resampled datasets, through bootstrapping within populations, recalculated the test statistic and compared this bootstrapped value to the original value (Manly, 1991). As this was a very time consuming approach, only 20 of such resampled datasets were created. The calculations of the Rho-statistic were done using the program SPAGeDi (Hardy, Vekemans, 2002).

Results

The presence of male sterility

Among the 178 plants analysed, 24 were scored as male sterile, all of which were asexual triploids. Of the 178 plants, 75 were diploid sexuals, 102 were triploid asexuals and one was a tetraploid asexual; therefore, the male sterility trait was present in about one quarter of the asexuals from these populations. The frequency of the male sterile plants however differed between the populations: In the population sample from Brno 35% of the asexuals were male sterile, in the sample from Znojmo 27% and in the one from Kyov 12%. However, the expression of male sterility was not always constant; many plants that produced mainly male sterile capitula, occasionally produced capitula with some florets bearing pollen, while on the other hand, some plants that produced mainly male fertile capitula occasionally had some male sterile florets in a capitulum. In 2003, one plant that had previously produced four male sterile capitula and one partially male sterile capitulum, also produced one fully male fertile capitulum. Nevertheless, it was possible to make a clear distinction between plants that predominantly produced male fertile capitula and those that predominantly produced male sterile capitula.

Clones, haplotypes and male sterility

The frequency distribution of pairwise genetic distances between individuals, based on four microsatellite loci, was clearly bimodal (Figure 1). Based on this frequency distribution we chose a threshold of six mutation steps for assigning individuals to clones. Using this method, 45 different clones were distinguished among the asexuals, 28 of which were found only once. Within the clones that were found more than once, all members typically had the same pollen phenotype (Table 1). The only exception to this is one member of clone number five that repeatedly produced pollen, while the other seven members of the same clone were male sterile. The association between clone and male sterility was strongly significant (999 permutations, $p = 0.001$). In contrast, there was no association between male sterility and the seven chloroplast haplotypes that were found (Table 2); the chloroplast haplotype associated with male sterility in sexuals (Van der Hulst *et al.*, 2004) was not found in the studied populations. The results from the microsatellite and chloroplast analyses therefore already suggest nuclear rather than cytoplasmic inheritance of male sterility in the asexuals.

Table 1: Clone frequencies in male fertile male and sterile asexuals dandelions.

Clone nr.	Male fertile	Male sterile
1	10	
3		2
4		3
5	1	7
10	2	
15		2
20	13	
23		2
25	2	
31	2	
32		2
33	3	
34	5	
37	2	
41	2	
42	3	
43	3	
Unique	22	6
Total	70	24

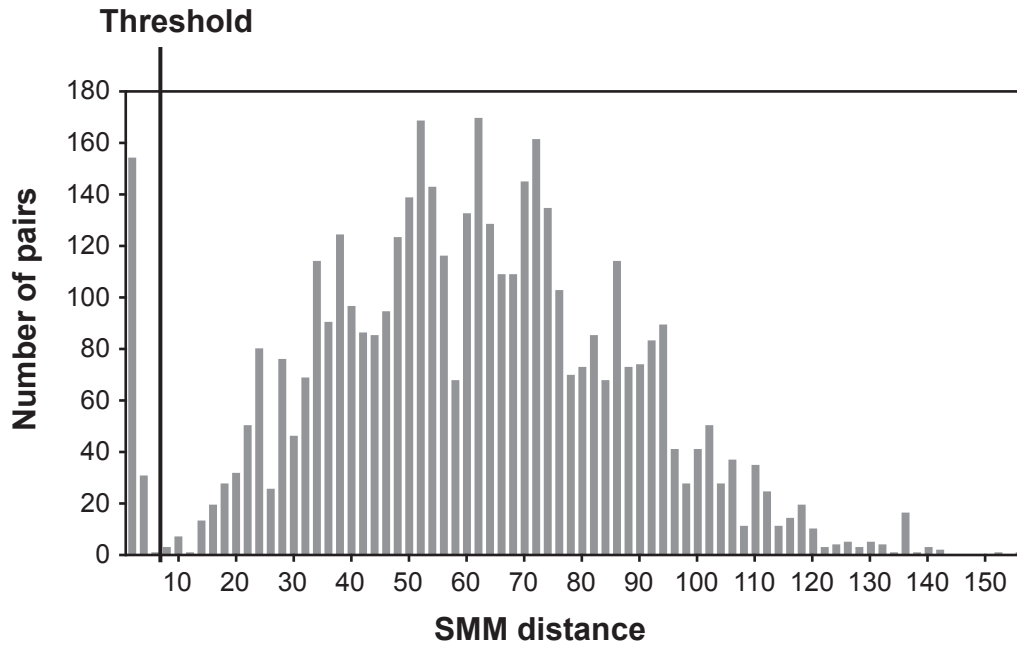


Figure 1: Frequency distribution of pairwise genetic distances between asexual dandelions. The number of mutation steps under the Stepwise Mutation Model, needed to transform one genotype into the other is used as a distance measure. A threshold of 6 is used as the maximum distance allowed between individuals to belong to the same clone.

Crosses

Further evidence for nuclear inheritance of male sterility is provided by the crosses we were able to make between partially male sterile pollen donors and male fertile sexual diploids. The three successful crosses, out of more than twenty attempts, yielded very low seed set: 5, 17 and 76 viable brown seeds were produced, of which, respectively, 5, 7 and 25 seeds germinated. Microsatellite analysis showed that part of the progeny had resulted from selfing (0, 1 and 23 respectively), so that the three crosses resulted in thirteen F1 hybrids: eight diploid, three triploid and two tetraploid individuals. Two of the three crosses indicated nuclear inheritance of male sterility, as part of the offspring was male sterile, apparently inherited from their father: in the first cross two diploids and one triploid and in the second cross four diploids were male sterile. The data from the third cross were inconclusive as the only two plants that resulted from the cross were both tetraploid and male fertile. The number of offspring in the crosses was too low to perform any test for Mendelian inheritance of the male sterility trait.

Reproductive characters

Male sterile clones produced more seed heads than pollen producing ones. However, the seeds produced by male sterile clones were lighter rather than heavier compared to seeds of male fertile clones. There was no difference in the number of seeds per head, the percentage of inviable seeds and the germination rate (Tables 3 and 4). The number

Table 2: *Chloroplast haplotype frequencies in male fertile asexual, male sterile asexual and sexual dandelions.*

Haplotype	Sexual	MS Asexual	MF Asexual
1	28	22	53
2	25	1	14
3	1	1	3
4	3		1
5	1		1
6	2		
Total	60	24	72

of produced seed heads was significantly higher in 2003 than in 2002. There were no differences among the three populations for all measured variables, nor were there any interaction effects. There also were no differences between years or among clones nested within male sterility/population. Individuals nested within clones, showed differences for seed weight and germination rate.

Genetic differentiation

The male sterile asexuals were not more differentiated genetically from the sexuals than the pollen producing asexuals were. The multilocus Rho estimate for the divergence between the sexuals and the male sterile asexuals ($Rho = 0.137$) was only slightly higher than that between the sexuals and the male fertile asexuals ($Rho = 0.135$). This small difference between the two Rho estimates proved not significant when tested using bootstrapping ($p = 0.67$). However, both Rho estimates were significantly different from zero (1000 permutations, $p = 0.001$, for both values), indicating that both the male sterile and the pollen producing asexuals were genetically differentiated from the sexuals. Nevertheless, the relatively low value of the Rho-statistic indicates that most of the genetic variation was shared between the sexual and asexual populations.

Table 3: *Seed and germination characteristics of male sterile and male fertile dandelions. Values are averaged over populations, with genotypes nested within populations and individuals within genotypes. Between brackets is the standard deviation over populations.*

Variable	Male fertile	Male sterile
# of heads, 2002	3.03 (0.35)	4.30 (0.94)
# of heads, 2003	7.45 (1.41)	9.99 (2.21)
seed weight (mg)	5.90 (0.25)	4.92 (0.53)
# of seeds per head	173.56 (23.1)	179.38 (2.77)
% inviable seeds	8.84 (5.33)	7.43 (1.69)
% germinated	74.68 (4.04)	61.99 (6.28)

Table 4: ANOVAs, comparing seed and germination characteristics between male sterile and male fertile dandelions (MS), between populations (POP) and years (YEAR), with genotypes (CLONE) nested within MS by POP and with individuals (IND) nested within genotypes. Interactions that were both non-significant and uninteresting were removed from the ANOVA model, to increase the power (the significant effects in the simplified model were also significant in the full model). Number of seed heads and percentage of inviable seeds were log-transformed; the percentage of seeds that germinated was arcsine-transformed prior to analysis. (*: $p \leq 0.05$, ***: $p \leq 0.001$).

Number of seed heads produced	Mean Sq.	Err. Mean Sq.	d.f.	F
MS	0.421	0.065	1/167	6.52*
POP	0.002	0.065	2/167	0.03
YEAR	4.417	0.065	1/167	68.44***
MS x POP	0.007	0.065	2/167	0.11

Seed weight	Mean Sq.	Err. Mean Sq.	d.f.	F
MS	16598.9	3961.4	1/29.3	4.19*
POP	2616.2	3973.5	2/28.8	0.66
MS x POP	926.1	3983.9	2/28.9	0.23
CLONE (MS x POP)	3951.0	2510.6	30/14.4	1.57
IND (CLONE (MS x POP))	2381.8	687.2	15/40	3.47***

Number of seeds per head	Mean Sq.	Err. Mean Sq.	d.f.	F
MS	790.5	4335.5	1/29.6	0.18
POP	7957.2	4329.4	2/28.7	1.84
MS x POP	6173.0	4338.9	2/28.9	1.42
CLONE (MS x POP)	4347.6	4307.9	30/15.8	1.01
IND (CLONE (MS x POP))	4282.1	2310.4	16/45	1.85

% inviable seeds	Mean Sq.	Err. Mean Sq.	d.f.	F
MS	0.000	0.231	1/29.6	0.00
POP	0.233	0.233	2/29.3	1.00
MS x POP	0.030	0.233	2/29.3	0.13
CLONE (MS x POP)	0.229	0.096	30/13.3	2.39
IND (CLONE (MS x POP))	0.095	0.078	15/40	1.21

% germinated	Mean Sq.	Err. Mean Sq.	d.f.	F
MS	0.648	0.222	1/29.2	2.92
POP	0.022	0.222	2/28.5	0.10
MS x POP	0.168	0.223	2/28.6	0.76
CLONE (MS x POP)	0.221	0.178	30/14.3	1.24
IND (CLONE (MS x POP))	0.172	0.070	15/39	2.47

Discussion

Mode of inheritance

The results of the crosses, the microsatellites and the chloroplast haplotyping all indicate that male sterility in the asexual dandelions studied has a nuclear inheritance. First, there was a close correspondence between multilocus microsatellite genotype and the male sterile phenotype. Second, the PCR-RFLPs show no correspondence between the chloroplast haplotype and male sterility, and the chloroplast haplotype that is indicative for a male sterility inducing cytoplasm (Van der Hulst *et al.*, 2004) was not found in the populations studied here. Third, the crosses between sexual dandelions and predominantly male sterile asexuals resulted in male sterile offspring in two out of the three crosses.

The nuclear inheritance of male sterility in asexual dandelions found in our study does not match the findings of Malecka (1971) who argued, based on anther morphology, that male sterility in two asexual dandelion microspecies was cytoplasmic. She argued that the pattern of the tapetal layer degeneration was characteristic for cytoplasmic male sterility. Whereas we can exclude cytoplasmic inheritance in two of our crosses, where male sterile offspring was obtained, it cannot be excluded in the third; the only hybridogeneous offspring of this cross were two pollen-producing individuals. These two plants were both tetraploid and therefore presumably received the complete nuclear genome of their male sterile, triploid father and a haploid set from their mother. As the offspring was producing pollen, the male sterility of the father may have been cytoplasmic and not transmitted to the offspring. Alternatively, the male sterility may have been restored by alleles present in the motherplant.

Fitness benefit

Male sterile dandelions reallocate resources into the production of more seed heads: Averaged over the two years that the production of seed heads was monitored, the male sterile plants produced 38% more seed heads than the pollen producing ones. Seeds produced by male sterile clones were lighter than those of pollen producing clones, but, as we found no difference in germination rate, seed weight might have little effect on viability. The increased seed production of the male sterile clones therefore gives them a large fitness benefit that makes the widespread occurrence of pollen production in asexual dandelions particularly puzzling. Apart from any non-equilibrium scenarios in which male sterility simply did not yet go to fixation, there are two explanations for why most asexuals produce pollen. The first explanation is that the produced pollen gives the male fertile asexuals a fitness benefit through the pollination of coexisting sexuals. The second explanation is that balancing selection maintains clonal diversity, which includes diversity in pollen production.

Concerning the functionality of the pollen produced by the asexual dandelions; several studies have already shown that this pollen can successfully pollinate sexual

dandelions, which then results in gene flow between the sexuals and asexuals (for an overview, see Verduijn *et al.*, 2004). The occurrence of gene flow between sexuals and asexuals is also apparent in the present study from the low values of the Rho statistic (which is like F_{st} bound between 0 and 1). However, it seems unlikely to us that these hybridisations are frequent enough to balance a 38% higher reproductive output of the male sterile individuals as found in our study. This would require that the production of new clonal lineages through pollen produced by asexuals has to be equal to the same 38% per generation. This requires a rate of gene flow between the sexuals and asexuals that is unrealistically high; in a study of a dandelion population containing both sexuals and asexuals, Verduijn *et al.* (2004) found that less than two percent of the offspring produced by sexuals was polyploid.

Balancing selection can maintain variation in pollen production among clonal lineages if it is not pollen production in itself but clonally linked traits that are under selection. Under the Frozen Niche Variation hypothesis (Vrijenhoek, 1979) clonal lineages coexist in separate ecological niches. Under such a scenario, the male sterile lineages may have a higher production of seed heads, but may still not be able to outcompete the pollen producing lineages in their respective ecological niche. In dandelions, niche differentiation between two clonal lineages has been shown by Solbrig and Simpson (1974), who showed that one of the two clones was better at competition, while the other had a higher seed set and was therefore assumed to be better at colonisation. Meirmans *et al.* (Chapter 5) found that although clonal lineages differed from each other in seed production, they did not differ in their habitat, measured by the vegetation growing around the individuals. It is therefore uncertain whether the high clonal diversity that is generally found in dandelions (Van der Hulst *et al.*, 2003) is maintained by Frozen Niche Variation.

Genetic differentiation

Male sterile asexuals were not more diverged from the sexuals than the pollen producing asexuals were. Such a difference was expected as gene flow between sexuals and asexuals is thought to take place mainly through the pollination of sexuals by pollen produced by asexuals (Verduijn *et al.*, 2004). Because of their lack of pollen production, male sterile asexuals are not expected to take part in the gene flow between sexuals and asexuals. As most clonal lineages originate from hybridisations between sexuals and asexuals, the Rho-statistic for differentiation from the sexuals can also be seen as an indicator for the average age of the clonal lineages. In that case, the lack of difference between the Rho statistics indicates that the male sterile clones are not older than the pollen producing ones. A difference in the age of male sterile and pollen producing clones would be expected if it takes time for a specific clone to accumulate the mutations necessary for male sterility, as suggested by Maynard Smith (1978).

The observed lack of a difference in relatedness with the sexuals can be explained by the ability of male sterile plants to sometimes produce small amounts of pollen. One

individual even produced a fully male fertile capitulum, even though previously it had only produced male sterile ones. In addition, one member of an otherwise male sterile clone repeatedly produced male fertile capitula. The crosses between the sexuals and partially male sterile asexuals indicate that partially male sterile plants can successfully act as pollen donors, though only few of the crosses were successful. Gene flow between partially male sterile asexuals and sexuals is therefore possible and repeated production of new male sterile clones would prevent a high amount of genetic differentiation between the male steriles and the sexuals. Limited gene-flow even does not exclude the possibility of a single mutational origin of the male sterility trait in the studied populations, despite the fact that male sterility was found in individuals with twelve different microsatellite genotypes and three different chloroplast haplotypes. However, we think that independent evolutionary origins and genetic causes are more likely, given that there was variation in the morphology of male sterile capitula in different individuals; in some male sterile individuals the capitula had distinctly brown coloured anther tubes, while in the other male sterile individuals the capitula had yellow anther tubes (P. Meirmans, pers. obs.).

Conclusions

Male reproductive output of parthenogenetic hermaphrodites is determined by the trade off between the reallocation of resources into the female function, leading to male sterility, and the ability of the male gametes to fertilise sexual relatives, leading to the production of new clones. Furthermore, the evolution of male sterility in asexuals is constrained by the rate of mutation to male sterility. However, the argument that there has not been enough time for the asexuals to accumulate the necessary mutations for male sterility (Maynard Smith, 1978), does not hold for the dandelions under study here. The mutations have occurred, possibly even several times, but they did not go to fixation, despite a substantial fitness benefit due to the reallocation of resources. This fitness benefit and the observation that male sterility is present in several different clonal lineages, should give the male sterile dandelions plenty of evolutionary potential, which makes the widespread occurrence of pollen producing asexual dandelions seem quite paradoxical. However, if the high clonal diversity in dandelions is maintained by Frozen Niche Variation, it might be difficult for male sterility to go to fixation.

Acknowledgements

This work would not have been possible without the help of Louis Lie who counted, weighted and germinated the seeds. We also thank Jan Kirschner for help in sampling and identifying the dandelions, Ludek Tikovsky for taking care of the plants in the greenhouse, Rob Bregman for help in collecting the seeds and Peter Kuperus for assistance in the lab. We thank Stephanie Meirmans for valuable advice on the manuscript. Ron van der Hulst triggered the study of male sterility in dandelions.

General discussion: Overcoming a low cost of sex in dandelions

8



Patrick G. Meirmans

How big is the cost of sex in dandelions?

In the introduction (Chapter 1), I argued that the realised cost of sexual reproduction is strongly species specific and that it is therefore important to study the costs of sex before proceeding to the possible benefits of sex. So before I discuss how my work gives insights about the coexistence of sexual and asexual dandelions, I will review which costs of sex are applicable to dandelions and which are not. To this end, I use results from some parts of my work and from the work of others to estimate important parameters determining the realised cost of sex.

1) *The cost of males / the cost of genome dilution*

Joshi and Moody (1998) presented a model enabling them to specify and separate the cost of males and the cost of genome dilution in hermaphrodites (see also Chapter 1). I will take their model and estimate parameters using my own data and those of other studies on dandelions, to calculate the contributions of these two costs of sex in dandelions.

The model of Joshi and Moody (1998) is directly applicable to dandelions since it assumes that both sexuals and asexuals are hermaphrodites, that the two types may differ in their investment in male function and that male output of asexuals may not necessarily be as successful in fertilising sexuals as the male output of the sexuals. The investment in male function of sexuals is expressed in their parameter k . This parameter can be estimated for dandelions from the effects of male sterility in asexual dandelions (Chapter 7). Male sterile dandelions produced 38% more seeds than pollen-producing dandelions. The other way around, pollen-producing dandelions produce 27% less seeds, so that the investment in male reproductive output in sexuals (k) is estimated to be 0.27. The parameter n in the model of Joshi and Moody stands for the male output of asexuals relative to that of sexuals. In dandelions, most asexuals still produce pollen and only a small percentage is male sterile. In Chapter 7, I estimated that between one and five percent of asexuals are male sterile. Taking the upper limit of this value plus accounting for the fact that, in general, asexuals make less pollen than sexuals (P.G. Meirmans, pers. obs.), I estimate the overall value for n in dandelions to be about 0.80. The last parameter value to estimate is c , which expresses the fertilisation success of asexuals relative to that of sexuals. The pollen of asexual dandelions is known to be largely infertile, though a part of it is able to fertilise ovules of sexuals. Several studies from crossings between sexuals and asexuals have shown that a large part of the offspring stems from selfing of the sexuals, so that only a small part is hybridogenous. Tas and Van Dijk (1999) reported the latter to be about 12%, and they also observed that the seed set resulting from the crosses was low, about 22% of the usual seed set of crosses between sexuals. I therefore set the value of c to $0.12 * 0.22 = 0.03$.

Using these parameter values for the Joshi and Moody (1998) model, it is possible to plot the resulting values of the cost of males and the cost of genome dilution as a

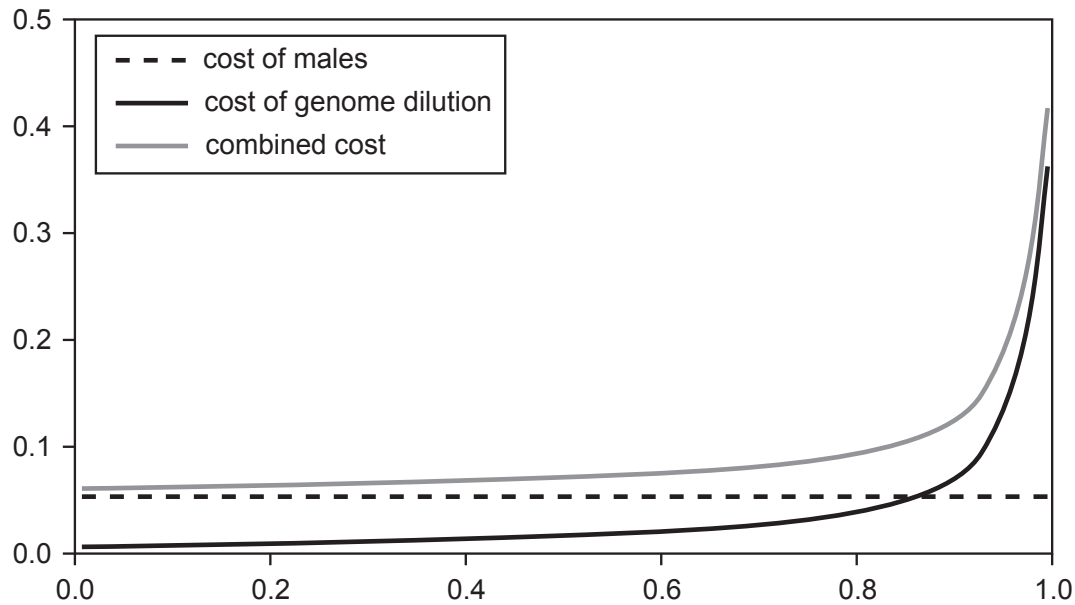


Figure 1: The cost of males and the cost of genome dilution for dandelions as a function of the frequency of asexual in a population, based on the model of Joshi and Moody (1998), with parameters estimated for dandelions ($k = 0.35$, $n = 0.80$, $c = 0.03$).

function of the frequency of asexuals in the population (Figure 1). In Figure 1, the costs of sex are given as the *per capita rate of egg loss* that sexuals suffer relative to asexuals. This way of presenting the cost might be counterintuitive when considering that the cost of sex usually is presented in terms like “two-fold”, which would be a value of 0.5 here. The benefit of presenting the cost in this way is that the two types of costs can more easily be combined. It is apparent from Figure 1 that the cost of males is not influenced by the frequency of the asexuals, while the cost of genome dilution increases with an increasing frequency of asexuals. Both costs are generally low for dandelions, except for high frequencies of asexuals, where the cost of genome dilution becomes rather large; this is because more pollination by asexuals takes place when sexuals are rare.

2). The cost of sexual selection.

This cost is probably not applicable to dandelions as sexual reproduction takes place via a pollen vector, which reduces the impact of sexual selection as the individuals do not directly exhibit mate choice. The cost related to the cost of sexual selection, the cost of having a combined male/female genome (intersexual ontogenetic conflict), is probably not applicable to dandelions. Though it is possible that being hermaphrodite puts a constraint on the female fitness of dandelions, the cost of this constraint is probably not realised as it is a long-term cost: it may take quite some time before mutation and subsequent selection have optimised female fitness in asexuals.

3). *The cost of finding a mate.*

This is a complex cost in plants as it involves many different aspects of their life history, such as nectar production and the showiness of flowers, but also reproductive assurance. The first two components of the cost of mating are probably not applicable to dandelions as asexual dandelions still produce showy flowers and, judging from the large numbers of visiting insects, also a considerable amount of nectar. The lack of reproductive assurance probably constitutes a large cost to sexual dandelions. On rainy days and dark, overcast days, dandelion flowers do not open and several days of bad weather in a row may cause that sexual flowers are not pollinated at all (Sterk, 1987b). Asexual flowers also do not open during bad weather, but this does not affect their seed set. This “cost of rain” can be very conspicuous in the field as it can cause sexual individuals to have a circle of white sterile seeds in a seed head (P.G. Meirmans, pers. obs.) or, in extreme cases, all seeds in the head are sterile. It is tempting to use this cost of rain to explain patterns of geographical parthenogenesis in the distribution of sexuals and asexuals (bad weather in the north), but also distribution patterns on a smaller scale (sexuals have better seed set on warmer spots). However, these patterns probably have more fundamental causes; geographical parthenogenesis is a well established pattern in the distribution of many asexual complexes (Bierzychudek, 1987), few of which have a “cost of rain” like dandelions.

4). *The cellular mechanistic cost of meiosis and syngamy.*

This cost is mainly applicable to unicellular organisms, where the process of meiosis takes up considerably more energy and time than it would take to undergo simple mitosis. The energetic costs of meiosis constitute probably only a negligible part of the total energy budget of a dandelion.

5). *The cost of recombination and segregation.*

This is one of the most difficult costs to qualify, and even more difficult to quantify. In general, there is little information available about the occurrence, nature and amount of epistatic interactions between genes in dandelions. Epistatic interactions are broken up by sexual reproduction and genetic mixis, which, depending on the nature of the epistatic interactions, might be highly costly. I found a possible example of epistatic interactions in dandelions: the male sterility trait that I studied in Chapter 7, and which generally gave a fitness benefit in asexuals, provided a disadvantage to the individual after crossing it into a different genetic background (see Box 1). Segregation and recombination also affects heterozygosity, which may be costly when heterozygotes are more fit than homozygotes. The idea that fixed heterozygosity is beneficial for asexual dandelions is, however, not confirmed by genetic marker studies; asexual dandelions had no higher heterozygosity than expected, judging from the values of F_{is} that were generally around zero. Furthermore, there was no correlation between heterozygosity and fitness (from

data from Chapters 5 & 7, results not presented). However, one should be very careful in drawing conclusions about heterozygosity on fitness-loci using results from neutral genetic markers (David, 1998).

6). *The cost of a balanced set of chromosomes.*

In dandelions, asexual reproduction makes it possible to be triploid and reproductive. Ploidy level has been shown to cause differences between the diploid and triploid dandelions in cell size, leaf number, leaf length and timing of flowering (De Kovel, 2001). Some of these differences turn out to be advantageous for the triploids, such as the ability to produce more or longer leaves, but it is questionable whether all differences can be seen as advantages. If higher ploidy level would generally result into better performance, one would assume that tetraploid dandelions should have an even bigger advantage than triploids. However, tetraploid individuals generally have low seed set and apomixis seems to be incomplete (Verduijn *et al.*, 2004b). Overall, it seems that being triploid per se might be advantageous in dandelions, and therefore there might be a cost for sexuals for not being triploid.

7). *Cost of hybridisation and inbreeding.*

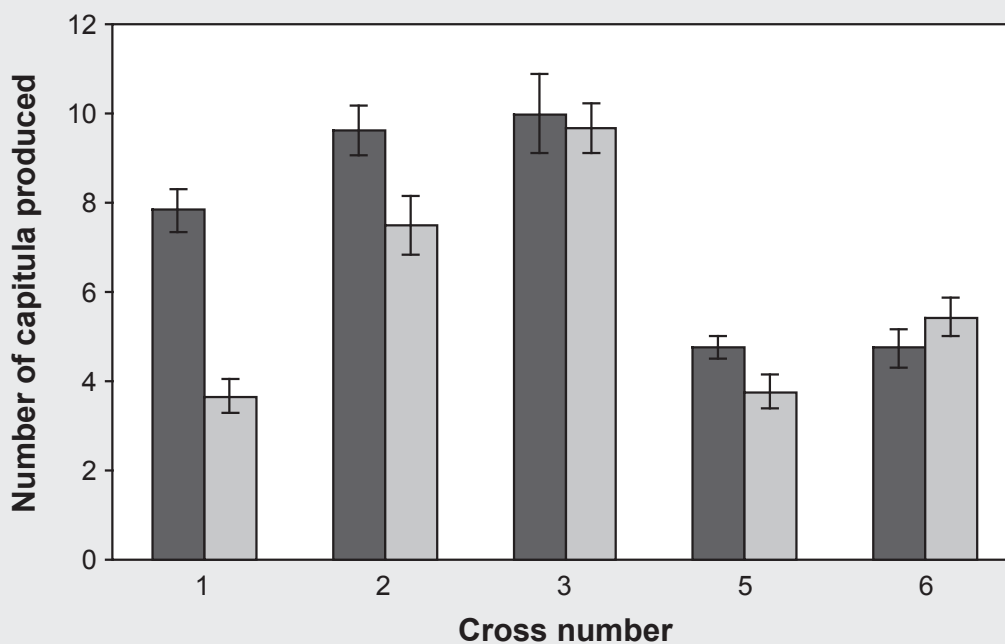
Diploid (sexual) dandelions may suffer from pollinations with pollen from triploid (asexual) dandelions. Such pollinations have been found to trigger selfing in the sexuals (Morita *et al.*, 1990a), which leads to inbreeding depression. As there is normally little inbreeding in dandelions as a result of the self-incompatibility system, recessive deleterious mutations will only very slowly get purged from the population, and inbreeding depression is therefore expected to be severe. Pollinations of sexuals with pollen from asexuals also result in a high amount of polyploid offspring (Verduijn *et al.*, 2004b), which lowers the frequency of diploid sexuals in the population. This latter type of cost was also included in the model of Joshi and Moody (1998).

8). *Sexually transmitted diseases and transposons.*

In animal-pollinated plants, sexually transmitted diseases are transferred via pollinators (e.g. anther-smut fungus in *Silene* (Van Putten *et al.*, 2003)). No sexually transmitted diseases are known for dandelions (Sterk, 1987b), although only little attention has been given to parasitism in dandelions. Asexual dandelions attract pollinators in a similar manner than sexuals since they have the same showy flowers as the sexuals and still produce pollen and nectar; they should therefore be equally susceptible to sexually transmitted diseases as sexuals. As there is nothing known about the effect of transposons in sexual and asexual dandelions, it is hard to establish whether there is a cost of sex resulting from their existence. However, the “cost of transposons” is only realised when a new transposon has entered the population, in that case, meiosis aids in the spread of the transposon through the population (Loxdale, Lushai, 2003a); it is unlikely that this is the case in dandelions.

Box 1: Effects of male sterility, originating from a triploid asexual background, in diploid sexual dandelions

Previously (Chapter 7), some asexual dandelions were found that were partially male sterile, i.e. they only rarely produced pollen. The pollen from these plants was used to pollinate sexual mothers and two of these crosses resulted in male sterile sexual diploids (see Chapter 7). Here, I look at the effect of the male sterility trait after segregation by making further crosses with these male sterile diploids using diploid pollen donors. I test for a difference in the number of produced capitula between male sterile and male fertile (pollen producing) offspring of five of the latter crosses.



Average number of capitula produced by male fertile and male sterile offspring from five different crosses, error bars indicate standard error.

Male sterile offspring produced significantly less capitula than pollen producing offspring (Anova with pollen type nested within cross $F_{5,234} = 9.10$, $p \leq 0.001$; no effect of cross $F_{4,5} = 4.45$). This indicates that the male sterility trait, previously shown to be advantageous in asexual dandelions (Chapter 7), is disadvantageous when crossed into a different genetic background. Possibly, the genes underlying the trait are involved in epistatic interactions with other parts of the genome of the clone from which they originate: in this clone the negative side effects of the male sterility gene are compensated by the effects of other loci. Because of the strict linkage between all loci within an asexual genome, such epistatic interactions can easily evolve in asexually reproducing lineages but are quickly broken down by segregation and recombination.

A low cost of sex in dandelions

The above review shows that there indeed is an overall cost to sex in dandelions, but that it is hard to put a number to it, as most realised costs are not quantifiable. Nevertheless, the realised cost of sex in dandelions is probably much lower than usually assumed in studies on the maintenance of sex. Often, only the cost of males or the cost of genome dilution are addressed; I found both to be relatively low in dandelions, given the framework of Joshi and Moody (1998). Nevertheless, both costs still provide a fitness advantage for the asexual dandelions: under the low cost of sex that increases with increasing frequency of asexuals that is shown in Figure 1, the frequency of asexuality will increase from 1 to 99% in about 120 generations (compared to less than 15 generations under a fixed two-fold cost). When considering other costs of sex as well, such as the cost of mating associated with bad weather, the replacement of sexual dandelions by asexuals should be even quicker. Thus, there still is a paradox of sex in dandelions, even though it could be smaller than in other species. This implies that the benefits of sex that enable the persistence of sexuals as well as the coexistence of sexual and asexual dandelions do not have to be as powerful as for other species suffering from a higher cost to sex. This is an important factor since it has been claimed that one of the major drawbacks of all models for the maintenance of sex is that the parameter values needed to overcome the presumed two-fold cost are rather unrealistic (West *et al.*, 1999).

An explanation for coexistence

Acknowledging that there is a cost to sex in dandelions, albeit a slight one, requires an explanation for how sexual and asexual individuals can coexist in nature. A large part of this thesis is devoted to searching for an explanation for the coexistence.

Ecological differentiation

In my work, I repeatedly found ecological differentiation between sexual and asexual dandelions. In Chapter 2, I investigated frequencies of sexuals and asexuals across populations on a regional scale. In Chapters 3 & 5, I analyzed the distribution of sexuals and asexuals within a single population and in Chapter 4 I looked at the changes in the ratio of sexuals and asexuals over time. All these studies showed ecological differences between the two types, though these differences were mostly quite small. The differences were similar in all studies: asexuals prefer more disturbed habitats than sexuals, or put differently: asexual dandelions are found in habitats that are in an earlier stage of succession than the habitats of sexual dandelions.

Considering the differentiation observed in my studies, it is easy to draw a rough picture of the dynamics taking place in a hypothetical dandelion metapopulation. The original (natural) habitat of dandelions of section *Ruderalia*, the section under study here, probably consisted of a metapopulation of temporarily available patches. When

an empty patch became available for dandelions, the asexuals would be first to invade the empty patch, but over time, when succession proceeded, the sexuals would establish themselves in the patch and replace the asexuals. When succession proceeded even further, eventually the local dandelion population in the patch would go extinct, until some disturbance event made the patch available to dandelions again.

Nowadays, dandelions mostly occupy man-made habitats that are kept in a constant stage or cycle of succession due to human activities. Thus, anthropogenic disturbance may nowadays keep the ratio of sexual and asexual dandelions more stable in many patches. I found evidence for this in the study of sexual and asexual dandelion populations in the Neuchâtel area (Chapter 2), where dandelions sampled in heavily disturbed vineyards were fully asexual, while those found in relatively undisturbed hay meadows were fully sexual. I believe that the ratio of sexuals and asexuals in those fields may have been constant for many years, as also the management of these fields had hardly changed over the last decades (according to the farmers). This hypothesis should however be tested by revisiting all studied populations after some years. Accordingly, I suggest that if the management of a certain field changes, the ratio of sexual and asexual dandelions in this field should change accordingly. Such dynamics are apparent in Chapter 4, where a decrease in the amount of disturbance in the sampled field coincided with an increase in the frequency of sexuals and a decline in population density over time.

Differences in seed quality between sexuals and asexuals

Studies comparing colonising abilities between closely related taxa have often looked at the seeds of species, assuming that there is a trade-off between seed quality and seed quantity (Turnbull *et al.*, 2000). Making a large number of low-quality seeds helps colonization of other patches, but the seedlings that grow out of those seeds are competitively inferior to the seedlings from high-quality seeds that have more resources available for initial growth. I doubt whether such a trade-off underlies the difference in ecological niche between the sexual and asexual dandelions, because the reproductive advantage of the asexuals appears to come at no additional cost. The reproductive advantage of the asexuals stems from the costs of sex per se; therefore, it does not cost the asexuals any resources to make more seeds which can be of equal quality as the seeds produced by sexuals.

Seed quality of sexual and asexual dandelions was studied by De Kovel (2001) as a part of a study on life-history characteristics of dandelions grown in the greenhouse. She did not find any significant differences between sexuals and asexuals for most of the characters she studied, and the differences she did find (e.g. sexuals produced more ovules per inflorescence) may simply be attributable to the hand-pollination of the plants; hand-pollination of sexual dandelions usually leads to a much lower percentage of developed seeds than open pollination in the field. The asexuals do not require pollination and their seed production in the greenhouse is therefore likely to be close to the seed production in the field. The low success of hand-pollinations of the sexuals may cause them to reallocate the resources not spend on seed ripening for other purposes, such as the production of a

higher number of ovules in subsequent inflorescences. In order to compare the seeds of sexual and asexual dandelions from open-pollinated dandelions, I did a morphometric analysis of seeds collected from the mixed sexual/asexual population in the Odenwald (see Box 2). I found that the seeds of asexuals were significantly larger than the seeds of sexuals, and that there was a difference in shape; the seeds from sexuals were more slender than those from asexuals. Though it is unlikely that the difference in shape may have a large fitness effect, seed size has previously been found to positively effect germination in dandelions (Tweney, Mogie, 1999). The observed difference in size therefore could indicate a higher quality of the asexual rather than the sexual seeds. The difference in size also does not suggest better dispersal capacities for the asexuals as dispersal capacity is inversely related to seed size (Ford, 1985). The ecological difference between the sexuals and asexuals is therefore presumably not caused by a trade-off between seed quantity and seed quality.

The origin of the niche differentiation

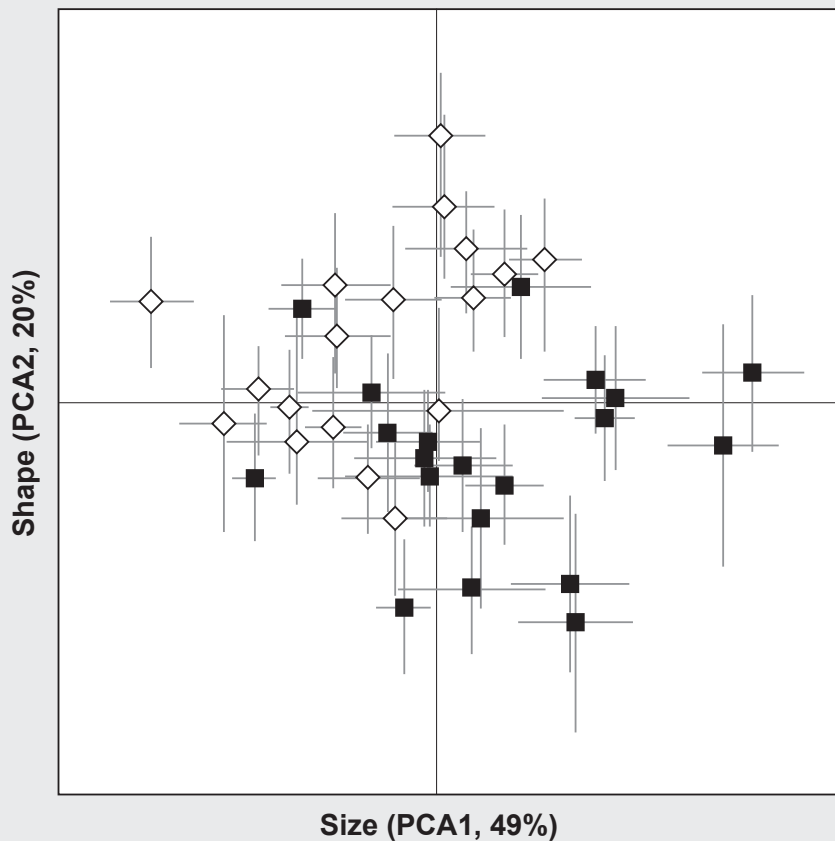
As I described above, I found a difference in ecological niche between the sexual and asexual dandelions. Although the mechanisms underlying the niche differentiation are not fully known, it is of interest to know how the differentiation arose. Here I discuss three different explanations: 1). The difference evolved through character displacement during Tangled Bank interactions between sexuals and asexuals. 2). The difference is caused by Red Queen processes, in combination with metapopulation dynamics. 3). There is no evolutionary explanation, but the difference is a direct effect of the difference in ploidy level.

Under the first explanation, the differences have evolved as a result of the competition between the two types. Case and Taper (1986) describe how such character displacement can develop quite quickly as a result of Tangled Bank mechanisms. The Tangled Bank is one of the few hypotheses for the maintenance of sex that actually allows for coexistence between sexuals and asexuals. However, I argued in Chapter 5 that the Tangled Bank does not seem to operate in dandelions as it assumes *intraspecific* competition for scarce resources, while my studies (Chapters 2, 4 & 5) indicate that sexuals are better at *interspecific* competition, at least when succession is viewed as an increase in interspecific competition. Another drawback of the Tangled Bank model is that it only works under low clonal diversity, while the clonal diversity in dandelions is in general very high (Van der Hulst *et al.*, 2003; Chapters 4, 5 & 7). Furthermore, the high rate of gene flow that takes place between the sexual and asexual dandelions (Chapters 3 & 4), which is the major cause of the high clonal diversity, would very quickly eradicate any genetically based ecological differences between the sexuals and asexuals. In conclusion, it is unlikely that the ecological difference evolved as a character displacement under the Tangled Bank mechanism.

The second explanation for the observed difference in competitive ability between sexuals and asexuals involves the Red Queen hypothesis. The Red Queen is often

Box 2: Seed morphology of sexual and asexual dandelions

In spring 2000, I collected open pollinated seed heads from a mixed population of sexual and asexual dandelions from the Odenwald, Germany. I randomly chose seed heads from 18 sexual and 20 asexual individuals, and from every seed head, 10 seeds were picked. Using a flatbed scanner and digital imaging software, I measured four morphological characters: seed length, seed width, pyramid length (top part of seed) and rostrum (part connecting seed and pappus-parachute). A Principal Components Analysis was performed on the data, Anova's were used to test the difference between sexual and asexual dandelions for the first two PCA-axes.



First two axes of a Principal Components Analysis of seed morphology of sexual (white diamonds) and asexual (black squares) dandelions. Error bars represent standard deviations.

Seeds produced by sexual and asexual dandelions were different for both the first PCA axis ($F_{1,36} = 10.2$, $p = 0.003$), representing size, and the second PCA axis ($F_{1,36} = 13.8$, $p = 0.001$), representing shape. The differences indicate that seeds produced by sexuals were smaller and more slender than those produced by asexuals. This is different than expected from the better colonising capabilities of the asexuals, as small, light seeds aid dispersal and large seeds provide seedlings with a competitive advantage, in which case the seeds of the asexuals should have been smaller than those of the sexuals.

explained using interactions with coevolving parasites that give sexuals a benefit over asexuals through their production of polymorphic offspring. Though the influence of parasites has been shown to be important for the maintenance of sex in another diploid/triploid-sexual/asexual complex, the freshwater snail *Potamopyrgus antipodarum*, no work has so far been done on Red Queen dynamics in dandelions. The observed preference of sexual dandelions for relatively undisturbed habitats could be explained with the Red Queen if the parasites were mainly present in such habitats. This could be the case in the above metapopulation system of temporarily available habitat patches. If the dispersal of parasites is slower than that of the hosts (possibly because most parasites do not have parachutes), the asexual dandelions will outcompete the sexuals in the patch until the parasite arrives. After the arrival of the parasite, the sexuals could have a higher fitness due to Red Queen mechanisms. This is an interesting idea that has not gained much attention in the literature (but see Johnson, 2000), and might be worth studying. A problem with this hypothesis for dandelions is that I also found an association of sexuals with relatively stable spots *within* a single field (Chapter 5), a pattern that could not be accounted for under this metapopulation scenario.

In general, not enough is known about parasites in dandelions to conclude that parasite-driven Red Queen dynamics are likely in dandelions. However, the mechanism of the Red Queen model does not only work with parasites, but also with other coevolving antagonists (Bell, 1982). Interspecific competition could be one kind of such an antagonistic interaction, though as far as I am aware the effect of interspecific competition on the maintenance of sex has not been studied in detail. Importantly, high clonal diversity might not be problematic under this type of interaction, since interspecific competition with a multitude of species could involve a large number of “interaction” loci. With more loci, the contrast between the number of combinations that is possible under sexual reproduction and the number of combinations present in a population of asexuals become more pronounced. Problematic with these types of antagonists, however, is whether coevolution would be tight enough for sex to be advantageous.

The third explanation may be that the observed niche differentiation between sexual and asexual dandelions is caused by the difference in ploidy-level between the two reproductive types. Polyploidy is in general known to have large effects on morphology and ecology of polyploids relative to their diploid ancestors (Ramsey, Schemske, 2002). Also for dandelions, polyploidy has been shown to have effects on certain morphological characters (De Kovel, 2001). Niche differentiation has been found between diploids and polyploids in many polyploid complexes (Petit *et al.*, 1999), though those differences might have evolved after the polyploidization events and it is difficult to untangle the effects of polyploidy itself. One advantage of seeing niche differentiation between sexual and asexual dandelions as a direct effect of polyploidy is that it would solve problems connected to the high rate of gene flow between the two types. This high rate of gene flow leads to a constant creation of new clonal lineages, which leads to high clonal diversity

under which most models explaining the maintenance of sex may not work. Furthermore, gene flow limits the extent to which a genetically based ecological difference can evolve between the sexuals and the asexuals; this is not the case with ecological differences that are the result of polyploidy.

In conclusion, I think it is most likely that the observed niche differentiation is a direct effect of polyploidy itself. This explanation has the least difficulties; it is still applicable given the high rate of gene flow and the resulting high clonal diversity. Alternatively, Red Queen processes, either driven by parasites in a metapopulation system or by interspecific competition, may also explain the observed niche differentiation, however it is not well enough known whether these processes actually work as suggested.

More differences between sexual and asexual dandelions

Although the nature of the observed niche differentiation was consistent among the studies included in this thesis, the difference was also consistently weak. In most cases, only a small part of the variation in mode of reproduction was explained by the ecological variables measured. This low explanatory power could be the effect of large sampling errors inherent in the analysis of ecological variables, in particular using vegetation relevés. Alternatively, the low explanatory power may be due to the fact that there are other ecological factors that allow coexistence between sexual and asexual dandelions.

Several studies have shown that there are indeed other factors of importance for the distribution of sexual and asexual dandelions, on small as well as on and large geographic scales. Verduijn *et al.* (2004a) showed that within a dandelion population from the Netherlands, the sexuals preferred slightly warmer habitats than the asexuals. This preference had previously been suggested based on the geographic distribution of sexuals in the Netherlands; in an extensive survey, sexuals were only found in thermophilic sites (Roetman *et al.*, 1988). Also the overall geographic distribution of sexuals and asexuals in Europe, with sexuals becoming increasingly rare with increasing latitude (Den Nijs, Sterk, 1980; Den Nijs *et al.*, 1990), suggests an other explanation than a colonisation/competition trade-off: there is no obvious North-South trend in the amount of disturbance. Of course these patterns may have a similar origin than the pattern observed in my study: polyploidy itself could make the asexuals better resistant to cold (Bierzychudek, 1987), or Red Queen processes could be more important in warmer areas, where parasites may be more abundant (Bell, 1982).

Clonal diversity

High rate of gene flow leads to high clonal diversity

There is a high rate of gene flow between the sexual and asexual dandelions. I found, using different kinds of molecular markers, that almost all genetic variation was shared

between the sexuals and asexuals (Chapters 3, 4 & 7), confirming the results from Menken *et al.* (1995). The influence of this gene flow on the genetic population structure of dandelions is striking: sexuals and asexuals that grow close together within a population are more similar than sexuals and asexuals growing further apart (Chapter 3). The high rate of gene flow also leads to a high level of clonal diversity (Chapters 4, 5 & 7). Such high levels had previously been found for strictly clonal populations (Van der Hulst *et al.*, 2000; Van der Hulst *et al.*, 2003).

The gene flow between sexual and asexual dandelions almost certainly takes place through pollinations of sexuals with pollen produced by asexuals. Verduijn *et al.* (2004b) showed that in a mixed population about two percent of the seeds produced by sexuals were polyploid, and thus most likely fathered by asexuals. Although this number is relatively small, it indicates that an extremely large number of new clones is created annually in a large mixed dandelion population; such populations usually contain hundreds of thousands of sexuals that all produce several capitula with hundreds of seeds. However, many of the new clones may have problems with getting themselves established in the population; it has previously been found that newly created clones have lower fitness than already established clones (De Kovel, De Jong, 2000).

The high clonal diversity in dandelions may also partly be maintained by Frozen Niche Variation (Vrijenhoek, 1979; Vrijenhoek, 1989). Ecological differences between clones have been found previously by Solbrig and Simpson (1974), and was also indicated in my study: clone frequencies changed over a 14 year period of ecological change in the Odenwald population (Chapter 4). This change in clone frequencies could not be explained by drift, and therefore is likely to have been the result of differential selection on the different clones. A more intensive long term monitoring of clone frequencies in a population is however required to find out more about the dynamics of the clones.

Clone concepts

As explained previously (Chapter 1), estimations of clonal diversity are important in any study on the maintenance of sex and the coexistence of sexuals and asexuals in general. In order to be able to compare across studies, it is important to have a common idea and use of what one understands under a “clone”, as well as universal, unbiased, statistical methods for estimating clonal diversity (Chapter 6). During my work, I realized that different scientists use the term often in different ways, which might result in unnecessary confusion. A definition of the word “clone” as found in dictionaries usually involves the idea of genetic identity between the members of a clone. If genetic identity however is used as a basis for a clone concept, the concept will turn out to be biologically unrealistic and unpractical (Loxdale, Lushai, 2003b).

Genetic identity is biologically unrealistic as two different individuals in any eukaryote species will never be completely identical. The genome size of eukaryotes is simply too large for this, despite the low error-rate of DNA-replication. In species with large individuals, cells *within* an individual are usually not all identical (Ainsworth,

2003), so a clone concept based on genetic identity *between* individuals is untenable. In addition, there is also a more practical problem connected to the use of a clone concept based on complete identity: the ability to distinguish clones depends too much on the marker system that is used to distinguish them. Highly variable DNA markers, such as microsatellites, AFLP, SSCP, ISSR and RAPD are more likely to uncover small differences between individuals, but they are also more prone to errors. PCR artefacts and scoring errors can cause small differences between members of the same clone (or between different amplifications performed on the same individual), and under a strict clone concept these will be different clones, which will inflate estimates of clonal diversity (Chapter 6).

At the risk of sounding overambitious, I would like to suggest a clone concept based on evolutionary history, which I think is more realistic. The proposed concept may seem obvious to some people who are already using it, but I have also noticed that others are vehemently against it. I simply define a clone as a collection of individuals belonging to an evolutionary lineage that has not engaged in sex. The “birth” of a clone under this concept would therefore be the last sexual event before clonal reproduction started. This concept has the advantage that it is equally applicable to parthenogenetic organisms and organisms with vegetative reproduction, and allows members of a clone to differ genetically (Mes *et al.*, 2002). Such genetic differences may even be apparent in relatively young clones when markers with a high mutation rate, such as microsatellites, are used (Chapters 4 & 7).

A practical advantage of the proposed concept is that clonal diversity is not an ever-increasing function of the number of genetic marker loci used. When using a clone concept based on strict identity, the problem is that adding loci would increase the number within-lineage differences found, as well as the number of errors and artefacts. Therefore, the number of clones will increase with every added locus, until in the end every individual is assigned to a different clone. There is no such problem under the proposed clone concept. When using a low number of loci, the variation may not be enough to distinguish all clones present, but adding loci will increase resolution. Ultimately, the resolution should be high enough and adding more loci should not lead to the identification of more clones. This idea provides the background for the computer program I wrote (Chapter 6), which assists in assigning individuals to clones.

Under the proposed concept, some of the “ancient asexuals” (Judson, Normark, 1996), such as bdelloid rotifers, could become single clones. This might be counterintuitive to many since these asexual organisms are morphologically diverse and commonly divided into a number of different species. To me, this would not necessary mean a drawback of my suggested clone concept, but rather an example of the evolutionary potential of clones - despite their asexual mode of reproduction (see also Loxdale, Lushai, 2003b).

Synthesis

The paradox of sex as it was originally described is in drastic need of redefinition. Not only is the presence of sexual reproduction not paradoxical in the absence of asexual reproduction, also the size of the paradox can wildly differ between species in which both sexuals and asexuals are present. Combining data from different sources, I found that although there is indeed a cost of sex in dandelions, and thus a paradox of sex, this cost is relatively low. As far as I am aware, dandelions are the first species for which the realised cost of sex has been described with such detail. The low cost of sex in dandelions means that the explanation for the coexistence of sexuals and asexuals does not have to be very powerful. Therefore, the ecological differentiation between the sexual and asexual dandelions that I found, even though it is weak, may already explain their coexistence to a large extent. As other factors have previously been shown to be important for the distribution of sexual and asexual dandelions, the sexuals may have ample opportunities to overcome the reproductive advantage of the asexuals. I repeatedly found that asexual dandelions prefer more disturbed habitats than sexuals, indicating that asexual dandelions are better at colonisation and sexuals are better at competition. The origin of this difference is not known, but it is possibly caused by the difference in ploidy level between the two reproductive types. Alternatively, the niche differentiation could result from Red Queen processes.

The genetic studies show that the amount of gene flow between the sexual and asexual dandelions is very high. This gene flow has an important effect on the genetic population structure of sexual and asexual dandelions and thus influences the adaptability of both reproductive types, giving the asexuals large evolutionary potential. The gene flow between sexuals and asexuals however also puts a relatively low upper limit on the evolution of genetically based ecological differences between the types. The constant creation of new clonal lineages that is the result of the gene flow is the main cause of the very high clonal diversity that is found in dandelions. As clonal diversity is an essential parameter in studies of asexual organisms, it is important to have a commonly employed concept of what constitutes a clone and universal methods to estimate clonal diversity. I therefore suggested a clone concept, which is not based on genetic identity but rather on asexual reproduction of evolutionary lineages. I also described methods to analyze clonal diversity based on this concept.

The low cost of sex and the high rate of gene flow between sexual and asexual dandelions are important issues to keep in mind for future research on the reproductive systems in dandelions. On the other hand, they can also be seen as the major strength of dandelions as a model system for studies on sex. This thesis only looked at dandelions of section *Ruderalia*, most likely dandelions from other sections will differ in the amount of sex taking place and thus in the clonal diversity of the asexuals in that section. From an evolutionary perspective, it may be worthwhile to study a wider taxonomic range of dandelions, to look for differences in clonal diversity, ecological diversity and rates of

sex and recombination. This may reveal whether it is a coincidence that the most widely spread section of *Taraxacum*, sect *Ruderalia*, is one of the few sections in which both sexuals and asexuals are present.

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Curriculum Vitae

Patrick Gerardus Meirmans was born on January 27, 1974 in the small town of Beinsdorp in the Haarlemmermeer. When he was four years old, he moved to the nearby village of Nieuw-Vennep, where he lived for the remainder of his youth. From 1986 to 1992 he biked every day to his high school in Lisse (Gymnasium, Fioretti college). After graduating from high school in 1992, he started his study of Biology at the Universiteit of Amsterdam. In 1997 he stepped into the office of Hans den Nijs, looking for a Master project somewhere abroad, preferably in Europe. Hans suggested him to go to Neuchâtel, Switzerland, for half a year to do research on diploid sexual and triploid asexual dandelions under the supervision of Francois Felber, Francois Bretagnolle and Francois Calame. He finished his study of Biology in 1998, six years to the day after he had started it. During and after his graduation, he worked outside of Biology for a year as an editorial assistant for the news service of Het Financieele Dagblad. In 1999, he started his PhD-research on sexual and asexual dandelions at the Universiteit van Amsterdam under supervision of Hans den Nijs and Steph Menken, later joined by Peter van Tienderen. Since November 2004, he is working as a postdoctoral fellow at the Canadian Forest Service in Quebec City.



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