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Microevolutionary processes in apomictic genus *Taraxacum*.

Ph.D. Thesis

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ABSTRAKT

Rod *Taraxacum* představuje druhově velmi početnou a morfologicky variabilní skupinu vytrvalých rostlin se složitými evolučními vztahy. Tato komplexnost je způsobena retikulární evolucí zahrnující diploidní (sexuální) a polyploidní (apomiktické) druhy. V rodě převažuje obligátní asexuální rozmnožování, ačkoli některé sexuální druhy mají rovněž široký areál rozšíření. Ze své povahy mají apomiktické druhy (jedinci) jen velice omezené možnosti k získaní variability.

V předložené práci jsem se zabýval studiem genetické variability apomiktických a sexuálních druhů s cílem popsat procesy probíhající v populacích apomiktických klonů. Za populaci apomiktického klonu je považována morfologicky homogenní jednotka rozlišována v taxonomii tohoto rodu na úrovni tzv. apomiktického mikrodruhu. V práci byly použity jednak v součastnosti známe (vědecky popsané druhy), a součastně výrazné (morfologicky stabilní) morfotypy, které dosud validně popsány nebyly, a to ze dvou sekcí *T.* sect. *Taraxacum* a *T.* sect. *Erythrosperma*. Pro získání co největšího množství informací pro vysvětlení procesů, které mohly vést k pozorované struktuře klonů, byly použity tři typy molekulárních markerů – SSR, AFLP, cpDNA.

Výsledky mé práce ukazují, že apomiktické klony pampelišek jsou geneticky vysoce homogenní, přestože nejsou výlučně tvořeny pouze jediným klonem. Populace apomiktických klonů jsou tvořeny i) klonálními genotypy, ii) odvozenými klonálními genotypy (lišící se nahromaděnými somatickými mutacemi) a iii) klonálními liniemi. Klonální linie pocházejí z hybridizace mezi asexuálními a sexuálními jedinci. Klony apomiktických mikrodruhů jsou geneticky dobře charakterizovatelné a navzájem vyhraněné. V oblastech, kde apomiktičtí a sexuální jedinci tvoří smíšené populace, dochází k hybridizaci a tím ke vzniku nových apomiktických klonů. Opakovaná hybridizace nově vzniklých apomiktických klonů může následně vést k vytvoření nového apomiktického mikrodruhu, pokud se klon dokáže rozšířit na větší vzdálenosti. Hybridizace je zde interpretována a chápána jako proces zvyšující variabilitu v populacích apomiktů, který umožňuje získat nové vlastnosti a tak přispět k pozitivní selekci daných taxonů. S přispěním experimentálních dat z této dizertace byla potvrzena genetická homogenita jednoho dříve rozlišovaného a vzácnějšího morfotypu – *Taraxacum pudicum*, který byl proto popsán jako nový druh pro vědu.

Klíčová slova: *Taraxacum*, apomixie, klon, variabilita, mutace, hybridizace
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ABSTRACT

Taraxacum represents abundant and widespread genus of perennial herbs well known for its high evolutional and morphological complexity. This is caused by reticulate evolution including diploid (sexual) and polyploid (apomictic) species. Obligate apomixis is prevalent in the genus and sexually reproducing species with wide distribution are known for only some of the groups. Thus in general, apomictic taxa have restricted sources for acquiring of any variability.

In this thesis, I have investigated genetic variability of apomictic and also sexual taxa with the aim to describe the processes ongoing on the level of populations of apomictic clones. Population of apomictic clone represents here a morphological unit, traditionally recognized as microspecies in the taxonomy of dandelions. I used scientifically recognized microspecies as well as undescribed morphotypes (stable in their morphology) from two sections: *T.* sect. *Taraxacum* and *T.* sect. *Erythrosperma*. Three different molecular markers (SSR, AFLP, cpDNA) were used to gain all the possible information on processes leading to the observed genetic structure of the clones.

Results of my work showed that apomictic clones of dandelions are genetically highly homogenous, although not always uniclonal. Populations of apomictic clones consist of i) clonal genotypes, ii) clonal mates with accumulated somatic mutations and iii) clonal lineages resulting from hybridisation events among apomicts and sexuals. Apomictic clones—microspecies—are genetically well separated from each other with specific fingerprint. In regions with sympatric occurrence of apomicts and sexuals, new apomictic clones are formed in hybridisation process. Recurrent hybridisation of neo-apomictic clones may lead to formation of new apomictic microspecies, which after successful spreading may become widespread. Hybridisation is seen also as process for increasing the variability and possibility to gain a new positively selected trait. Results of this thesis contributed also to scientific description of one new apomictic microspecies of dandelions – *Taraxacum pudicum*, which was found to be genetically uniform and thus of a clonal origin.

Key words: *Taraxacum*, apomixis, clone, variability, mutation, hybridisation
Number of pages: 142
Language: English

DECLARATION

I hereby declare that this thesis has been worked out by myself together with listed coauthors. All literary sources cited in this thesis are listed in the References section.

AUTHOR CONTRIBUTIONS

CHAPTER 1   Introduction
ĽM wrote the text.

CHAPTER 2   The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts
Published in PLoS ONE.
RJV and ĽM conceived and designed the experiments. ĽM performed the experiments and analyzed the data. ĽM, BT, MK and RJV contributed reagents/material/analysis tools. ĽM and RJV wrote the paper. BT and MK edited manuscript. BT phenotyped the plant material. MK – contributed to optimalization of AFLP.

CHAPTER 3   Genotypic variability of obligate apomicts is enriched by the gene pool of sexuals in contact zones between sexual-apomictic dandelions (*Taraxacum* sect. *Erythrosperma*)
BMC Evolutionary biology (submitted).
RJV conceived the idea of the study, and RJV and ĽM designed the study. Plant material was collected by RJV and ĽM or was kindly provided by colleagues, RJV phenotyped the plant material. ĽM did the molecular work and performed the statistical analyses. MK contributed to AFLP analyses and helped with the lab work. ĽM wrote the first draft, and MK and RJV improved subsequent versions of the manuscript. All authors read and approved the final manuscript.

CHAPTER 4   *Taraxacum pudicum,* a new apomictic microspecies of the section *Erythrosperma* from Central Europe
Manuscript prepared to be submitted to Preslia.
RJV discovered the described species. RJV did comparative cultivation, flow-cytometric analyses, counted chromosome numbers, tested microsatellites for the species and made scientific description. ĽM cultivated F_1 offspring of the species and studied genetic diversity of the species, its F_1 offspring and its relatives.

Where is the place for apomictic taxa in taxonomic hierarchy of apomictic genera? Let's take a look on apomictic dandelions

Manuscript prepared to be submitted to Taxon.

ĽM and RJV conceived the idea of the review. ĽM wrote the first draft and RJV improved the subsequent version.

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CONTENTS

CHAPTER 1

General introduction

Ľuboš Majeský

Reproductive strategies in plants and their consequences

Sexuality versus asexuality

One of the main driving forces in evolution of living nature is reproduction – the ability of formation of new living entity, on which evolution can act. It is the will of living organisms to leave the offspring that will carry parental genes and so will hold the continuity of the lineage until the time when some stochastic incident will cause the disappearance of that lineage. Disappearance of lineage will delete the lineage–specific genetic constitution and throw "out of the game".

During evolution several mechanisms evolved, in which living organisms give the birth of a new individual. Basically, there are two major pathways: a sexual and an asexual one. Sexuality enables the organisms to create a new genetic constitution by process of fusion of haploid gametes coming from both partners; female and male. During reductional–meiotic division leading to formation of haploid gamete, crossing-over ensures random redistribution of parental alleles into gametes – i.e., reshuffle parental alleles. After syngamy the new genetic constitution is formed resembling parental genomes.

Instead of recombining the both male and female gemomes into a new one, plenty of organisms reproduce asexually by avoiding meiosis and syngamy. Such organisms form clonal copies of themselves. Asexual reproduction can be split into vegetative and generative; vegetative means reproduction through vegetative parts as buds, rhizomes, tillers, while generative means reproduction by spores or seeds without requirement of syngamy of male and female gametes for embryo formation (Asker & Jerling, 1992). This type is often referred as apomictic reproduction.

Several types of apomictic reproduction are recognized as: adventitious embryony – embryo arises from somatic tissues in ovule external to sexually derived megagametophyte; and gamethophytic apomixis – embryo sacs arise from unreduced initial cells either from somatic cells in the ovule—apospory, or from unreduced megaspore mother cell— diplospory (Asker & Jerling, 1992). In adventitious embryony (sporophytic apomixis) asexual reproduction coexists with normal sexual reproduction while in gametophytic apomixis sexual pathway is omitted. In apomicts, embryo is followed by parthenogenetic development – no requirement of fusion of male and female gamete – into a new maternal–like offspring (Asker & Jerling, 1992). The fate of central cell, developing into nourishing endosperm tissue, can be double: in autonomous apomicts, central cell develops without fertilization (mainly in family *Asteraceae*), however, many apomictic species require fertilization of central cell or polar nuclei to trigger endosperm formation—pseudogamy (e.g. *Hypericum*, *Rubus*, *Ranunculus*, *Panicum*, etc). Because in diplospory meisosis is malfunctioned, diplosporic apomicts tend to be obligate (Asker & Jerling, 1992).

Taxonomic distribution and origin of apomixis

Apomixis was documented in about 400 plant genera from 40 families (Carman, 1997). Apomixis is very rare among gymnosperms and only one case of apomixis is known within order *Pinales*. An unique type of apomixis – male apomixis evolved in Saharan Cypress (*Cupressus dupreziana*), an endangered species living under extreme desert conditions. This species produces embryos entirely from unreduced pollen and female provides only nutrition (Pichot et al., 2000; 2001). In angiosperms, apomixis is frequent and was documented within all major clades, with almost equally frequent occurrence among monocots and among dicots (Asker & Jerling, 1992; Carman, 1997).

The presence of apomixis among different clades and higher taxa makes obvious that apomixis evolved independently and repeatedly. However, the largest numbers of apomictic taxa are found within three families only: *Asteraceae, Poaceae* and *Rosaceae* (Richards, 2003). Within these families both types, i.e. gametophytic and sporophytic apomixis are present (Carman, 1997; Ozias-Akins & van Dijk, 2007). It can be hypothesized that within some families a predisposition for development of different type of apomixis may be present (e.g. Carman 1997).

Apomixis is a multi-step developmental pathway consisting of several different elements that are (most probably) encoded by multiple different genes (Ozias-Akins & van Dijk, 2007). Therefore, it is extremely tricky to uncover and well understand the origin of apomixis. Prevailing majority of all known apomicts of flowering plants are polyploids of putative hybrid origin. The exception from the apomixis-polyploid linkage is the genus *Boechera* with known diploid apomicts (bearing supernumerary small chromosomes, Kantama et al. 2007). These diploid apomictic *Boechera* species/lines are products of interspecific hybridisation (Schranz et al., 2005).

Carman (1997) hypothesis assume conflicting behaving of signals coming from different genetic backgrounds. Under this hypothesis, apomixis arose as the consequence of anomalies during the expression of female developmental pathway – megagamethogenesis. In genome of hybrid or polyploid individual the duplicated genes from different genetic background do not respond equally, but follow their own signals. This leads to asynchrony gene expression, which in turn may lead to skipping the „normal" developmental program and to premature formation of embryo sac. Asynchronous signals could have negative effect on newly established apomicts and probably several mutation were required to overcome these negative effects for stabilizing apomixis as a new reproduction link (Carman, 1997).

Another possibility, mutually not exclusive with Carman theory is that parthenogeny developed as mechanism preventing the fertilization of unreduced gametes in newly established polyploid hybrids, thus avoids the intolerable high polyploidisation. Apomixis may be selectively favoured in newly established polyploid hybrids to bridge their problems with decreased fertility (Whitton et al., 2008a).

Anyway, evolution towards apomixis should be very rare event because several independent mutations are required to develop more or less simultaneously, otherwise such mutations have deleterious effect on organism (van Dijk, 2003). Diplosporic apomixis consist of two/three traits controlled by separate loci: i) formation of unreduced megaspore (diplospory), ii) autonomous embryo development (parthenogeny) and iii) in autonomous apomicts also autonomous endosperm development. Mutation leading just to production of unreduced megaspore would cause the rapid increase of the ploidy level and parthenogeny

alone will lead to formation of haploid offspring. Both these processes occurring separately would be very disadvantageous and ultimately will lead to strong selection against them

In the light of above statement, Noyes (2008) obtained interesting results. He observed that single mutation in the gene *SWI* (called *DYAD*) in *Arabidopsis* mutants disrupt normal meiosis, what causes formation of functional unreduced egg cell that can be fertilized by haploid pollen and results in triploid progeny. This is a first step towards the complex apomictic pathway, which makes the "mutational" origin of apomixis probable.

All apomicts within seed plants are considered to be of young evolutionary origin, the youngest formed during Pleistocene (Asker & Jerling, 1992; Hörandl, 2006). After retreat of the ice sheat spatially separated species come into contact, what led to a massive hybridisation between them and formation of hybrid swarms. In such newly established genomes disturbances in expression of developmental pathways and asynchrony in expression of duplicated genes may lead to establishment of apomixis (Carman, 1997).

Coexistence and maintenance of sex in apomictic complexes

Maintenance of sexual reproduction in nature and its overdominance is quite surprising especially when compared with possibilities coming from asexual reproduction. Evolutionary biologists often mention the "cost of sex" as the key disadvantage of sexually reproducing species (Maynard–Smith, 1978). This cost paid by sexuals refers to the fact that sexually reproducing females produce males and females while asexual females produce only females in twice quantity than sexual. Therefore, asexual population has higher growth rate than sexual population (Maynard–Smith, 1978). It seems to be apparent that maintenance of male function is a disadvantageous and sources used for keeping the male function could be utilized more effectively. Especially this paradox is more obvious in asexuals with autonomous apomixis, while they do not need pollen they still produce it. Mutations leading to male sterility will have no negative effect on seed production and should increase the reproductive potential (van Dijk, 2003). However, keeping the male function in apomicts make sense for formation of new clones formation (Mogie, 1992). Only apomicts that produce viable pollen grains may cross with sexuals and establish new apomictic clones/lineages in the case that pollen grain will carry all genes for apomixis. Keeping the male function has also another advantage for apomicts, which is purging mutation load acquired during the asexual life history of particular clone. Accumulation of mutations is considered to be one of the main penalties coming from the asexuality (Kondrashov, 1982). Because of meiosis is malfunction apomicts cannot purge deleterious mutations, which are accumulated within clonal lineage. This may lead ultimately to extinction of clone. In sexuals, the deleterious mutations have lower chance to be transferred into next generations, because of selection on haploid gametes (Mable & Otto, 1998). Another consequence of apomixis is the reduced variability; e.g. *Taraxacum hollandicum* (Battjes et al., 1992), *Taraxacum albidum* (Menken & Morita, 1989), *Rubus alceifolius* in introduced areas (Amsellem et al., 2001), which may influence survival of clone in temporally fluctuating natural conditions. Apomicts with "trapped" genetic diversity are considered to be highly adapted to particular conditions, but are less flexible in adaptation to changing environment (in comparison with sexuals). For these reasons, apomicts are often considered to be evolutionary *dead ends* with little or no evolutionary potential

(Asker & Jerling, 1992; van Dijk, 2003). This reflects also a placement of apomictic lineages on the terminal branches of phylogenetic tree (Neiman et al. 2010).

Besides the above discussed disadvantages of apomixis, there are also advantages of apomixis, which gave at least theoretical power to asexuals to exceed sexuals under certain conditions. These advantages may balance disadvantages of clonality. Because the meiosis is skipped in asexuals and the whole maternal genome is transferred into new generation, there is no threat of breaking down potentially favourable genetic constitution. Conservation of genotype may bring benefits in stable environments, while in variable environments it may result in several disadvantages (Vepsäläinen & Järvinen, 1979; Beck & Agrawal, 2010). Avoidance of meiosis in apomicts keeps fixed heterozygosity, while in sexuals heterozygosity is broken down in haploid gametes (Richards, 2003; Meirmans Ph.D. thesis, 2005). Because apomicts do not need mating partner for offspring production, they may succeed in extreme conditions, which would act on others as eliminative. This independence on mating partner makes form apomicts good colonizers (Dupont, 2002; van Dijk, 2003; Hörandl, 2008) able to colonize empty niche by one individual.

Despite of the apparent short-term advantages of apomicts, the long-term disadvantages play role in the maintenance of sex within apomictic genera. There is any 100%-asexual genus in flowering plants (Asker & Jerling 1992). Sexual reproduction is under some condition much more favourable than clonal copying. Sexual reproduction may be beneficial for organisms in ecological interaction when selection operate to preserve variable genotypes preventing an adaptation of parasites – "Red Queen" hypothesis (Hamilton 1980). Under this theory sexually reproducing species would be able to escape parasitism better than clonally reproducing species.

Another hypothesis for the maintenance of sex within asexual complexes is the "Tangled Bank" hypothesis under which sex may be advantageous in case of intensive competition for multiple resources (Bell 1982). Polymorphic genotypes (products of sexual reproduction) have better chance to succeed in competition for resources than clonal genotypes, because they can settle unoccupied niche while all members of clonal lineage compete for the same resource/niche. Beck and Agrawal (2010) showed in the experiment with rotifer *Brachionus calyciflorus*, that sex is more advantageous in heterogeneous environments.

Both reproduction pathways have their "pros and cons" and their retention is the result of specific genetic/ecological interactions. Creation of new allelic combinations, which may be beneficial and their positive selection can be the reason why sex is preserved beside asexual reproduction in nature. However, it can be disputed whether there is some difference in evolutionary potential between apomicts and sexuals, because apomicts as well as sexuals may be widely distributed – e.g sexual vs. apomictic species within genera *Rubus* (Alice & Campbell, 1999) and *Taraxacum* (Kirschner & Štěpánek 1994, 1996; Uhlemann et al. 2004), strictly endemic – e.g. *Taraxacum nigricans*, *T. alpestre* (Štěpánek et al. 2011) *Sorbus portae-bohemicae* and *Sorbus albensis* (Lepší et al., 2009), invasive – e.g. *Hieracium* subg. *Pilosella* in New Zealand (Trewick et al. 2004) or *Hypericum perforatum* in Australia (Buckley et al. 2003).

Genetic variability of apomicts

As mentioned above, apomicts have lower capability of acquiring variability and decreased variability is often considered as the drawback of apomicts. Copying of maternal genotype lead to offspring with identical genotype in apomicts. For consideration of variability within apomicts it is important to realize the hierarchical system existing within clone, between clones and within population.

Populations of apomicts are often multiclonal showing unexpectedly high diversity (van der Hulst et al. 2000) due to fixed heterozygosity, which is sometimes even equal with their sexual relatives (Menken et al. 1995). However, when looking on clone as vivid evolutionary unit within apomictic population, there are several sources from which clonal variability can be enriched.

Richards (1996) provided nice overview of possible sources of genetic variability in obligate apomicts of the genus *Taraxacum*. Although it is focused on dandelions, it can be used for all apomictic genera/species in general.

Mutational changes in DNA sequences seem to be major source of variability in apomicts. Increased variability due to accumulation of mutations was proposed to be responsible for divergence from strictly clonal genotypes within apomictic lineage in several well studied apomictic genera (species complexes); e.g. *Potentila* (Paule et al. 2011), *Ranunculus* (Paun et al., 2006a), *Taraxacum* (Mes et al. 2002).

Because male function is fully functional in majority of apomicts, apomicts can enter sexual process as pollen donors (fathers). In the case when pollen from apomict bears whole genetic constitution for apomixis, new apomictic clone is established. In such neo-apomictic clone part of the genetic variability will be derived from sexual mother and this will increase total variability of apomictic genetic pool. Described gene flow from apomicts to sexuals is possible in regions with sympatric occurrence of apomictic and their sexual relative species (van Dijk 2003, Verduijn et al. 2004).

Other sources of variability can be: i) recombinations during restitutional meiosis (van Baarlen et al. 2000); ii) somatic recombinations (Richards 1996). Recent studies showed that epigenetic changes such as the level of methylation induced by different stress factors may be heritable (Verhoeven et al. 2010a) and also de novo methylation variation may be generated in hybridisation process (Verhoeven et al., 2010b). Thus also epigenetical variation can contribute to the total variability in apomictic species.

Genus *Taraxacum* WIGG.

Distribution, reproduction and taxonomy

The genus *Taraxacum* WIGG. ranks to the order *Asterales* LINK, family *Asteraceae* BERCHT. & J. PRESL and subfamily *Cichorioidae* CHEVALLIER (Stevens, 2001). The genus consists of more than 2500 perennial herbs with a worldwide distribution spanning both hemispheres (Kirschner & Štěpánek, 1994). Species of the genus grow in range of habitats, from anthropogenic disturbed to evolutionary old biotopes, through dry steppes to marshes, from subtropics to alpine and arctic biotopes. The proposed evolutionary centre of the genus are mountains of the Central Asia and the Middle East—many sexual species with primitive morphological characters are native to these areas—from where the genus gradually expand to the rest of the world (Richards 1973).

Species of *Taraxacum* form polyploid series; apomicts are mostly triploids and tetraploids, whereas sexuals are mostly confined to diploids (Kirschner & Štěpánek 1996, Vašut 2003). Strong reticular evolution was observed within both sexuals and apomicts, which considerably complicates a study of the genus phylogeny (Wittzell 1999, Kirschner et al. 2003, Záveská et al. 2009).

Taxonomic treatment of the genus is based on the grouping of morphologically similar (and also evolutionary related) species into sections. Section consists of one or more sexual species and a clump of apomictic polyploids, traditionally recognized as microspecies. Polyploid apomictic dandelions are either of an autopolyploid origin or a result of hybridisation (Kirschner & Štěpánek 1996). To date, there are about 55-60 recognized and accepted sections within the genus *Taraxacum* (Kirschner & Štěpánek 1997, 2004, 2008, Uhlemann et al. 2004).

Apomixis within the genus *Taraxacum*

Apomixis in dandelions is a type of meiotic diplospory referred as *Taraxacum*-type (Asker & Jerling, 1992) and is considered to be obligate (van Dijk, 2003). However, Małecka (1971; 1973) reported deviation from the obligatory apomixis within the section *Palustria*. In *Taraxacum*, three dominant loci are involved in the regulation of apomixis (Tas & Van Dijk, 1999; R.J. Vašut, unpubl. results), two of them are already known, i.e. *DIPLOSPOROUS* (*DIP*) and *PARTHENOGENESIS* (*PAR*) (Vijverberg et al., 2004; 2010; van Dijk et al., 2009) loci. The Diplospory (*Ddd*; *DIP*) is inherited as a dominant trait having at least two genes playing the role of enhancers or *cis*-regulatory elements (Vijverberg et al., 2004; 2010). Genetic fine-mapping revealed lack of recombination suppression in the *DIP* region (ibid.). Physical mapping positioned the *DIP* region to a distal arm of the one of the NOR chromosomes displaying differences in an amount of repeats along the *DIP* region (R.J. Vašut, unpubl. results). Apomictic genes are hypothesized to have longer asexual history than other parts of the genome through, which a considerable amount of (deleterious) mutations accumulated in a close proximity to apomictic loci (van Dijk, 2003; van Dijk et al., 2009; R.J. Vašut, unpubl. results).

Population structure

Typical population of apomictic dandelions (plants growing on lawn, meadow or pasture) comprise a set of different apomictic clones/microspecies. In such a population there are many unique and few overrepresented genotypes. Some genotypes may be derived from clonal genotypes and differ only a little bit due to accumulated somatic mutations. Such derived clonal genotypes represent the clonal mates and together with the maternal apomictic clone they form the "clonal" network (Mes et al., 2002). Members of this network have undergone only asexual reproduction from their most common recent ancestor. However, such view is more realistic when we are looking on the population as a set of individuals of one apomictic clone – microspecies (as done e.g. in Mes et al., 2002).

Except mutational derived genotypes, populations consist of genotypes showing larger differences, which cannot be satisfactorily explained by accumulation of mutations. These genotypes are derived from hybridisation event(s) (e.g. van der Hulst, 2000).

Both processes – accumulation of mutations and hybridisation – were observed and confirmed in apomictic and mixed populations of dandelions (e.g. Menken et al., 1995; van der Hulst et al., 2003; Mes et al., 2002). In sympatric populations where both reproduction types coexist, gene flow between apomicts and sexuals is probable (Menken et al., 1995; Meirmans et al., 2003; Verduijn et al., 2004), but in fully asexual populations, occurring behind the range of sexual diploids, the gene flow is unlikely to occur. Thus, the genotypic diversity in these regions that can not be explained by the accumulation of mutations may represent an ancestral polymorphism (Mes et al., 2002) or represent migrants over large distances from regions where the gene flow is possible (van Dijk et al., 2009). Dandelion seeds – achenes are well suited for wind dispersal, although the simulation study dealing with dispersal ability stated that majority of seeds fall in a close proximity to the mother plant – 10 m (Tackenberg et al., 2003). However, long distance seed dispersal is likely (Tackenberg et al., 2003) and the presence of clonal genotypes among particular microspecies across large distances can be a result of an effective dispersal mechanism (Menken et al., 1995; van Dijk et al., 2009).

Why to study dandelions?

Apomictic dandelions have been under the intensive study from the begging of the last century on the different levels: cytological, population, genetic, ecological, and taxonomic. However, there still remain unanswered questions related to: evolutionary history of the genus, appearance of apomixis and coexistence of the different reproductive systems.

The genus *Taraxacum* provides a good system for the study of the evolution of apomixis and maintenance of sex in mixed populations, because of: i) nearly obligate apomixis in the genus, ii) cytotype (and partially also spatially) separated reproduction types, iii) intersectional and intercytotype crossability, iv) self–incompatibility of diploid sexuals. However, the genus has also several restrictions – strong reticulated evolution and great phenotypic plasticity, what makes the problem in considering species entity and searching for clear plesiomorphies and synapomorphies in molecular genetics suitable for the reconstruction of relevant phylogeny of the genus.

Knowledge of the population structure, evolutionary potential and behavior of apomictic genes/individuals in a complex natural environment will greatly enrich understanding of the nature, coexistence of diverse reproductive pathways and their mission in evolution. Detection of genes involved in the regulation of apomixis may help in breeding programmes to creation of the apomictic economically important plants from related families.

Aims of the Thesis

The main aim of this thesis was to try to understand and describe the microevolutionary processes ongoing in apomictic and mixed apomictic-sexual populations of dandelions with emphasis on: heterogeneity/homogeneity of apomictic clones, degree of variability in apomictic clones, origin of variability within and among apomictic clones, structure of apomictic clone and possible links between apomictic clones and sexuals. Populations of apomictic clones were conceived as a set of individuals selected on the basis of phenotype – morphological criteria and represent scientifically described microspecies and also not described microspecies. For apomictic clone I consider all individuals that reproduced apomicticaly since their last common ancestor. Thus variation among members of such clone should not be of sexual origin.

To meet the aims of the thesis I used several well established molecular techniques, which are described in details in particular chapters. Only Chapter 5 represents summary overview of obtained results, their comparison with similar studies of different apomictic genera and their implications for the taxonomy of apomicts.

The thesis consists of the following parts:

CHAPTER 2

The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts

This chapter describes and evaluates the amount, sources and pattern of distribution of a molecular diversity in apomictic accessions. The possible origin of apomictic clones is discussed.

CHAPTER 3

Genotypic variability of obligate apomicts is enriched by the gene pool of sexuals in contact zones between sexual-apomictic dandelions (*Taraxacum* sect. *Erythrosperma*)

This part brings evidence about the gene flow and formation of neo-apomictic clones in hybridisation between the well established local apomictic clones and widespread sexuals in mixed populations.

CHAPTER 4

Taraxacum pudicum **sp. nova, a new species of the** *Taraxacum scanicum* **group (sect.** *Erythrosperma***)**

Chapter four provides scientific description of the new apomictic microspecies from the group of lesser dandelions – *T.* sect. *Erythrosperma*. The new microspecies is described based on well characterized morphology and ecology as well as based on molecular evidence.

CHAPTER 5

Where is the place for apomictic taxa in taxonomic hierarchy of apomictic genera? Let's take a look on apomictic dandelions

The last part provides an overview of taxonomic treatments of apomictic taxa in different plant genera with frequent occurrence of apomixis. Several aspects of apomictic life together with often used concepts of species are discussed. Second part of chapter is focused on the genus *Taraxacum* and represents overview of the literature about evolution and taxonomy. The results presented in this Ph.D. thesis were used to support present taxonomic treatment of the genus *Taraxacum*.

CHAPTER 2

The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts

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Abstract

Dandelions (genus *Taraxacum*) comprise a group of sexual diploids and apomictic polyploids with a complicated reticular evolution. Apomixis (clonal reproduction through seeds) in this genus is considered to be obligate, and therefore represent a good model for studying the role of asexual reproduction in microevolutionary processes of apomictic genera. In our study, a total of 187 apomictic individuals composing a set of nine microspecies (sampled across wide geographic area in Europe) were genotyped for six microsatellite loci and for 162 amplified fragment length polymorphism (AFLP) markers. Our results indicated that significant genetic similarity existed within accessions with low numbers of genotypes. Genotypic variability was high among accessions but low within accessions. Clustering methods discriminated individuals into nine groups corresponding to their phenotypes. Furthermore, two groups of apomictic genotypes were observed, which suggests that they had different asexual histories. A matrix compatibility test suggests that most of the variability within accession groups was mutational in origin. However, the presence of recombination was also detected. The accumulation of mutations in asexual clones leads to the establishment of a network of clone mates. However, this study suggests that the clones primarily originated from the hybridisation between sexual and apomicts.

Introduction

Asexual reproduction through seeds (i.e., apomixis) occurs in less than 1% of flowering plants (Whitton et al., 2008). Although asexual organisms are expected to be evolutionary dead ends (Maynard Smith, 1978), apomictic plants are known to occur in numerous phylogenetic groups across all flowering plants (Asker & Jerling, 1992). In some genera, such as the genus dandelion (*Taraxacum*), the widespread distribution of apomictic clones suggests that they are temporarily ecologically successful (van Dijk, 2003).Asexual reproduction thus offers a low-cost alternative to sexual reproduction. Although apomixis gives plants temporal ecological and evolutionary benefits, sexuality is generally playing the dominant role in their reproduction, a concept that is referred to as the Paradox of Sex (Bell, 1982).

The lack of recombination is expected to direct apomicts towards their extinction (Maynard Smith, 1978). The most ancient asexuals are found among bdelloid rotifers and darwinulid ostracods, which appear to have persisted for tens of millions years (Welch et al., 2009). However, the age of genes involved in the regulation of apomixis and their evolutionary origins are still unknown. The majority of plant apomicts are polyploids of putative young Pleistocene origin, with diploid sexuals as their closest relatives (Richards, 1973; Asker & Jerling, 1992; Carman, 1997). The association of polyploidy and apomixis may be a consequence of asynchronous expression of duplicate-genes controlling megagametogenesis, which causes regular meiosis to malfunction (Carman, 1997) and/or process of hybridisation and polyploidisation might favour mutation, leading to parthenogenesis to avoid sterility or loss of fitness in hybrids (Whitton et al., 2008). The spread of apomicts could occur directly through apomictically raised seeds or indirectly through pollen, when genes for apomixis are transferred into new genetic backgrounds derived from sexuals. Gene flow among apomicts and sexuals keeps apomictic genes present for long periods of time, allowing the genes to avoid mutation and thus decreasing the mutation load (van Dijk, 2003; Whitton et al., 2008; van Dijk et al., 2009).

Apomicts have significant advantages over sexuals in colonising new areas (Maynard Smith, 1978). A vast majority of asexuals occur over wide areas, e.g., *Taraxacum*, *Hieracium*, *Rubus*, and *Poa*, with significant geographic parthenogenesis in their distribution (van Dijk, 2003; Hörandl, 2006). Another advantage of apomixis is the high proportion of loci fixed in heterozygous conditions compared to that of sexuals (Paun et al., 2006a; Lo et al., 2009). In contrast, asexuality has far-reaching penalties, such as a lack of diversity, the limited possibility of acquiring heritable variability (e.g. Richards, 1996) and an increased mutation load leading to the extinction of clones (van Dijk, 2003), which give apomicts an adaptive disadvantage. However, the short-term advantages of apomixis have become of interest to the agricultural industry. Fixing the heterozygous genetic condition of plants via apomixis and revealing the nature of the genetic control of apomixis are important goals for plant breeding research (van Dijk, 2008).

The genus *Taraxacum* Wigg. (*Asteraceae*, *Cichorioideae*) consists of perennial herbs that are widely distributed throughout the world (with exception of Antarctica). The putative centre of origin is in Central Asia in a region that includes the Himalayas (Richards, 1973). Apomictic dandelions in Europe are believed to be of young evolutionary origin (Kirschner et al., 2003), with an explosive spread in the late Holocene period (Richards, 1973). Apomixis in *Taraxacum* is obligate meiotic diplospory, which is the type that is most similar to sexual

reproduction among apomixis systems (Asker & Jerling, 1992). Diplosporous plants undergo part of meiosis, in case of meiotic diplospory the anaphase II during megasporogenesis (spore formation from the Megaspore Mother Cell) is skipped resulting in development of two unreduced megaspores (unlike to sexual reproduction that results in development of four reduced megaspores). In both, sexual and diplosporous plants one of the megaspores further mitotically divides to form an embryo sac (megagametophyte). Aposporous plants differ from both above types in formation the embryo sac directly from cells of sporophyte by mitosis (i.e., meiosis is completely omitted). In dandelions, apomictic reproduction is regulated by three dominant loci (Tas & van Dijk, 1999), (Vašut, unpublished results). Two loci are already identified–*DIPLOSPOROUS* (*DIP*) and *PARTHENOGENESIS* (*PAR*) (van Dijk et al., 2003; Vijverberg et al., 2004; Vijverberg et al., 2010).

Species of *Taraxacum* form polyploid series; apomicts are mostly triploids or tetraploids, whereas sexuals are mostly confined to diploids (Kirschner & Štěpánek, 1996; Vašut, 2003). Sexual species show extensive reticulate evolution, which was detected in apomicts as well (Wittzell, 1999; Kirschner et al., 2003; Záveská et al., 2009). *Taraxacum* species are classified into morphological groups (sections) that contain one or more sexual species and polyploid clumps of apomictic accessions (traditionally either classified as microspecies or not recognised). Apomicts are either of autopolyploid origin or are the result of hybridisation (Kirschner & Štěpánek, 1996). Therefore, apomicts have the potential to reveal evolutionary processes within the genus in detail.

Fully asexual or mixed sexual apomictic *Taraxacum* populations comprise individuals with extended genotypic variability; such genotypes of these populations can be widely distributed or can exist as local clones (Menken et al., 1995; van der Hulst et al., 2000; 2003; Rogstadt et al., 2001; Meirmans et al., 2003). Sexual recombination and mutational differentiation are considered to be main sources of this variability (van der Hulst et al., 2000; 2003). Interploidy gene flow can occur within populations (Mártonfiová, 2006; Mártonfiová et al., 2007). This gene flow was suggested to be responsible for the presence of shared allozyme polymorphism and unique alleles present in populations of different cytotypes (Menken et al., 1995) and for the spatial structure of cytotype distribution (Meirmans et al., 2003). Only a few studies have examined the variability within clones or within morphologically uniform accessions (e.g. Hughes & Richards, 1988; Menken & Morita, 1989). Mes et al. (2002) used Internal Transcribed Spacer (ITS), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR, microsatellites) markers to characterise apomictic clones from sect. *Naevosa*. He stressed that individuals from a single clone with sufficiently long asexual histories may differ genetically due to mutation accumulation.

We consider that the apomictic clones are genotypes that underwent only clonal reproduction since their most recent common ancestor, and thus, genotype diversity within the clone has a detectable mutational background. There are two different approaches to the analysis of population genetics of apomictic plants (e.g. Mes et al., 2002; van der Hulst et al., 2003): i) plants are sampled randomly from the population, and ii) a plant sample is selected based on morphological criteria (e.g. Battjes et al. 1992; Reisch, 2004). In this study, we adopted the second approach and applied it to nine apomictic accessions of *Taraxacum officinale* agg. sampled in regions of sympatric occurrence of sexuals and apomicts, allowing us to study the mutation load and the formation of novel genotypes in greater detail. Morphology was the key criterion for accession assignment. In present study, we addressed

following questions: 1) What is the pattern of genotypic variability within and differentiation among apomictic clones? 2) What is the source of intraclonal and interclonal variability? 3) What is the origin of apomictic clones? 4) What is the detection ability of molecular markers? To answer these questions, we used three types of molecular markers. Microsatellites are flexible tools for population studies (e.g. Heuertz et al., 2004; Paun & Hörandl 2006; Lo et al., 2009; Symonds et al., 2010). The development of microsatellites for Taraxacum (Falque et al., 1998; Vašut et al., 2004) enabled them to be used in population studies (combined with other markers) to detect clones and to investigate population structures and gene flow (e.g. Mes et al., 2002; van der Hulst et al., 2003). Microsatellites are suitable for population genetics due to their polymorphism, high mutational rate and co-dominant nature (e.g. Goldstein & Pollock, 1997; Hardy et al., 2003). In contrast, dominant AFLP markers are firmly established as valuable tools for a wide range of evolutionary and biosystematic studies (e.g. Mráz et al., 2007; Kolarčik et al., 2010; Benediksby et al., 2011; Kitner et al., 2012). Both types of marker systems are commonly used for measuring population genetic structure and diversity, providing congruent and robust results (Mariette et al., 2001; Mariette et al., 2002a,b; Gaudeul et al., 2004; Meudt & Clarke, 2007). To compare the fingerprints acquired from nuclear markers with information that is inherited matrilineally, the trnL-trnF region in cpDNA was sequenced.

Materials and Methods

Plant Material and DNA Extraction

We studied total of 187 individuals from two morphological series of apomictic accessions of *Taraxacum officinale* agg., i.e., *T*. sect. *Taraxacum* (syn. *T*. sect. *Ruderalia*) (Kirschner & Štěpánek, 1987, 2011). The first group comprises a complex of six morphologically closely related accessions (*T. amplum* agg. – *AMP* group, each phenotyped accession denoted as *amp1– amp6*); the second one contains three morphologically divergent accessions (*OSP* group, denoted as *O*, *S*, *P*). Term ''accession'' is used in sense of morphologically homogeneous phenotypic unit. All accessions were phenotyped according to the taxonomic microspecies concept and only confirmed phenotypes were included in this study; a complete list of the individuals studied (including taxonomic identification) is provided in Table S1.

We sampled one individual per one locality across the wide geographic range of Central Europe (Figure 1). The plant material was documented by depositing herbarium specimens into herbarium of the Department of Botany, Palacký University in Olomouc, Czech Republic (OL).

Apomictic reproduction was confirmed by either emasculation or Flow Cytometric Seed Screen (FCSS) (Matzk et al., 2000). To sequence the trnL-trnF region, four sexual diploid plants were added. Ploidy levels were confirmed by flow-cytometric analysis of relative DNA content using an inner diploid control.

Genomic DNA was extracted from voucher specimens or fresh leaves, following CTAB (Cetyl Trimethyl Ammonium Bromide) protocol of Doyle & Doyle (1987) with minor modifications.
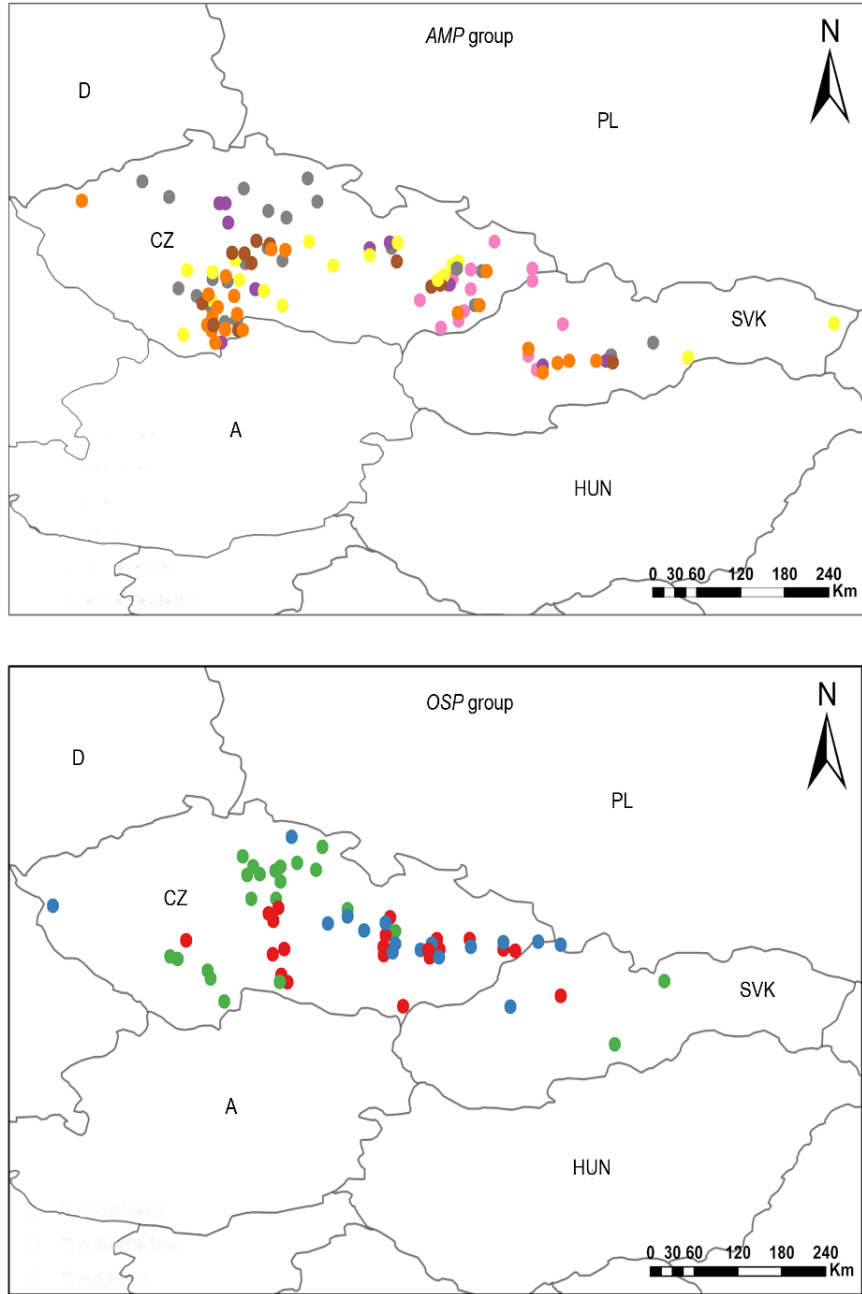
Figure 1. Geographical distribution of studied individuals of apomictic *Taraxacum* accessions. *AMP*-group: yellow circle – *amp1*, orange – *amp2*, purple – *amp3*, brown – *amp4*, pink – *amp5*, grey – *amp6*; *OSP*-group: red circle – *O*, green – *S*, blue – *P*. Country codes: A – Austria; CZ – Czech Republic; D – Germany; HU – Hungary; PL – Poland; SK – Slovakia. For taxon abbreviations see Table S1.

Microsatellite Genotyping

All 187 individuals were genotyped for six microsatellite loci: MSTA44B, MSTA53, MSTA78 (Falque et al., 1998); and MSTA93, MSTA131, MSTA133 (Vašut et al., 2004). The PCR amplifications were performed in total volume of 15 µl with 0.2 mM of each primers, 0.2 mM dNTPs, 1X PCR reaction buffer (containing 1.5 mM of MgCl2 in final volume) and 0.42 U of GoTaq DNA Polymerase (Promega).

AFLP Fingerprinting

Ninety-six individuals including all nine apomictic accessions (see Table S1) were analysed by AFLP (Amplified Fragment Length Polymorphism) for three primer combinations (*EcoRI*-AGC/*MseI*-CAAT, *EcoRI*-AAT/*MseI*-CAAC, and *EcoRI*-AGC/*MseI*-CGATG). AFLP analyses followed the protocol of Vos et al. (1995) with the modifications of Kitner et al. (2008).

cpDNA Sequencing and Sequence Alignment

The trnL-trnF region was sequenced in four samples from each apomictic accession and in the diploid sexual. A PCR reaction was performed in a total volume of 25 µl with 10 ng of template DNA, 2 mM of e and f primers (Taberlet et al., 1991), 0.2 mM dNTPs, 1X PCR reaction buffer (containing 2 mM of MgCl2) and 1 U of Pfu DNA Polymerase (Fermentas). The reaction conditions were as follows: 95°C for 2 min; 30 cycles with 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min; followed by 5 min at 72°C. The PCR products were sequenced using an Applied Biosystems 3730xL capillary sequencing system. Sequences were edited in BIOEDIT (Hall, 1999), alignment and haplotype identification was performed in MEGA 5 (Tamura et al., 2007). Sequence data are deposited in GenBank (accession numbers JQ696774-JQ696810).

Fragment Analyses of Microsatellite Genotypes and AFLP Profiles

PCR products were separated by denaturising polyacrylamide gel electrophoresis (PAGE) and visualised by silver staining. The 30–330-bp AFLP DNA ladder (Invitrogen) was used to size the microsatellite alleles. If only one allele was observed for a locus, then the individual genotype was considered to be an absolute homozygote; when two different alleles were observed, the third was coded as missing data. In the case of AFLP, the profiles were visually checked and coded as a binary matrix. To avoid the genotyping errors and to retain reproducibility of the analyses, several control levels were included during the entire study: blind samples, double samples and repetitions. This allowed estimation of the error rate, which was calculated as the difference between all markers and the markers used in the final matrix.

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Microsatellite Data Analyses

Microsatellites data were analysed as microsatellite genotypes based on the number of repeats. They were also scored using a binary matrix, where the presence/absence of a fragment of a particular size was coded as 1/0. This approach was used to perform hierarchical AMOVA with ARLEQUIN 3.5 (Excoffier & Lischer, 2010) based on pairwise genetic distances. To determine whether differences among genotypes are due to mutations or recombination, a character compatibility test was performed using module JACTAX from package PICA 4.0 (Wilkinson, 2001). Character compatibility test is based on the assumption that pairs of loci should have fully compatible variation in the absence of recombination. If the ancestral condition for a pair of binary loci is 00, under the assumption of clonality only two of the three possible character states might be expected: 01 and 11. Presence of the fourth character state (10) is evidence of incompatibility and suggests the action of recombination. Incompatibility is inferred when all four combinations of two binary states are observed within matrix. In compatibility analysis the number of incompatibilities (*MIC* – the matrix incompatibility count) between each pair of multilocus genotypes is computed. After removing of genotypes with the highest number of incompatibilities only fully compatible genotypes should be left in the matrix differing only due to mutations ($MIC = 0$) (Mes, 1998; van der Hulst et al., 2003).

From genotype data several locus and multilocus statistics were computed using SPAGEDI software (Hardy & Vekemans, 2002): the number of genotypes (*NG*), overall number of alleles, the number of different alleles (*NDA*), the means allele size (*MAS*), the range of allele size, and gene diversity (*Ge/Ge*). To characterise polymorphism and amongpopulation differentiation, locus and multilocus estimates of *F*- and *R*-statistics were calculated. The contribution of stepwise mutations (SMM) vs. nonstepwise mutations (IAM – infinite allele model) to population differentiation was tested (i.e., whether the observed R_{ST} is significantly larger than its value after permuting allele sizes among alleles within populations) (Hardy et al., 2003). P values were obtained after 999 random permutations.

AFLP Data Analyses

Basic population statistic indices such as the mean number of bands (*NB*) and the number of polymorphic bands (NPB) at the 5% level, number of private markers (*PrB*; restricted to a given population), number of diagnostic markers (*DB*; present in all individuals in a population) were calculated in FAMD (Schlüter & Harris, 2006). Polymorphism (*P*), Nei's gene diversity (*Hj*), number of different genotypes (*NG*) and genotype diversity (*GD*) were calculated using the R-script of AFLPDAT (Ehrich, 2006).

To evaluate the distribution of genotypic variation, AMOVA was performed as described for SSRs. To explore the relationship within and among groups, Principal Coordinate Analysis (PCoA) was performed in NTSYS-PC version 2.02 (Rohlf, 1998) (Jaccard similarity matrix), and inspected on a 3D plot. An unrooted neighbourjoining tree (Dice coefficient of similarity) was constructed by FREETREE (Pavlíček et al., 1999) and visualised in TREEVIEW (Page, 1996) (bootstrap support with 1,000 replications; (Felsentein, 1985). Computing of split network based on SplitDecomposition method (uncorrected P-distances,

Hamming distances), was done in SPLITSTREE 4 (Huson & Bryant, 2006; robustness tested by 1,000 bootstrap replicates).

To determine different genetic groups, Bayesian clustering approach was used as implemented in the programs STRUCTURE 2.2 (Falush et al., 2007) and BAPS 3.2 (Corander et al., 2006). The difference between STRUCTURE and BAPS is in the treatment of *K* (number of clusters). Whereas STRUCTURE uses the Markov Chain Monte Carlo (MCMC) algorithm to cluster genetically similar individuals and to estimate the likelihood of the data for different numbers of groups (*K*), in BAPS frequency of alleles and the number of genetically different groups are taken as random variables and the program estimates one optimal partitioning. Computation in STRUCTURE was set up for the recessive allele model and the admixture model with correlated allele frequencies. The *K* was set to 1–11 with 10 replicate runs for each *K* using the 1,000,000 MCMC iterations following the period of 100,000 burn-in iterations. The computation was carried out on the freely accessible Bioportal of the University of Oslo (www.bioportal.uio.no). The R-script STRUCTURE-SUM-2009 (Ehrich et al., 2007) was used to summarise the output files: calculation of similarity coefficients between replicate runs (*SC*), means of the posterior log probability [mean*L(K)*], and a quantity based on the second order rate of change of the likelihood function with respect to *K* (ΔK) (as denoted in Evanno et al., 2005). Additionally, two programs, CLUMPP (Jakobsson & Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004), were used to summarise the STRUCTURE outputs and to figure the clustering graphically. For analyses in BAPS, module "clustering of individuals" was used. *K* ranged from 1 to 11, and the analysis was repeated ten times.

Results

Microsatellites

The total number of scored alleles over six microsatellite loci was 2842 in 186 individuals. The ranges of allele sizes and allele numbers per locus are summarised in Table 1. Different levels of control (including repeated PCRs and double samples) confirm high reproducibility of microsatellites data (error rate ,1%). The number of different alleles and genotypes observed per locus was low; the range was between 8 (MSTA133) and 16 alleles (MSTA44B). Low numbers of SSR genotypes were detected with high levels of allele sharing across investigated accessions. The majority of different alleles observed per loci in all six *AMP* accessions were also present within genotypes of *amp1*. However, the genotypes were always accession specific (except for locus MSTA131, *amp2* and *amp4* shared the same genotype). Most of genotypes were clonal or differ in only one or two alleles in a few repetitions. No variability was observed among all genotyped individuals of *S*, with only one genotype detected over all of the loci. For *O*, *P*, *amp4*, two genotypes were observed for only one locus, and all other loci were without genotype variation. The rest of the species show higher genotype variability within genotyped loci. Individuals of *amp1* showed the highest genotype and allelic variability (Table 2). The overall gene diversities *Ge* and *Ge* within the dataset were high and very similar over all loci, from *Ge* = 0.81 (loci MSTA133, MSTA53) to *Ge* = 0.89 (MSTA44B) (Table 1, Table 2). However, in loci MSTA93 among *P* and for MSTA133 within *amp4*, only one allele was detected; thus, *Ge* =0 (Table 2).

The values of R_{ST} and F_{ST} were similar (Table 1, Table 3). However, the test of significance of mutational model favours IAM model and the use of F_{ST} for description of variability for multilocus and majority of locus estimates (data not shown). Nevertheless, locus estimates for MSTA133 (P<0.05) and MSTA78 (P = 0.059) within AMP+OSP and MSTA78 (P<0.01) within *OSP* suggested that some loci may undergo also stepwise mutations. As expected from the nature of the plants used in this values. In multilocus estimates, F_{IS}/R_{IS} = -0.5400/-0.5744 for the *AMP+OSP* group (Table 3). A similar situation was observed for locus estimates (Table 1). The values of R_{ST} and F_{ST} were similar, and high (P<0.05; Table 1, Table 3), suggesting that the majority of SSR diversity is present among apomictic accessions. The multilocus values for the *AMP+OSP* dataset were F_{ST}/R_{ST} = 0.3293/0.3076 (Table 3).

Table 1. Allelic diversity of six nuclear microsatellite loci for *Taraxacum*.

All populations

Locus	Allele size	K	G_e	F_{is}	F_{st}	R_{st}
MSTA131[1]	167-203	10	0.8185	-0.5908***	0.2655***	0.2983***
MSTA133[1]	260-312	8	0.8051	-0.5397***	0.2924***	0.4879***
MSTA53[2]	228-234	11	0.8122	-0.5402***	0.3070***	0.4230***
MSTA44B[2]	165-199	16	0.8933	-0.5690***	0.3065***	0.2169***
MSTA78[2]	150-182	12	0.8383	-0.5397***	0.2534***	0.4415***
MSTA93[1]	278-317	10	0.8151	-0.4108***	0.5501***	0.3385***

Allele size, size range of PCR products in number of nucleotides; K, total number of alleles; G_e, gene diversity; F_{IS}, Wright's inbreeding coefficient; F_{ST}, relative differentiation based on allele identity; R_{ST} relative differentiation based on allele size.
*** – significant value, P < 0.001. [1] (Vašut et al., 2004); [2] (Falque et al., 1998)

No monomorphic alleles were observed for loci MSTA78 and MSTA53 across the analysed samples, which should be linked the *DIP* locus. AMOVA showed that 88.4% of the variation was present among apomictic accessions and only 11.6% within accessions. When one hierarchical level is added (*AMP*, *OSP*), the pattern of the variation remains unchanged with 22.9% of variation between *AMP/OSP* groups.
A character compatibility test was applied on only *amp1*, *amp2*, *amp3*, *amp5*, *amp6*, *S*, *OSP*, and *AMP*. For others apomictic accessions, fewer than four required genotypes were present (Table 4). In *amp5* and *S*, no incompatibility (no genotypes that would be in disagreement

with fully asexual differentiation) was found, and in *amp2*, *amp3*, *amp6*, only one genotype cause matrix incompatibility; after its removal, *MIC* = 0 (Table 4). In the *OSP* group, two genotypes had to be removed to *MIC* =0. For *amp1*, 9 genotypes caused 201 incompatibilities. Investigation of the whole *AMP* group led to the deletion of 42 genotypes from a total of 46 to reach *MIC* = 0 (Table 4). This result appears to be inconsistent with only mutational differentiation and a fully asexual history of the genotypes.

Table 3. Multilocus estimates of F- and R-statistic for all six microsatellite loci for studied apomictic *Taraxacum* accessions.

	F-statistics		R-statistics	
	F_{is}	F_{st}	R_{is}	R_{st}
All Loci [*AMP+OSP*]	-0.5400***	0.3293***	-0.5744***	0.3076***
All Loci [*AMP*]	-0.5147***	0.2881***	-0.5616***	0.2132***
All Loci [*OSP*]	-0.6126***	0.3534***	-0.6706***	0.5935***

***– significant value, P < 0.001.

Table 2. Descriptive statistics for nine apomictic *Taraxacum* accessions based on six SSR loci. For taxon abbreviations see Supplemental Table 1.

	amp1						amp4						O					
N	23						12						21					
	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B
MAS	32	65,1	121,2	66	41,2	68,9	31	64	127,5	65,5	42,3	67	30,5	68,3	124	63,4	44,3	66,5
NDA	6	7	6	8	8	10	1	2	2	2	4	3	2	3	2	4	3	2
NG	9	7	7	7	7	8	1	1	1	1	2	1	1	1	1	2	1	1
G'_e	0,7582	0,7262	0,677	0,648	0,603	0,7362	0	0,5217	0,522	0,522	0,703	0,6857	0,5122	0,6774	0,512	0,693	0,677	0,5122

	amp2						amp5						S					
N	23						14						23					
	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B
MAS	30	64	120,6	67,8	41,1	65,3	31	69,6	123,4	67	43,6	68,7	28	68,7	120,3	63	42,3	72,5
NDA	5	2	4	4	3	5	4	4	4	3	4	3	3	3	2	3	3	2
NG	2	1	3	3	2	2	3	2	3	2	2	2	1	1	1	1	1	1
G'_e	0,6952	0,5111	0,553	0,56	0,532	0,6952	0,5751	0,5645	0,575	0,553	0,698	0,5416	0,6765	0,6765	0,451	0,677	0,677	0,5111

	amp3						amp6						P					
N	18						36						17					
	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B	MSTA133	MSTA131	MSTA93	MSTA78	MSTA53	MSTA44B
MAS	31,1	66,5	120,6	65,3	41,9	67	29,5	70,1	124,8	67	41,5	67,7	28,7	68,3	124	62,7	45	62,5
NDA	5	3	3	3	4	5	2	6	4	6	3	6	3	3	1	3	3	2
NG	3	2	2	1	3	2	2	4	4	5	2	3	1	1	1	1	2	1
G'_e	0,7303	0,679	0,532	0,679	0,584	0,7146	0,5066	0,5647	0,183	0,702	0,44	0,7024	0,68	0,68	0	0,68	0,544	0,5152

N, sample size; *MAS*, mean allele size in number of repetition of repeat motif; *NDA*, number of different alelles; *NG*, number of different genotypes; G'_e, gene diversity counted for loci.

AFLPs

Three primer combinations produced a total of 162 unambiguously scorable markers, of, which 129 were polymorphic. The error rate corresponds to 2%. An observation of clonality (allowed difference of three markers, calculated from error rate: 2% = 3.24) showed that most of the clonal genotypes in *O* and *S* had one AFLP phenotype among ten individuals, while the most diverse in *amp3* and *amp1* had 10 different AFLP phenotypes observed among eleven individuals (Table 5). The highest *GD* was detected (with both markers) in the *amp1* accession, and among ten AFLP phenotypes, only seven also had different SSR genotypes. In *amp3*, ten AFLP phenotypes were detected, but only four also had different SSR genotypes. The difference occurred in one or two alleles in one repetition. *Amp4* contained five AFLP phenotypes, and only one also had a different SSR genotype in one repetition.

Table 4. Character compatibility test for studied apomictic *Taraxacum* accessions.

	amp1	*amp2*	*amp3*	*amp4*	*amp5*	*amp6*	*O*	*P*	*S*	*AMP*	*OSP*
N	23	23	18	12	14	36	21	17	23	126	61
NG	15	5	8	2	8	8	2	3	4	46	9
MIC	201	5	2	-	0	16	-	-	0	743	51
E	6	4	7	-	8	7	-	-	4	4	7

N, number of samples; *NG*, number of genotypes; *MIC*, matrix incompatibility count; *E*, number of genotypes left at *MIC* = 0. For taxon abbreviations see Supplemental Table 1.

Polymorphism was low, ranging from the highest value of 19.1% for *amp1* to the lowest value of only 1.9% for *O*. For each apomictic accession, private and diagnostic markers were detected, only for amp1 no diagnostic marker was observed (Table 5). Gene diversity *Hj* was very low: $Hj = 0.007$– *O*; $Hj = 0.008$– *S* and $Hj = 0.028$– *P*. The highest gene diversity was observed for *amp1* ($Hj = 0.056$) and *amp3* ($Hj = 0.053$). The distribution of genotypic variability revealed that the majority of the variability, 86.6%, occurred among apomictic accessions, while only 13.4% occurred within accessions. The addition of one more hierarchical level (*AMP*, *OSP*) did not change the variability distribution, with 51.6% variability among apomictic accessions and 37.6% among groups (Table 6).

Table 5. Genotypic variability indices for AFLP analyses across investigated apomictic *Taraxacum* accessions.

Accession	*amp1*	*amp2*	*amp3*	*amp4*	*amp5*	*amp6*	*O*	*S*	*P*
N	11	11	11	11	11	11	10	10	10
NB	65	64	67	64	65	64	77.5	72	78
NPB	31	10	21	15	20	9	3	5	13
P (%)	19.1	6.2	13	9.3	12.3	5.6	1.9	3.1	8
PrB	6	3	6	2	8	5	10	3	12
DB	0	2	6	1	3	4	10	3	7
H_j	0.056	0.025	0.053	0.030	0.042	0.021	0.007	0.008	0.028
NG	10	3	10	5	3	3	1	1	3
GD	0.98	0.47	0.98	0.62	0.56	0.35	0.00	0.00	0.38

N, number of samples; *NB* – mean number of bands; *NPB* – number of polymorphic bands; *P* – polymorphism; *PrB* – number of private bands; *DB* – number of diagnostic bands; H_j, gene diversity; *NG*, number of genotypes; *GD*, genotype diversity. For taxon abbreviations see Supplemental Table 1.

Principal coordinate analysis clearly discriminates all studied apomictic accessions. The first two axes of the PCoA plot discriminate between the *AMP* and *OSP* groups and place all accessions into separate groups. The third axis stressed this discrimination (Figure 2). The first three axes explain 50.6% of the variability. In the neighbour-joining tree (bootstrap support in range 61–99), apomictic accessions were grouped into nine clusters (Figure 3). All accessions are placed on highly supported branches in SplitDecomposition network (goodness of fit 85.6; Figure 4) without reticular network connections between branches. The topology of the network suggests different genetic pools between *AMP* and *OSP* and a star-like structure suggests a common origin of *AMP* group. Four AFLP-*amp1* and ten AFLP-*amp4* phenotypes were placed in the centre of this structure. The Bayesian clustering method implemented in BAPS suggested optimal partition of the samples into 8 clusters (probability equals 1) (Figure 5). The division into clusters followed microspecific insertion; only amp1 was clustered with *amp2*. The result of STRUCTURE was unambiguous. The mean *L(K)* increased up to two and then flattened out. Additionally, ΔK showed a maximum value for *K* =2 (*SC* = 1). Because a high similarity coefficient (*SC* = 0.8) was also observed for *K* = 3, this clustering was inspected. For the *K* =2, STRUCTURE identified clusters corresponded with the division of the *AMP* and *OSP* groups. For *K* =3, two different clustering outcomes were gained (Figure 5).

Table 6. Distribution of molecular variance (AMOVA) compared among molecular markers and different hierarchy of studied groups of apomictic *Taraxacum* accessions.

	Marker system	Among groups (%)	Among apo accessions (%)	Within apo accessions (%)	F_{ST}*
All apomictic accessions	AFLP		86.58	13.42	0.866*
	SSR		88.44	11.56	0.884*
AMP+OSP	AFLP	37.57	51.62	10.81	0.827*
	SSR	22.85	66.91	10.24	0.867*
AMP	AFLP		78.09	21.91	0.781*
	SSR		81.02	18.98	0.810*
OSP	AFLP		92.45	7.55	0.925*
	SSR		97.83	2.17	0.978*

Values of F_{ST} correspond to the "Among apomictic accessions" differentiation; *, significant value, $P < 0.05$.

cpDNA (trnL-trnF)

The length of the final alignment was 384 bp. Three samples were discarded from the alignment (1-*amp1*, 1-*amp6*, 1-*P*) because of high background noise in the sequences. Only five haplotypes were observed within accessions (Table 7, Table S1). Two of them were accession specific (cp*1b* for *amp2* and cp*3* for *P*), while two appeared only once and were individual specific (cp*1c* and cp*2* within *amp1*). The most common haplotype, cp*1a*, was shared among the rest of the accessions and diploids. The differences between each haplotype and the most common haplotype, cp*1a*, were as follows: substitution for cp*1b*, one deletion; for cp*1c*, nine substitutions and two deletions; and for cp*3*, the insertion of 9 bp, six substitutions, and one deletion.
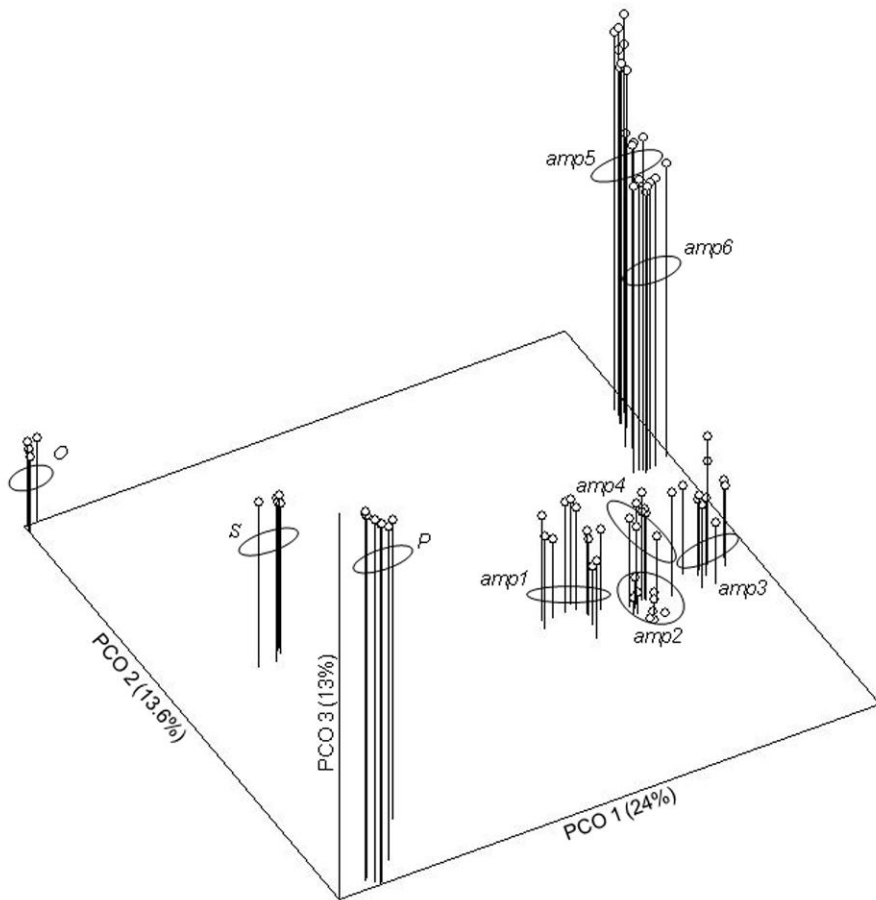
Figure 2. PCoA 3D plot. Principal coordinate analysis (based on Jaccard's similarity coefficient) of 96 apomictic *Taraxacum* individuals. For taxon abbreviations see Table S1.

Table 7. Comparison of cpDNA haplotypes (*trnL-trnF*) in apomictic *Taraxacum* accessions.

haplotype	064	097	098	099	100	101	102	103	104	105	106	160	337	338	349	353	354	360	362	364	370	377	381	accession presence
cp*1a*	A	*	*	*	*	*	*	*	*	*	G	G	G	T	C	T	G	G	A	A	G	C	T	*amp1, amp3, amp4, amp5, amp6, O, S, 2x*
cp*1b*	·	*	*	*	*	*	*	*	*	*	A	·	·	·	·	·	·	·	·	·	·	·	·	*amp2*
cp*1c*	*	*	*	*	*	*	*	*	*	*	·	·	·	·	·	·	·	·	·	·	·	·	·	*amp1*
cp*2*	·	*	*	*	*	*	*	*	*	*	·	T	C	C	T	*	C	·	G	G	C	T	*	*amp1*
cp*3*	·	A	T	T	A	C	A	A	A	T	·	T	C	C	·	·	·	A	·	G	·	T	*	*P*

Numbers refer to variable position observed in final alignment; * – represent insertion/deletion; · – represent match with cp*1a* haplotype; 2x – diploid sexual. For taxon abbreviations see Supplemental Table 1.

Discussion

Apomictic dandelions (with diplospory as the prevailing reproduction mode) are widely distributed in temperate zones of the Northern hemisphere. These apomicts usually predominate in populations in cooler regions and the genus as a whole is successful in colonizing great areas. In this study, we tested whether morphologically uniform phenotypes (i.e., clones, microspecies) are genetically uniform or diverged. Our data showed that apomictic accessions are genetically highly homogeneous and that its low genotypic variability can be explained by the accumulation of mutations during their asexual history. Many of the observed genotypes were clonal and accession specific and therefore accessions could be considered to represent apomictic clones. However, the variability among accessions is much higher than within accession variability. It originates from the recombination events – sexual process between apomictic pollen donor-apomictic father and sexual mother. The pattern of variability and its distribution is in correspondence with the morphological and taxonomic differentiation of accessions into apomictic microspecies.
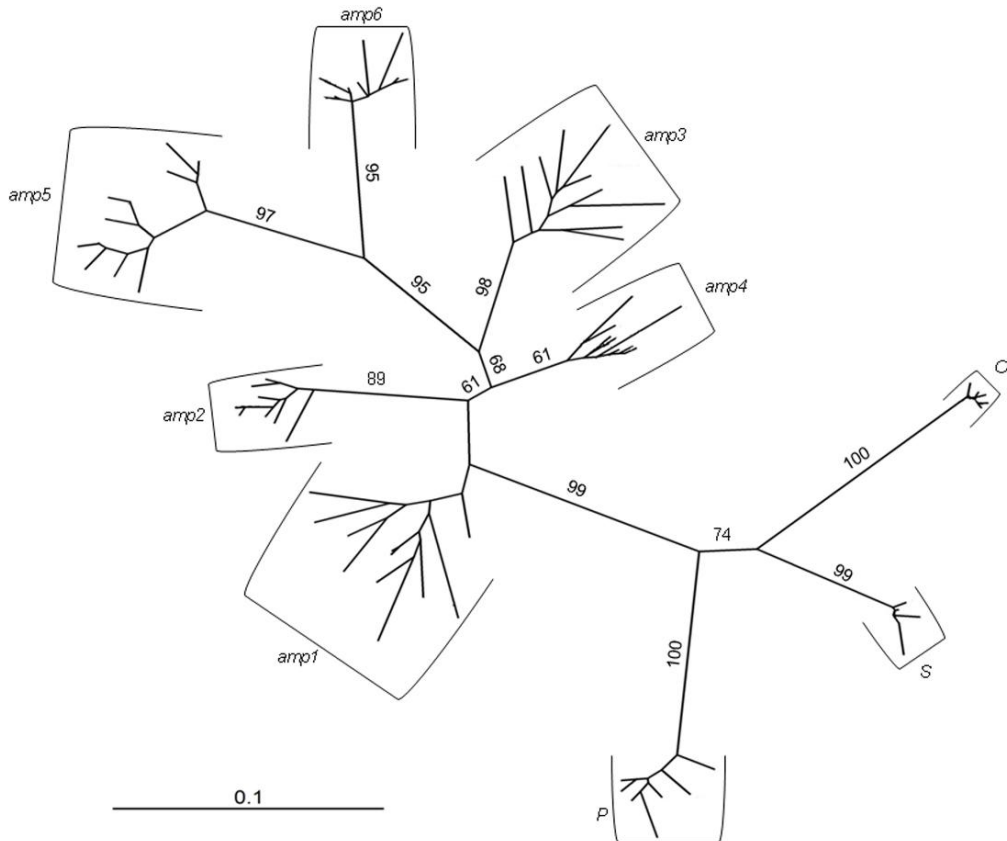
Figure 3. **Unrooted Neighbor-joining tree.** Neighbor-joining tree based on Dice coefficient of similarity (bootstrap values < 50 are shown above the branches) depicting division of nine apomictic *Taraxacum* accessions into well supported agamospecific clusters (based on AFLP data of 96 individuals). For taxon abbreviations see Table S1.

Source of Genotypic Variation in Apomictic Clones

Although obligate apomicts undergo only clonal reproduction, the possibility to generate genetic variability still exists. Richards (1996) discussed processes of mutational changes to DNA and their accumulation (including changes to genes involved in regulation of apomixis) and gross changes at the level of chromosomes including somatic recombination or disjunctional accidents. The mutation accumulation and the recombination during female meiosis are well supported by experiments (van Baarlen et al., 2000; Mes et al., 2002). Our data suggest that intraclonal diversity of *Taraxacum* apomicts is caused by mutation load within a single clone but that interclonal diversity is most likely of sexual origin. We found that all of the investigated apomictic accessions contained nearly identical genotypes or a low number of different genotypes (Table 2, Table 4, Table 5). The genotypic differentiation within accessions, in addition to *amp1*, is mutational rather than recombinational in nature (Table 4). Considering the sampling strategy and the sampling area (Figure 1), the detection of such high clonality with mutational diversity is a good representation of the asexual history of accessions (Tibayrenc et al., 1991). Apomictic genotypes in the absence of sexual partners become frozen for hybridisation (Richards, 1973; van Dijk, 2003). Mutations therefore start to play an important role in generating the variability in clonal lineages. Such an evidence was made in asexual *Ranunculus carpaticola*, which has high allelic variation of mutational origin (Paun et al., 2006a). Similarly, populations of hexaploid apomictic *Potentilla argentea* have high variability within AFLP phenotypes indicating its mutational origin (Paule et al., 2011).

Genotypic Diversity of Apomictic Dandelions

We found a perfect correlation between genotype fingerprints and phenotypes. All of the observed genotypes were accession specific, with no genotype shared among them. A similar pattern of genotypic diversity was observed within both markers examined in this study. With AFLPs, we detected nearly as many AFLP phenotypes as observed individuals for the *amp1* and *amp3* accessions (Table 5). Despite the high mutation rate of microsatellites (10^{-2}–10^{-6}; Schlötterer, 2000), the accession always displayed fewer genotypes for SSR loci than individuals examined (Table 2, Table 4). Microsatellites detected higher clonality (low number of genotypes) within studied accessions when compared to AFLPs. A comparable pattern is known to occur in apomictic populations of aposporous *Crataegus douglasii* (*Rosaceae*) complex, where lower number of genotypes than AFLP phenotypes was detected by 13 SSR loci (Lo et al., 2009). In contrast, *Ranunculus carpaticola* had as many genotypes as individuals for two SSR loci (Paun et al., 2006a). This contrasting pattern can be explained by the differences in mutational rates of SSR loci and the differences in marker resolution (Loxdale & Lushai, 2003). The overall genotypic diversity and polymorphism observed with AFLPs was low (Table 5). A possible explanation is the recent origin of the investigated accessions, with a minimum gained genotypic diversity, as was also proposed for *Taraxacum albidum* (Menken & Morita, 1989) and for the apomictic *R. carpaticola* (Paun et al., 2006a). However, the observed multilocus and locus allelic diversity assessed by SSRs was high (Table 1, Table 2) due to fixed heterozygosity in apomicts (Tibayrenc et al., 1991).

The terminology used for clones varies among geneticists, plant systematists and molecular biologists. For taxonomists or ecologists a clone usually refers to morphologically identical offspring of vegetative (or apomictic origin), whereas strictly genetic view differs in considering each unique genotype or AFLP phenotype as distinct clone even they are morphologically identical. Our data support the hierarchy of apomictic clones discussed by van Dijk et al. (2009) who reflects different origin of clones that can be consisted of several clone mates differing in their genotypes. We similarly consider a group of individuals sharing the same AFLP phenotype and the same SSR genotype to be an asexual clone. Individuals having the same AFLP phenotype and different (but similar) SSR genotypes (or vice versa) are considered to be clone mates. They differ from each other due to different mutation loads. The lowest level in this hierarchy represents asexual lineages, i.e., individuals having unique AFLP phenotypes and SSR genotypes. This asexual lineage has its origin in clonal genotypes with variability from three possible sources: i) somatic recombination (Richards, 1996), ii) recombination during restitutional meiosis [77], or iii) the sexual process (van der Hulst, 2003; Fehrer et al., 2005). This hierarchy takes into consideration both, the biological background of apomicts (reproduction, morphology, ecology etc.) as well as the nature of markers.

Figure 4. SplitDecomposition network. Network of 96 apomictic individuals of *Taraxacum* based on 162 AFLP markers (uncorrected P-distances; goodness of fit 85.6). Bootstrap values .< 50 are shown above branches. Observed cpDNA haplotypes are also designated. For taxon abbreviations see Table S1.

Distribution of Genotypic Diversity

Genotypic variability in the morphological groups of apomictic dandelions that we studied had similar distributions for both types of markers. Analysis on several hierarchical levels showed that diversity is distributed mainly among accessions, whereas higher homogeneity was observed within accessions (Table 6).

Morphological groups *OSP* and *AMP* clearly form two separate pools of apomictic genotypes, which were confirmed by testing the relatedness of genotypes using several clustering methods (Figure 2, Figure 3, Figure 5). Estimated population differentiation for AFLPs/SSRs corresponds to morphological homogeneity of accessions and their genotypic variability. Apomictic accessions are highly differentiated, and are fixed for different alleles (Table 1, Table 3, Table 5). Furthermore, nearly all accessions are characterised by private markers (restricted to the group) and diagnostic markers (present in all individuals of one group) (Table 5). The only exception is *amp1*, which does not have a diagnostic marker. The high differentiation values can be expected because any mutation that will be not repaired can become fixed and frequent in clone (Paun et al., 2006b). There are no comparable results for obligate diplosporous apomicts. However, the hexaploid facultative aposporous *Potentilla argentea* and *Ranunculus carpaticola* have stronger differentiation among populations of agamospecies than within populations (Paun et al., 2006a; Paule et al., 2011). Differences in the pattern of interpopulation differentiation can be the effect of short distance seed dispersal within these two genera. Genotypes are than concentrated on smaller geographic range and populations differ from each other. In contrast, dandelions are more effective in seed dispersal with effective spread of genotypes across wide area. In addition, reduced gene flow and differentiation through genetic drift contribute to a high diversification among populations (Hartl & Clark, 1997).

Some microsatellite loci are highly conserved and are thus shared among individuals from distant regions. Microsatellites linked to *DIPLOSPOROUS* locus (MSTA78 and MSTA53 – Vijverberg et al., 2004) share the same alleles (164 bp and 202 bp, respectively) in individuals analysed from the Netherlands, Denmark and Northern Germany and thus it was hypothesised that these alleles are linked to *DIP* in natural populations of apomictic dandelions (van Dijk & Bakx-Schotman, 2004; van Dijk et al., 2009). Our results do not support this hypothesis, as alleles for MSTA78 and MSTA53 vary considerably in our apomictic samples. Although in some regions might be the tight linkage between microsatellite alleles and *DIP*, generally speaking it does not appear to be a general rule. The first evidence apposing this hypothesis was provided by observing a 164 bp allele (MSTA78) in population samples of sexuals from France (van Dijk et al., 2009).
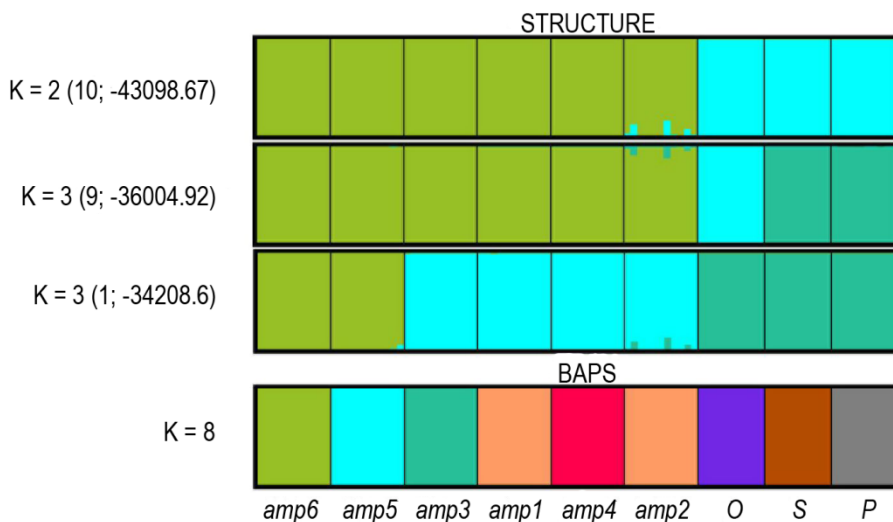
Figure 5. Bayesian clustering of apomictic *Taraxacum* accessions. Results of Bayesian clustering of nine apomictic *Taraxacum* accessions (96 individuals) and assignment into clusters using STRUCTURE and BAPS. Each individual is represented by a vertical bar, the color representing the assignment probability to different clusters. Clustering for K = 2 and K =3 (STRUCTURE) and K =8 (BAPS) are shown. The number of replicate runs producing the partition and the mean L(K) value are shown for STRUCTURE results only. Names of taxa are displayed below the graphic. For taxon abbreviations see Table S1.

History of Asexual Clones

Considering the role of recombinations/mutations in pattern of genotypic variability for different apomictic accessions, in *OSP* only two genotypes do not fit the expectation for genotypes differentiating by changes gained through clonal reproduction. While the results could suggest that genotypes within the *OSP* group differ purely by accumulation of mutations, the results from cpDNA revealed that *P* had a different origin from *S* and *O* (Table 7). This incongruence is caused by overall low differentiation of SSR genotypes observed within the *OSP* group. The similarity between genotypes within the *OSP* group can also be the result of different mutational behaviour of the SSR loci within a different genetic background. Based on cpDNA results, the *OSP* group appears to be of different genetic origin than the *AMP* group. The unique haplotype cp*3* of *P* suggests that there is also a different maternal origin of this accession, while *O* and *S* share the common cp*1a* haplotype. Although *O* and *S* share the same cpDNA haplotype with the *AMP* group, both SSRs and AFLPs clearly separate all of the groups.

SplitDecomposition network does not indicate a reticulate or recombination relationship between the accessions from *AMP* group (Figure 4). All accessions are on highly supported branches, and the star-like structure of the net suggests that the *AMP* group has a unique origin (common ancestor) and a consequent radiative spreading of the clones. The

possible scenario of the *AMP* group origin is the hybridisation between the sexual and apomictic pre*amp1* genotypes, in which an array of newly asexual genotypes arose and several lineages became fixed and further evolved into separate clones that share a common history. This scenario confirmed also the result of Matrix Compatibility analysis, in which nearly all genotypes are in congruence with recombinational/sexual difference of investigated accessions (Table 4). The *amp1* could represent the oldest clonal genotype within the group because it exhibits the highest variability, and it could represent the maternal haplotype for the whole group, with an SSR-allelic pattern shared within the *AMP* group and the position of *amp1* in the centre of the SplitDecomposition network. Three different cpDNA haplotypes were observed within *amp1* and four within *AMP*. The cp*1a* is the most common one; cp*1c* differs in single point deletion. Haplotype cp*2* could represent a hybridisation event with different sexual haplotypes (Table 7). The cp*1b* is specific for *amp2* and may also have a mutational origin from cp*1a*, when a single bp transition became fixed by apomixis within a clone.

The evolutionary history of *Taraxacum* shows intensive reticular evolution. Haplotypes in this study belong to group of derived haplotypes common among advanced sections (Wittzell, 1999). Apomictic lineages can originate from multiple hybridisations of ancestral apomictic and sexual generating arrays of novel genotypes (Richards, 1973; Kirschner & Štěpánek, 1996; van Dijk, 2003). Apomictic genotypes become fixed in the absence of gene flow and due to genetic drift (Hartl & Clark, 1997). Successful clones then spread over large areas and persist for a long time (Fehrer et al., 2005). A new asexual ''life history'' allows the clone to gain only a limited fraction of variability compared to its sexual relatives (Richards, 1996). In a situation where the frequency of the sexual process is low enough, mutations become the major source of genotypic variability (Brookfield, 1992). Clonal genotypes will produce an array of mutationally differentiated genotypes–clone mates– under such conditions (Mes et al., 2002; Paun & Hörandl, 2006; Paule et al., 2011). The number of mutations will be higher in the former clones than in the younger ones. The older an asexual genotype is, the higher the rate at which mutations accumulate; this leads to the formation of a mutant genotype network.

Conclusions

Our data demonstrate that there is both significant genetic similarity and significant differences among putative apomictic clones that were identified by phenotype. Unlike in previous studies, in which clonality was detected in a population sample of not-phenotyped accessions, we focused on the genetic structure within phenotyped apomictic accessions themselves. Both asexual life history and sexual recombination have an impact on the genetic variability of apomicts of *Taraxacum officinale* agg. From an evolutionary point of view, apomictic dandelions have undergone an asexual life history in recent evolutionary periods with preceding (sexual) hybridisation. The structure of their genotypic variability is tightly correlated with morphology. Correct genotyping is crucial requirement both in population biology and ecological studies (Drummond & Vellend 2012). Although detailed morphological characterization of each single individual should be the first methodological step used in genetics, population genetics, biotechnology and biosystematic studies for reliable genotyping/sorting of clones, it is evident from our results that genotyping can significantly assist in correct determination in genera where the determination is extremely difficult.

CHAPTER 3

Genotypic variability of obligate apomicts is enriched by the gene pool of sexuals in contact zones between sexual-apomictic dandelions (*Taraxacum* sect. *Erythrosperma*)

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Miloslav Kitner

Abstract

Populations of polyploid apomictic dandelions consist of a mixture of clonal accessions that are phenotypically and genetically almost uniform. Some of the Central European *Taraxacum* populations consist of both diploid sexuals and polyploid apomicts; thus, novel apomictic accessions can be formed via hybridisation among sexuals and apomicts. Groups of the xerothermic section *Erythrosperma* are formed by morphologically similar taxa. Some taxa are widespread, but many other morphotypes occur locally or at a single locality. We aimed to determine whether this pattern is a consequence of putative hybridisation.

We tested for dandelion hybridisations by genetically analysing 111 apomictic and sexual plants from sympatric and purely apomictic populations using a combination of nuclear and chloroplast DNA markers. The pattern of genotypic variability within phenotyped apomictic accessions suggested both intra-accession genetic diversification, generating a network of clone mates, and variability resulting from recent hybridisation, leading to the formation of neo-apomicts. These neo-apomicts possessed genetic variability from two sources: apomicts and sexuals. Neo-apomicts were partially free of the mutation load accumulated in maternal-apomictic clones and acquired new genetic combinations of chromosomes with sexual and asexual life histories.

Our results show the formation of neo-apomictic accessions from phenotypically distinct and widespread apomictic and sexual accessions in their contact zones. This process is apparently still ongoing on a regional scale and can be considered a diversity-increasing process of apomictic dandelions.

Introduction

The population structure of plants depends on many ecological and genetic factors. The amount and the pattern of genetic diversity is an important factor and provides the pool from which selection may operate. The amount of diversity depends on the effective population size, population structure, mating system, and stochastic processes occurring from the origin until the disintegration of any biological unit (entity). Additionally, the mating system is a crucial evolutionary force because it represents a point break – the formation of a novel entity – with a new genetic composition. There are several ways for organisms to reproduce. Generally speaking, the vast majority of species have their genetic constitution reshuffled by sexual reproduction, and thus, (positively) selected novel traits are formed in each new generation. However, many organisms—across all known clades—instead of the "mixing cards" strategy choose "playing the game with a single card", which is generally called apomixis. Except for vegetative propagation, apomixis is interesting from the evolutionary point of view due to its ability to clone the maternal genome via reproduction through seeds without the requirement of syngamy of female and male gametes (Asker & Jerling, 1992). Although there are other types of apomixis and apomixis evolved in different clades of flowering plants independently (van Dijk, 2003; Whitton et al., 2008), the consequence is always the same – the offspring is a copy of the maternal genome. Furthermore, nearly all apomictic species are polyploids and are often of hybridogenous origin (Asker & Jerling, 1992). Apomixis likely developed as a rescue system to overcome the decreased fertility due to unfavourable environments (e.g., apomicts in alpine biota – Hörandl, 2011) or due to recent hybrid origin (Whitton et al., 2008), or it is a genetic consequence of interspecific hybridisation, polyploidisation or genome duplication (Carman, 1997). Apomixis helps to stabilise newly formed hybrids and enables their rapid dispersal in the autopolyploid alpine species *Ranunculus kuepferi* (Cosendai et al., 2011) or the allopolyploid *Potentilla argentea* (Paule et al., 2011).

On the other hand, the drawback of asexual reproduction is reduced genetic variability in combination with the increase of the mutation load. Apomicts thus have lower evolutionary potential in the long term (van Dijk, 2003). This disadvantage can be partially overcome by gene flow between apomicts and sexuals in their contact zones, as apomicts can produce viable pollen grains and therefore enter the sexual process (van Dijk, 2003). This phenomenon may enrich the gene pool of apomicts (van Dijk, 2003; Menken et al., 1995), or it may lead to the formation of a novel apomictic hybrid (e.g., *Amelanchier* – Campbell & Wright (1996); *Boechera* – Dobeš et al. (2007)). Although hybridisation with sexual relatives enriches the genetic variability of apomicts, the genotypic diversity of apomicts is to some extent mutational in origin (Paun et al., 2006a; Paule et al., 2011; Majeský et al., 2012). The longer the asexual history of a clone, the higher the mutation load that accumulates within the clone.

Apomixis in the genus *Taraxacum* is strictly confined to polyploids—mostly triploids or tetraploids—which is the most frequent cytotype in the genus. Dandelions use obligate meiotic diplosporous apomixis. Sexual individuals are always diploids in natural populations, although the exception of tetraploid sexuals is known (Kirschner et al., 1994). *Taraxacum* species are classified based on morphology into sections that usually contain one or few sexual species and a large polyploid cluster of apomictic accessions that are traditionally classified as microspecies (Kirschner & Štěpánek, 1994). The population structure of apomictic and mixed *apo-sex* (apomictic-sexual) populations shows a mixed origin for the

observed diversity, i.e., gained through accumulation of mutations and through recombination (van der Hulst et al., 2003). In contact zones between apomicts and sexuals, gene flow is expected to contribute to the diversification of the apomictic gene pool and vice versa (Kirschner & Štěpánek, 1994; Meirmans et al., 2003). In purely apomictic populations, the population may consist of one or several apomictic genotypes with a network of clone mates that have mutational diversity (Mes et al., 2002; van der Hulst et al., 2003; Majeský et al., 2012). Migration of newly established apomictic genotypes from regions of sympatric occurrence of *apo-sex* individuals may enrich such purely asexual populations.

Apomictic dandelions can easily hybridise with sexual diploids, although the hybrids constitute only a minor part of the offspring from such crosses. Experimental crosses in *Taraxacum officinale* agg. using a triploid apomict (♂) × a diploid sexual (♀) revealed that the seed-set was significantly lower than in a diploid × diploid cross (22% on average) and that the vast majority of the offspring (89%) were diploids resulting from the selfing (Tas & van Dijk, 1999). Similarly, in experimental crosses of the European triploid apomictic *T. officinale* agg. (♂) × 3 different Asian diploid sexual species of sect. *Mongolica* (♀), all the diploid plants among the offspring (87.5% of all plants in F_1 offspring) were a result of selfing (Morita et al., 1990a). Self-incompatibility is a result of the mentor effect caused by unviable aneuploid pollen (Morita et al., 1990b; Brock, 2004). Considering that the germination rate of polyploid hybrids from such crosses is significantly lower than in a triploid apomictic father (Tas & van Dijk, 1999), the occurrence of such hybrids in nature—especially for a triploid father—is expected to be low. The formation of neo-apomicts can be bridged by hybridisation via tetraploids, as they produce significantly more triploids than do triploid fathers (Verduijn et al., 2004). In contrast, the natural cross of the North American diploid sexual species *T. ceratophorum* (♀) × introduced European apomictic triploids of *T. officinale* agg. shows a relatively high rate (33.2% in ca. 1/3 seed-set) of germinating seeds formed by the hybridisation (Brock, 2004). Although several studies demonstrated ongoing hybridisation of obligate apomicts with sexuals in dandelions in Asia, Europe and North America, no study has focused on hybridisation of a particular clone or group of similar clones.

Analyses of the origin of genotypic diversity in apomicts strongly depend on the methodology. If the population of apomicts is analysed without explicitly determining what represents the evolutionary unit of an apomictic clone, the analysis of population structure will reveal several clonal genotypes, some clones being frequent and some being local. That approach can well describe the overall nature of populations (van der Hulst et al., 2003). However, if a population of apomicts is analysed by the approach in which morphology (phenotype) can be a sign of pertinence to the clonal genotype and the population forms taxonomic (biological) operational unit, its genetic structure may reveal a set of well-discriminated clones, reflecting their morphological and genetic characteristics and relationships (Mes et al., 2002; Majeský et al., 2012). By using the second approach, it is possible to search for the nature of observed variability in populations more in detail, and, especially, the modes of hybridisation can be better defined.

The aim of the present study was to analyse the populations of obligate asexual apomictic dandelions, evaluate their genetic structure by different molecular markers and gain a deeper understanding of the possible ongoing microevolutionary processes within these populations. For the populations of asexuals, we used phenotyped apomictic accessions within the genus *Taraxacum*, treated at the level of microspecies.

Suitable molecular markers for this type of study should cover different parts of the genomes and provide qualitatively various information. We used three types of molecular markers: nuclear Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) DNA markers, and a selected sequenced region of chloroplast DNA (cpDNA). Microsatellites cover short noncoding and evolutionarily neutral parts of a genome, and they have a high mutational rate and codominant character. They allow us to observe the intensity of mutations and track the relationships between genotypes. AFLPs represent fingerprints of randomly amplified DNA fragments of different length, scattered throughout the genome. The information provided by a single AFLP marker is weak because of the dominant nature of the marker, but analysing a large number of markers overcomes this issue and also provides information on the relationships between the studied genotypes. cpDNA in angiosperms has (usually) matrilineal inheritance and can trace the history of at least half of the genome and, in apomicts, hypothetically the whole genome (if no hybridisation with sexuals occurs). The connection of the information from morphology and molecular data can shed light on the population structure of apomictic organisms in sympatry with sexuals.

Methods

Plant material and sampling strategy

We genotyped 102 apomictic individuals from the *Taraxacum scanicum* group and 9 diploid sexual individuals of the red-fruited section of lesser dandelions, i.e., *T*. sect. *Erythrosperma*. This section consists of one diploid sexual species (*T. erythrospermum*) and approximately 150 apomictic polyploid microspecies (Vašut, 2003), growing on xerothermic and dry biotopes. The *Taraxacum scanicum* group consists of 16 species that are morphologically similar (Doll, 1973; Øllgaard, 1986; Schmid, 2002;Schmid et al., 2004; Vašut et al., 2005). Some of the taxa have wide distribution areas, whereas others are distributed only locally or at a single locality (Schmid et al., 2004; Vašut et al., 2005; Marciniuk et al., 2009). The distribution of sexuals in Europe is disjunct, and sexual diploid species can be found in SW and SC Europe (Kirschner & Štěpánek, 1994; den Nijs, 1997). Plants used in the present study were collected along such contact zones in SC Europe. We sampled individuals based on the taxonomic concept of microspecies and morphology. The 102 apomictic individuals represented three microspecies (*T. prunicolor* – "PRU"; *T. cristatum* – "CRI"; *T. scanicum* – "SC") and six morphological series recognised by authors (*Prunicolor* derived – "PRU_d"; *Scanicum* similar – "SCs"; Morphotype 1 – "MOR_1"; Morphotype 2 – "MOR_2"; *T. pudicum* – "PUD" and *T. arachnitis* – "ARA"). We collected one to three samples per locality per apomictic accession. Plants were transferred and cultivated in the experimental field of the Department of Botany, Palacký University in Olomouc, Czech Republic. The plant material was documented by depositing herbarium specimens into the herbarium of the Department of Botany (*OL*). Apomictic reproduction was confirmed by Flow Cytometric Seed Screen (FCSS) (Matzk et al., 2000). For a complete list of the studied individuals (including taxonomic identification) and sampling areas, see the Additional file 1 and Fig. 1.
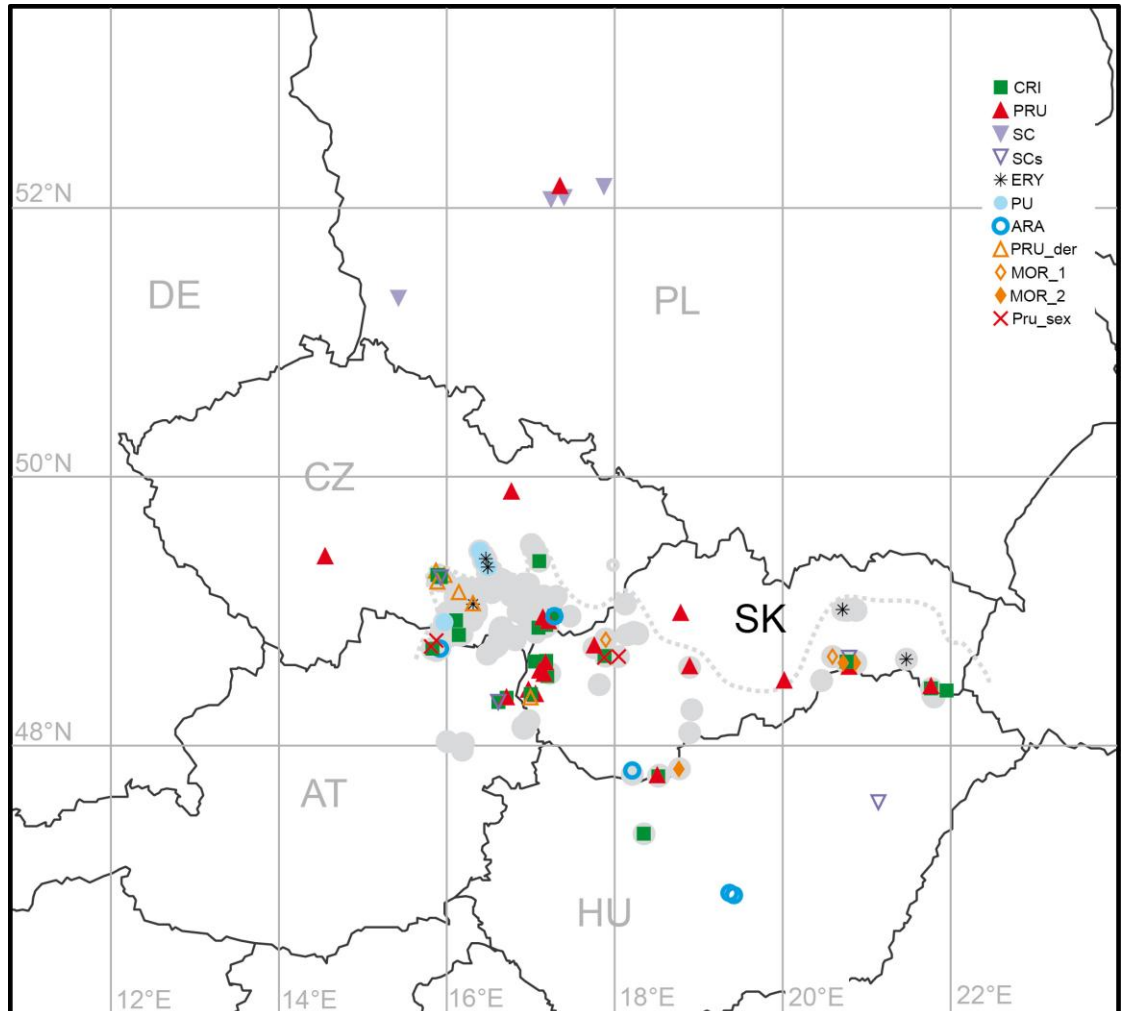
Figure 1. Geographical origin of the studied individuals of lesser dandelions (*Taraxacum* sect. *Erythrosperma*). Grey dots represent documented occurrences of diploid sexual species (*T. erythrospermum*) based on field observation and study of herbaria records (Vašut 2003, Vašut in prep.). This highlights the extent of distribution of sexuals in the studied region (grey circles represent extinct localities of sexuals behind the continuous distribution). Samples P_BELL (Northernmost Finland) and SC_TsM (the Netherlands) are not shown on this map. For taxon abbreviations in the legend, see Additional file 1.

DNA extraction, SSR and AFLP fingerprinting

Genomic DNA was extracted from voucher specimens or fresh leaves, following the CTAB protocol of Doyle & Doyle (1987) with minor modifications. All 111 individuals were genotyped for six SSR loci: MSTA44B, MSTA53, MSTA78 (Falque et al., 1998), MSTA93, MSTA131, and MSTA133 (Vašut et al., 2004), as specified in Majeský et al. (2012). Total of

75 individuals representing each accession and also diploid sexuals were AFLP-fingerprinted with seven primer combinations: *Eco*RI–AGC/*Mse*I–CAAC, *Eco*RI–AGC/*Mse*I–CAAT, *Eco*RI–AGC/*Mse*I–CAATC, *Eco*RI–AGC/*Mse*I–CGATG, *Eco*RI–AGG/*Mse*I–CAAC, *Eco*RI–ATC/*Mse*I–CAAC, *Eco*RI–ATC/*Mse*I–CAAT. AFLP fingerprinting was performed as described in detail by Kitner et al. (2008) and Kitner et al. (2012).

cpDNA sequencing and sequence alignment

The *trnL-trnF* region was sequenced in several samples from each apomictic accession and diploid sexual (see Additional files 1and 2). PCR, sequencing and sequence processing were performed as described by Majeský et al. (2012). Sequence data were deposited in GenBank (http://www.ncbi.nlm.nih.gov/) [accession numbers KC119514–KC119542].

Fragment analyses of microsatellite genotypes and AFLP profiles

PCR products were separated by denaturing polyacrylamide gel electrophoresis (PAGE) and visualised by silver staining. The 30-330-bp AFLP® DNA Ladder (Invitrogen) was used to size the microsatellite alleles. Genotypes were considered absolutely homozygous when only one allele per locus was observed. When two different alleles were observed, the shorter allele was considered to occur twice, except at loci MSTA53, MSTA78 and MSTA131, for which fixed alleles were observed. In this case, the longer allele was considered to be present twice. Microsatellite data were recorded as genotypes based on the length of the PCR product and as binary data based on the presence/absence of a fragment of a particular size. The AFLP profiles were visually checked and coded as a binary matrix. To avoid genotyping errors and to retain the reproducibility of the analyses, controls of blind samples, double samples and repetitions were integrated into our analysis.

Data analysis

Joined binary data from both markers, SSRs and AFLPs, were used to perform analysis of molecular variance (AMOVA) with ARLEQUINE 3.5 (Excoffier & Lischer, 2010). To visualise similarities among genotypes in hierarchical order, a neighbour-joining tree (Dice coefficient of similarity) was constructed by FREETREE (Pavlíček et al., 1999) and visualised in FIGTREE (http://tree.bio.ed.ac.uk/software/figtree/) with midpoint rooting (bootstrap support with 1000 replications). To inspect genotype clustering under a Bayesian approach, a matrix of joined data was subjected to the software BAPS 3.2 (Corander, 2006). In BAPS, the frequency of alleles and the number of genetically different groups are taken as random variables, and the program estimates one optimal partitioning. For analyses, the module "clustering of groups of individuals" was used. *K* ranged from 1 to 11, and the analysis was repeated ten times. Within the joined SSR-AFLP binary matrix, only individuals genotyped for both markers (75 individuals) were used. To determine the possible source of differences among genotypes (mutations vs. recombinations), SSR-binary data were used for character

compatibility testing using the module JACTAX in PICA 4.0 (Wilkinson, 2001). For more details about character compatibility, see van der Hulst et al. (2003).

From SSR genotype data, basic indices of variability, including the number of genotypes (*NG*), overall number of alleles, the number of different alleles (*NDA*), the range of allele size, and gene diversity (G_e), were calculated in SPAGEDI software (Hardy & Vekemans, 2002). For descriptions of accession differentiation, *F*- and *R*-statistics were calculated. Effective number of genotypes (*ENG*) and genotype diversity (*GD*) were calculated in GENOTYPE/GENODIVE software (Meirmans & van Tienderen, 2004).

Population statistics for AFLP, i.e., the mean number of bands (*NB*), the number of polymorphic bands (*NPB*) at the 5% level, the number of private markers (*PrB*; restricted to a given population), and the number of diagnostic markers (*DB*; present in all individuals in a population), were calculated in FAMD (Schlüter & Harris, 2006). Polymorphism (*P*), Nei's gene diversity (H_j), the number of different genotypes (*NG*) and genotype diversity (*GD*) were calculated using the R-script of AFLPDAT (Ehrich 2006).

Results and Discussion

Diversity of apomicts

Seven primer combinations yielded 171 AFLP markers. The error rate, calculated as the difference in the number of markers observed in the investigated individuals between repeated analyses, was used, as was the credibility of analyses as a guide for computing the clonality. The repeatability of analyses was calculated to be 98%. Genotypes differing in four markers were considered clonal. The basic statistical indices of diversity of particular microspecies are summarised in Table 1.

Table 1. Genotypic variability indices for AFLP analyses across investigated apomictic and sexual *Taraxacum* sect. *Erythrosperma* accessions.

	2x	*PRU*	*PRU_d*	*CRI*	*SC*	*SCs*	*PUD*	*ARA*	*MOR_1*	*MOR_2*
N	9	16	6	20	5	4	3	4	4	4
NB[1]	73	83	74	85	77	80	70	74	83	82
NPB[1]	72	68	49	27	66	47	37	36	8	40
P(%)[2]	42	40	29	16	39	28	22	21	5	23
PB[1]	2	1	1	2	0	2	0	1	2	3
DB[1]	0	0	0	1	0	0	0	0	1	2
Ge[2]	0.17	0.11	0.13	0.04	0.18	0.16	0.14	0.11	0.02	0.12
NG[2]	9	8	5	3	5	3	3	3	2	3
GD[2]	1	0.76	0.93	0.43	1	0.83	1	0.83	0.5	0.83

N, sample set; *NB*, number of bands; *NPB*, number of polymorphic bands; *PB*, number of private bands; *DB*, number of diagnostic bands; *Ge*, gene diversity; *NG*, number of genotypes (ER=4); *GD*, genotype diversity (ER=4). [1] Calculated in FAMD; [2] calculated in AFLPDAT.

Genotyping the 102 apomictic individuals at six SSR loci revealed the clonal genotypes as well as divergent genotypes. Testing for discrimination of variability (mutational versus recombinational) within apomicts was performed only for five out of nine apomictic accessions, for which more than four different genotypes – the lowest possible number for which the test can be performed – were observed (Table 2). The results of this test could thus have been affected by the small sample size in some accessions.

Based on the fingerprinting pattern of SSR alleles, we can infer three different sources of the analysed individuals: i) clonal origin, ii) hybridisation origin, and iii) repeated hybridisation. Within all genotyped apomictic accessions, overrepresented clonal genotypes were observed (Tables 1, 2, Additional file 3). More clonal genotypes were observed within microsatellites, in contrast to AFLP phenotypes, similar to our previous study (Majeský et al., 2012). However, the markers did not show contrasting patterns. Accumulation of somatic mutations could be considered the main source of intraspecific/intraclonal genotypic variability within the studied sample set, although not all the variability could be explained by this process (Table 2, Additional file 3).

Table 2. Descriptive statistics for 9 apomictic and one sexual *Taraxacum* sect. *Erythrosperma* accession based on 6 microsatellite loci.

	2x	*PRU*	*PRU_d*	*CRI*	*SC*	*SCs*	*PUD*	*ARA*	*MOR_1*	*MOR_2*
N	9	28	8	33	6	7	5	5	6	4
*NDA**	8/14/12/ 12/5/9	5/14/7/ 6/5/9	4/10/6/ 6/5/9	2/2/4/ 1/2/10	1/4/5/ 5/1/3	4/8/8/ 5/5/8	2/8/5/ 2/4/6	4/8/7/ 4/5/5	1/2/3/ 2/2/2	4/6/5/ 4/5/9
NG[1]	9	15	6	10	4	5	4	5	1	3
ENG[1]	9	4.2	5.3	1.9	3.6	4.46	3.6	5	1	2.7
GD[1]	1	0.79	0.93	0.48	0.87	0.91	0.9	1	0	0.83
*Ge**	0.89	0.61	0.71	0.40	0.50	0.86	0.65	0.76	0.43	0.80
MIC[2]	89	96	31	7	-	33	18	18	-	-
E[2]	0	9	0	8	-	4	0	0	-	-

N, sample size; *NDA*, number of different alleles in following order: MSTA 44B, MSTA 53, MSTA 78, MSTA 93, MSTA131, MSTA 133; *NG*, number of different genotypes (in multilocus estimate); *ENG*, effective number of genotypes; *GD*, genotype diversity; *Ge,* gene diversity; *MIC*, matrix incompatibility count; *E*, number of genotypes left in the matrix at *MIC = 0*. [2] Calculated in PICA 95; [1] calculated in GENOTYPE/GENODIVE; * calculated in SPAGEDI.

Individuals with one or few mutant alleles at some loci were considered clonal mates because changes caused by mutations are generally small. These were observed within nearly all apomictic accessions (Table 2, Additional file 3). The structure of the *"CRI"*, *"PRU"*, *"SC"*, and *"MOR_1"*, groups resembled the structure of clonal organisms, with the network of clonal mates differing due to accumulated mutations (Mes et al., 2002; Majeský et al., 2012).

Sexually derived apomictic genotypes were considered those that differed from the clonal genotype by a single allele in each fingerprinted locus, while the rest of the allelic composition was identical to the "maternal-apomictic". Hybridisation caused larger changes than accumulation of random somatic mutations and shifted genotypic diversity into a

different state. Cross-mating of apomicts with sexuals brought new alleles into the population of apomictic microspecies and increased the overall genotypic diversity. Within the investigated apomictic microspecies *T. prunicolor* (denoted "*PRU*"), a group of five individuals (*Ps_H2*, *Ps_H3*, *Ps_EUR*, *Ps_TL2*, *Ps_TO1*; denoted "*pru_sex*"; Additional file 3) could have been the descendants of a sexual process among the apomictic "*PRU*" genotype and a sexual partner. The allelic profile of the "*pru_sex*" group was shared with clonal genotypes and differed in one or two alleles at each locus (Additional file 3). Regarding the morphology, there were some differences between them and among the clonal "*PRU*" individuals (e.g., in the shape of the terminal lobe and the presence of a tooth on the distal margin of the lateral lobes). Hybridisation events also favoured the presence of different cp haplotypes (Additional files 2 and 3). In the hybridisation process, apomicts should play the role of father (pollen donor) and transfer, within partially reduced (diploid) pollen grains, all the apomictic genes (Tas & van Dijk, 1999). FCSS analysis of reproduction mode was performed only for two individuals and showed apomictic seed formation. The rest of the samples of "*pru_sex*" were available as herbarium vouchers only (Additional file 1). Within five genotyped individuals from "*pru_sex*", the observed genotypes were regionally differentiated. Geographically closer genotypes were more similar to each other than to the geographically more distant ones (Additional files 1 and 3). In the case of groups "*PUD*" and "*ARA*", the representatives possessed morphological homogeneity. Genetically, these groups represented more genotypes (Tables 1 and 2). Genotypic variability within these groups showed traces of asexual (accumulation of somatic mutations) and also hybridogeneous origin (Additional file 3).

For genotypes that passed several sexual events, were considered those that differed in more than one allele per genotyped locus but still shared allelic and morphological similarity with the maternal-apomictic genotype. While individuals of one hybridisation event differed in a few minor features, the more derived ones were in general more distinct, albeit bearing similar morphological patterns to the maternal-apomictic genotype. This prediction was observed within sample set of *T. prunicolor* ("*PRU*") and *T. scanicum* s. str. ("*SC*") microspecies. The group of "*prunicolor derived*" (denoted as *PRU_d*) and "*scanicum similar*" (denoted as *SCs*) genotypes most likely represented the result of repeated hybridisation of the "*PRU*" or "*SC*" genotype with a sexual partner.

The "*PRU_d*", "*pru_sex*" and "*SCs*" genotypes had diverged from apomicts by hybridisation and, together with maternal apomictic "*PRU*" and "*SC*" genotypes, represented a network of apomictic clones with clonal mates and with diverged neo-apomictic lineages that arose from a hybridisation event(s). Members of groups with hybridisation history also possessed different cpDNA haplotypes than the core clonal part of particular group (Additional files 1, 2 and 3).

The hierarchical structure of apomictic clones/microspecies was also apparent from the topology of the neighbour-joining tree. The tree lacked high bootstrap support for higher-level of branch organisation. Nevertheless, clustering of individuals followed their morphological classification, and derived apomictic genotypes ("*pru-sex*"; "*PRU_d*"; "*SCs*") appeared in clusters with maternal-apomicts ("*PRU*"; "*SC*") (Fig. 2). Short branches within the dendrogram represent low differentiation between apomictic individuals, while longer branches in other clusters are in accordance with overall higher differentiation within particular accessions. Under a Bayesian approach implemented in BAPS, the best partition for

investigated genotypes should be $K = 9$ (probability equal to 1). Only accessions of "*SCs*" clustered together with "*SC*" in one cluster. Other clustered separately (Fig. 2).

The molecular variability observed for the studied groups for both SSR and AFLP markers had distributions expected for asexual species. Higher variability was observed between accessions (60%) than within accessions (40%). The multilocus $F_{ST}/R_{ST} = 0.3709/0.2327$ implied high differentiation with fixed differences among studied accessions.
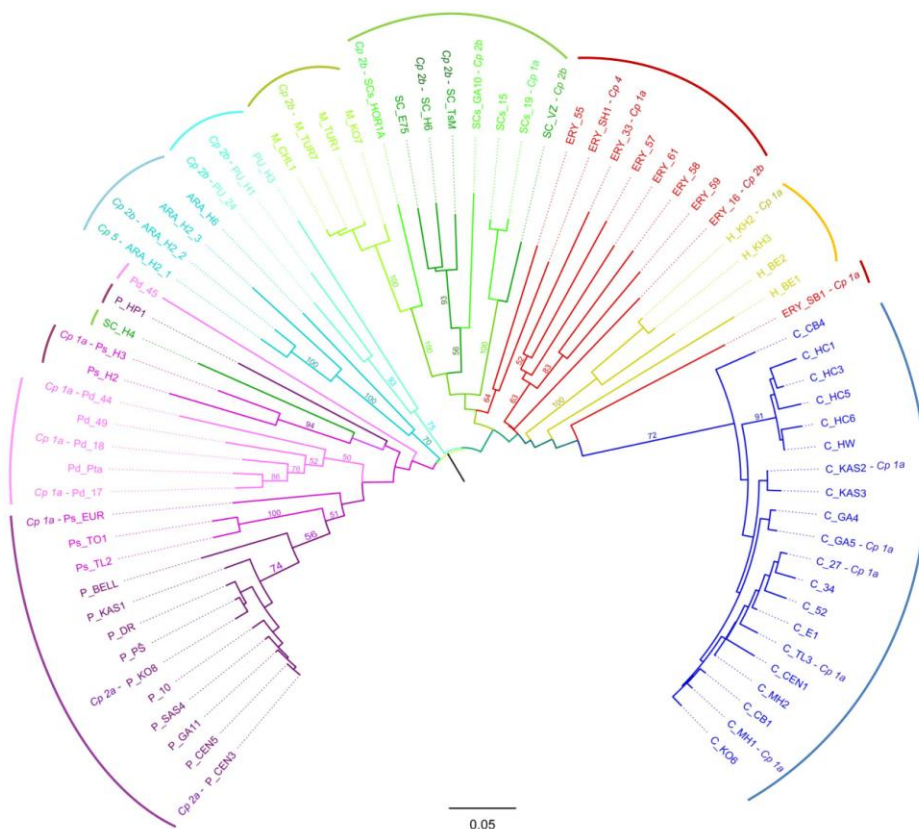
Figure 2. Midpoint-rooted neighbour-joining tree based on joined AFLP-SSR matrix.
Neighbour-joining tree showing hierarchical clustering of 66 apomictic and 9 sexual individuals, based on the Dice coefficient of similarity (bootstrap values >50 are shown above the branches). The cpDNA haplotypes observed for particular individuals are shown behind individual labeling. Result of BAPS clustering are designated as coloured lines (colours of BAPS clusters are as follows: blue: *"CRI"*; red: *"SEX"*; yellow: *"MOR_2"*; dark green: *"SC+SC_s"*; yellow green: *"MOR_1"*; turquoise: *"PU"*; light blue: *"ARA"*; pink: *"PRU_d";* purple: *"PRU+pru_sex"*). Colours of dendrogram branches correspond to accessions groups as follows: blue: *"CRI"*; red: *"SEX"*; yellow: *"MOR_2"*; dark green: *"SC"*; light green: *"SCs"*; yellow green: *"MOR_1"*; turquoise: *"PU"*; light blue: *"ARA"*; pink: *"PRU_d"*; light purple: *"pru_sex"*; purple: *"PRU"*. For taxon abbreviations, see Additional file 1.

Diversity of sexuals

As expected, we observed the highest genotypic diversity for diploid sexuals (Tables 1, 2). Within all three markers, the variability of diploids approached the variability observed for apomicts. Nearly all of the SSR-allelic diversity of apomicts was observed within the allelic diversity of diploid sexuals (Additional file 3), and the same held true for AFLP (data not shown) and for cpDNA haplotypes (Additional file 2). Diploids generate variability through the sexual process and recombination during meiotic division. This pool of genetic variability in regions with sympatric occurrence of sexuals and apomicts is available to apomicts and may result in enriched genotypic and phenotypic variability of apomicts throughout their hybridisations with asexuals (Menken et al., 1995).

Haplotype diversity

Sequencing of the *trnL-trnF* region revealed four haplotypes (Additional file 2, Fig. 2). Considering the chloroplast data, haplotype cp*1a* was common within investigated microspecies/accessions and also in the sexual diploid *T. erythrospermum* (Additional files 2 and 3). In our previous study (Majeský et al., 2012), the cp*1a* haplotype was the most common among apomicts and also within the sexual *T. linearisquameum*. Wittzell (1999) considered this haplotype (denoted haplotype *18a* in his study) to be the most common one within evolutionarily derived sections of European dandelions, particularly in the Central European region, which seems to be true also for our data. A majority of apomictic accessions possessed one cpDNA haplotype (Additional file 2), which is expected for clones with one history. Two different haplotypes were observed only for the *"ARA"* group (Additional file 2), which may indicate (together with the SSR allelic profile – Additional file 3) a hybridisation event among the *"apoARA"* genotype and a sexual diploid partner. The highest haplotype diversity was expected to be present within sexual diploids, which we did observe. Within four sequenced individuals, three haplotypes were present: cp*1a*, cp*2b* and cp*4*.

Specific alleles

For three genotyped loci, fixed alleles were observed. For locus MSTA131, an allele of 156 bp was present within genotypes of 101 out of 111 investigated individuals. Individuals with this allele were found within both apomicts and sexuals. For locus MSTA78, an allele of 165 bp was observed within 97 individuals, and for locus MSTA53, an allele of 202 bp within 51 individuals. All individuals bearing allele 202_{MSTA53} were fixed for allele 165_{MSTA78} (Additional file 3), and thus, these alleles were specific only for apomicts.

However, the linkage of allele 164_{MSTA78} to the *DIP* locus was not a general rule (van Dijk et al., 2009; Majeský et al., 2012), as we never observed this allele within genotyped sexuals. Except accession *"PUD"*, 97 individuals with allele 165_{MSTA78} were apomictic (Additional file 3). Apomictic accessions *"CRI"*, *"SC"* and *"MOR_1"* were uniform for another allele, 202_{MSTA53}, which was also linked to the *DIP* locus (van Dijk & Bakx-Schotman, 2004). Because the locus of MSTA53 is farther from the *DIP* locus than MSTA78 (van Dijk & Bakx-Schotman, 2004) and this is not in a segregation-suppressed region

(Vijverberg et al., 2010), this allele may not always be carried by apomicts, or both of these alleles may not be present in apomicts, as shown here for the accession *"PUD"* and in a previous study (Majeský et al., 2012).

Allele $156_{MSTA131}$, which was fixed within 101 out of 111 genotyped individuals, may be a taxonomic-specific marker for *Taraxacum* sect. *Erythrosperma*. This SSR allele was missing only in two diploid sexuals and eight apomicts, so it cannot be considered a "private fixed allele", only a "private allele", for lesser dandelions. In our previous study on dandelions of a different section *T.* sect. *Taraxacum*, no fixed allele or allele with similar size was observed in this locus. Similarly, in the study of Vašut et al. (2004), within twelve genotyped individuals of sect. *Erythrosperma* for the same locus, nine bore the allele of 157 bp, and another three individuals from different sections had also this allele. Differences in allele size determination might have come from differences in methods used for allele sizing, but we consider them to represent the same allele. For estimation of allele size, we used 30–330 bp AFLP DNA Ladder (Invitrogen) with 32 10-bp repeats. A 10-bp difference between two fragments was evenly divided onto 9 parts. The allele size was then estimated by eye. This method for allele size estimation is partially subjective and may cause differences of a few base pairs in length compared to estimations by different methods. The same holds true for allele 165_{MSTA78}, which we consider to represent allele 164_{MSTA78} in the study by van Dijk & Bakx-Schotman (2004).

Based on this study and our unpublished results, the presence of allele $156_{MSTA131}$ is specific for section *Erythrosperma*, may also occur sporadically in different sections, but will not be common to a majority of genotypes. It will be interesting to examine sect. *Erythrosperma* in its whole geographic distribution range for the presence of this allele to confirm our hypothesis.

Evolutionary consequences

Regions where apomictic dandelions coexist in mixed populations with sexual diploids provide a genetic pool of sexuals that are available for hybridisation with apomicts (den Nijs, 1997; van Dijk, 2003).

The gene flow in the direction *apomict→sexual* is interesting from the evolutionary point of view because it represents the process of formation of novel apomictic clones – neo-apomicts (Kirschner & Štěpánek, 1994) – that are partially purged of the deleterious mutation load, which is considered to be responsible for the *fatal destiny* of the clone (van Dijk, 2003). The possibility of hybridisation between sexual and apomictic dandelions was confirmed in both experimental and natural conditions in various taxonomic sections (see Table 1 in Verduijn et al., 2004). In a recent work on hybridisation opportunities within sect. *Erythrosperma*, Mártonfiová et al. (2010) reported a decreased rate of polyploid formation in 2x-3x crosses in comparison to *T.* sect. *Taraxacum* (syn. *T.* sect. *Ruderalia*), with no polyploids observed in the progeny of field-pollinated diploids. Polyploid progeny are formed at a low rate in nature, only ca. 2% or even 0% (for a comparison of several studies, see Table 1 in Verduijn et al., 2004), but a high number of polyploid offspring may still be theoretically created – up to 10,000 each year in a population of 2500 sexual individuals (Verduijn et al., 2004). Although the frequency of neo-apomict formation in nature is low, they play an important role in the evolution of the apomicts.

Within *T*. sect. *Erythrosperma* with sympatric populations in Central Europe (Kirschner & Štěpánek, 1994; den Nijs, 1997), we observed apomictic genotypes that were derived from clonal mating by hybridisation with sexuals. The important role of apomictic tetraploids in the formation of new triploid apomictic lineages via the 2x-3x-4x pathway was described by Verduijn et al. (2004). The structure of apomictic clones as described by Mes et al. (2002), van Dijk et al. (2009) and Majeský et al. (2012) was confirmed in the present study. This structure consists of i) a core clonal genotype, ii) a network of clonal mates with accumulated somatic mutations, and iii) asexual lineages, which represent the most diverged part of this structure. When apomicts act as pollen donors, the formation of new apomicts is possible only through unreduced or partially reduced pollen grains, because of lethal effects of accumulated mutations on haploid gametes (van Dijk et al., 2009). Two-thirds of the neo-apomictic genome will have apomictic history, and one-third will represent newly created sequences. This new apomict will be partially free of the mutational load of the maternal apomict and also gain new variability (van Dijk, 2003; van Dijk et al., 2009). As for its morphology, the new asexual lineage will be similar to the maternal apomict (this study; Majeský et al., 2012). Each subsequent hybridisation dilutes the original maternal genotype, which disappears in neo-apomicts (van Dijk, 2003). The different morphology of newly established apomictic clones could be the outcome of this process. Newly formed apomictic clones run the risk of extinction by demographic stochasticity (den Nijs, 1997; van Dijk et al., 2009). However, new clones originate continuously in mixed populations (present study, Menken et al., 1995; Meirmans et al., 2003). Due to the effective seed dispersal system of dandelions, the most successful genotype may expand over a large geographic range and become widespread (several examples can be found in Kirschner & Štěpánek, 1994). Sympatric populations allow apomicts to acquire genetic variability, which may be later positively selected for and bring some advantage to the neo-apomict. The evidence for ongoing hybridisation in mixed populations can be observed in the presence of abundant morphotypes, which are apomictic. However, it is not possible to assign them to any known morphological clone or microspecies (data not shown; Trávníček, personal communication).

Conclusions

Our previous study (Majeský et al., 2012) revealed that a sample of apomictic dandelions exhibits signs of ancient hybridisation. However, apomictic accessions in the absence of sexual relatives undergo only clonal reproduction, resulting in the formation of a network of related clone-mates. In the present study, we extended our sample of apomictic dandelions by studying apomicts in the contact zone with sexuals. Contrary to previous studies, based on "overall" population sampling, our approach, based on the concept of microspecies and morphology of sampled individuals, allowed us to draw the following conclusions. Although continuous mutation accumulation plays an important role in the microevolution of apomicts, the importance of occasional sexual reproduction via a sex-asex cycle (as described by van Dijk, 2003) was documented in our populations. Furthermore, this gap need not always be bridged via tetraploids as proposed by Verduijn et al. (2004). The evidence for such events is not extensive, but some deviated phenotypes clearly showed hybrid origin between defined apomictic accessions and unknown sexual individuals. In our study, 20 out of 102 (19.6%) apomictic individuals showed signs of hybrid origin between a defined apomictic accession and a sexual. It is thus evident that a sex-asex cycle at the contact zone between obligate apomicts and sexuals contributes considerably to the microevolution of agamic complexes.

CHAPTER 4

Taraxacum pudicum, a new apomictic microspecies of the section *Erythrosperma* from Central Europe

RADIM J. VAŠUT

ĽUBOŠ MAJESKÝ

Abstract

An apomictic lesser dandelion, *Taraxacum pudicum* Vašut et Majeský is described here as a new species. The species is triploid diplosporous apomict and belongs to the *T. scanicum* group. It occurs in SE part of Central Europe with the highest frequency in southern Bohemia and Moravia. Species characteristics, notes on ecology and chorology are given. The relationship to other taxa and also the controversy of describing apomictic species are discussed in this paper.

Introduction

Taraxacum is a large genus of temperate zones of both hemispheres (Handel-Mazzetti, 1907; Sterk, 1987). It comprises around 60 sections worldwide (Kirschner & Štěpánek, 1997; 2004; 2008; Uhlemann et al., 2004) and is known for complex taxonomy due to combination of different reproduction strategies. Whereas all diploids (with just few exceptional tetraploids) reproduce either by allogamy or rarely by autogamy (e.g., Kirschner et al., 1994), all polyploids reproduce by obligate diplosporous apomixis. Since its discovery (Raunkiaer, 1903), there is enormous number of described apomictic (micro)species to date. The plethora of described taxa, along with the fact that some authors described new species from single locality or even single herbarium specimen, caused that describing apomictic lineages as new microspecies is not generally accepted and is controversial. However, the recent biosystematic approach brought the science back into taxonomy of apomicts. It is obvious that considering each morphological deviation as a new species is a *dead end*. Although genetic stability over large geographical areas due to obligate apomixis (e.g. Majeský et al., 2012) supports the microspecies concept, there is still high potential for hybridisation and formation of novel (singular) apomicts. Therefore, further biological characteristics such as distribution, morphological stability in offspring, confirmed reproduction mode or genetic characteristics are required for considering an apomictic lineage of dandelions as the species. Unfortunately, the vast majority of European *Taraxacum* species were described till 70's of the 20th century and it takes enormous effort to purge the *Taraxacum* species list off such a vague taxa. So far, only for some species of the *Taraxacum officinale* agg. and *T.* sect. *Palustria* were such revisions done (Kirschner & Štěpánek, 1998a; Lundevall & Øllgaard, 1999).

In this paper we present description of a new species of the *Taraxacum* section *Erythrosperma*. The first author (RJV) recognizes this morphotype over 15 years, we learned about its distribution, morphological stability, genetic diversity, ploidy and its reproduction modes. Following the modern approach of describing apomictic dandelions (e.g. Uhlemann, 2007; Uhlemann et al., 2007; Trávníček et al., 2008; de Mera & Orellana, 2008; 2009; 2012; Štěpánek et al., 2011; Marciniuk et al., 2012, etc.) we are convinced that this distinct morphotype deserves classification of the species. We thus here provide its valid description.

Material and methods

The present study follows previous studies and the methods of field sampling, flow-cytometry protocol of Doležel et al. (1989) and chromosome spreads preparations are described in Vašut (2003). Flow-cytometric seed screen (Matzk et al., 2000) was applied to test the reproduction mode, and microsatellites and AFLPs to test genetic diversity (Vašut et al., 2004; Majeský et al., 2012; unpublished). Plants were cultivated by both authors for period more than 10 years (with gaps since 1998) to confirm morphological homogeneity among populations as well as in the offspring. The terminology for describing morphological characters of dandelions uses special terms. In this paper, the morphological terminology follows terms used in Dudman & Richards (1997) and Vašut (2003). Localities, from which plants were used for the analyses are marked with an appropriate initials, i.e. for flow-cytometric analyses by [FCM], for the flow-cytometric seed screen by [FCSS], for the

chromosome number counting by [CN] and plants included in the genetic diversity study by [GD]. Plants cultivated in garden/greenhouse by [cult.].

Description of new species

Taraxacum pudicum Vašut et Majeský, spec. nova

[— *Taraxacum pseudocristatum* nom. provis.]

[— *T*. aff. *slovacum* Klášt. in Vašut et al. (2004: 647)]

(Fig. 1-2)

Description: Plants delicate with rich leaf-rosettes. Leaves pale (greyish) green, without spots, usually 4–5x longer than wide, circa 3–10 (–15) cm long and circa 0.5–2.0 cm broad. The leaf blade narrowly ob-lanceolate to eliptic, usually broadest in the middle or in upper $^1/_3$, having 3 (–4) pairs of lateral lobes. Lateral lobes of inner leaves narrow, falcate, having the distal margin denticulate and convex, proximal margin usually concave and entire. Lateral lobes of outer leaves usually triangular or broadly falcate, with entire margins, which are convex on distal margin and concave on proximal margin. Terminal lobe of outer leaves distinctly triangular to, acute usually undulate on distal margin; proximal margin often has a pair of distinct tooth close to central vein. Terminal lobes of inner leaves tripartite, shortly lingulate, sometimes denticulate on distal margin, with extended narrow acute tip. Interlobia large with scarce narrow tooth. Petiole unwinged, green to pale purple, lanate at the base. Scapes short, usually shorter or as long as leaves, green by flowering, purplish by ripening, slightly lanate. Capitulum convex, (pale) yellow, 2.5 cm in diameter, outer strips grey-brown with pinkish fade. Inner bracts greyish-green, corniculate. Outer bracts usually 10–12, lanceolate to narrowly ovate, usually 5–7 mm long, 1.5–2.0 mm broad, greyish green, with narrow but distinct white hyaline margin (0.1–0.2 mm broad), erect or only slightly recurved, corniculate. Stigmas yellow-green, dark-green when dried, pollen present (irregularly sized). Achenes brown-purple, almost brown when dried, distinctly chestnut red when not fully ripe, distinctly narrow, only sparsely spinulose, 3.6–4.0 mm long and 0.5–0.8 (–0.9) mm broad, pyramid narrowly conical to cylindrical, (0.6–) 0.8–1.0 mm long, rostrum 5–6 mm long, pappus white. Diplosporous apomictic species.

Holotypus: South-western Moravia, district of Znojmo, along paths and roads in woods on sands between villages Kravsko and Bojanovice, ca. 2 km northwards from the centre of the village Kravsko, 370 m a. s. l., 48°56'30"N, 15°59'12"E. Leg. R. J. Vašut, 1. 5. 2000 (Fig. 1). Holotypus deposited in OL. [FCM, FCSS, CN, GD, cult.]

Etymology: *pudicum* = chaste, modest, pure, virtuous. The name reflects short stems and usually pale green appearance of plants and overall delicate habitus of plants. Plants, especially in cultivation have flower heads "hidden" in pale green leaves.

Karyology: *2n = 3x = 24* (counted from the holotype locality); 2n ~ 3x - distinguished by flow-cytometry (Vašut et al., 2004**).**

Figure 1. Holotype of *Taraxacum pudicum*. Bar = 5 cm.

Figure 2. Isotypes of *Taraxacum pudicum*. Plants with well developed inner leaves are shown.

Mode of reproduction: The species is apomictic. The mode of reproduction was tested on single population (Kravsko-Bojanovice) and it was confirmed to be a diplosporous apomixis by FCSS. Additionally, seeds of plants from five different populations were confirmed to be obligate apomicts too. These are marked in the list of known localities by [FCSS].

Similar species: Newly described species *Taraxacum pudicum* is quite distinct species in its morphology and when it is well developed, it usually cannot be confused with any other described species in Central Europe. However, there is occurring (even rarely than *T. pudicum*) an undescribed morphotype sharing some characters. This morphotype (named as *T. scanicum* agg. morphotype III – "ARA" in Majeský et al., unpublished) differs mainly in more toothed leaf-blade, broadened appendices on lateral lobes (similar to those ones of *T. danubium* A. J. Richards) and broader (up to 0.5 mm) white hyaline margins on outer bracts. However, general appearance of delicate habitus, pale green colour of whole plant, patent to adpressed outer bracts and narrow achenes suggested putative relationship to *T. pudicum*, which was also confirmed by genetic analyses (Majeský et al., unpublished).

Plants from extremely dry habitats, growing on rocky slopes, are usually very small and have very narrow lobes. The terminal lobe is sometimes denticulate on such plants, which might lead to mis-identification with *Taraxacum cristatum* Kirschner et al. (Vašut et al., 2005). However, this species differs in several distinct characters that are not influenced by ecological conditions. Especially achenes of *T. cristatum* differ in broader shape, slightly more spinulose upper part of the achene and the brown colour. Outer bracts of *T. cristatum* are distinctly and disorderly spreading (to recurved) and it has not visually that distinct hyaline margin as *T. pudicum*.

Taraxacum discretum H. Øllg. (Øllgaard, 1986) occurring in NW Europe is another superficially similar species to *T. pudicum*. However, based on field observations of the first author (Schiermonnikoog, the Netherlands), on the original descriptions (Øllgaard, 1986) and on Hans Øllgaard's observations (H. Øllgaard, 2001 in litt.), it clearly differs from *T. pudicum* in having narrow leaves, recurved outer bracts that are dark (greyish) green and longer achene-cone that is up to 1.2 mm long.

Taraxacum slovacum Klášt. (Klášterský, 1938) is species described from single herbarium specimen of juvenile plants of *T.* sect. *Erythrosperma*. Juvenile plants of *T. pudicum* somewhat resemble plants on holotype specimen of *T. slovacum*. The first author visited the type locality of *T. slovacum* and did search intensively for any morphotype that would remind juvenile plants from the holotype specimen. Unfortunately, any plant of *T. pudicum* was found on the type locality (Zádiel, Slovakian Karst, SE Slovakia), neither in the whole region, but diploid sexuals having similar morphology to the holotype plants were quite frequently found on the type locality. Based on these field observations, *T. slovacum* is very likely a distinct morphotype of sexual species *T. erythrospermum* and is not related to *T. pudicum*. First author published genetic data (Vašut et al., 2004) for the *T. pudicum* under name "*T.* aff. *slovacum* Klášt.", because data on *T. slovacum* from the field were not available by that time.
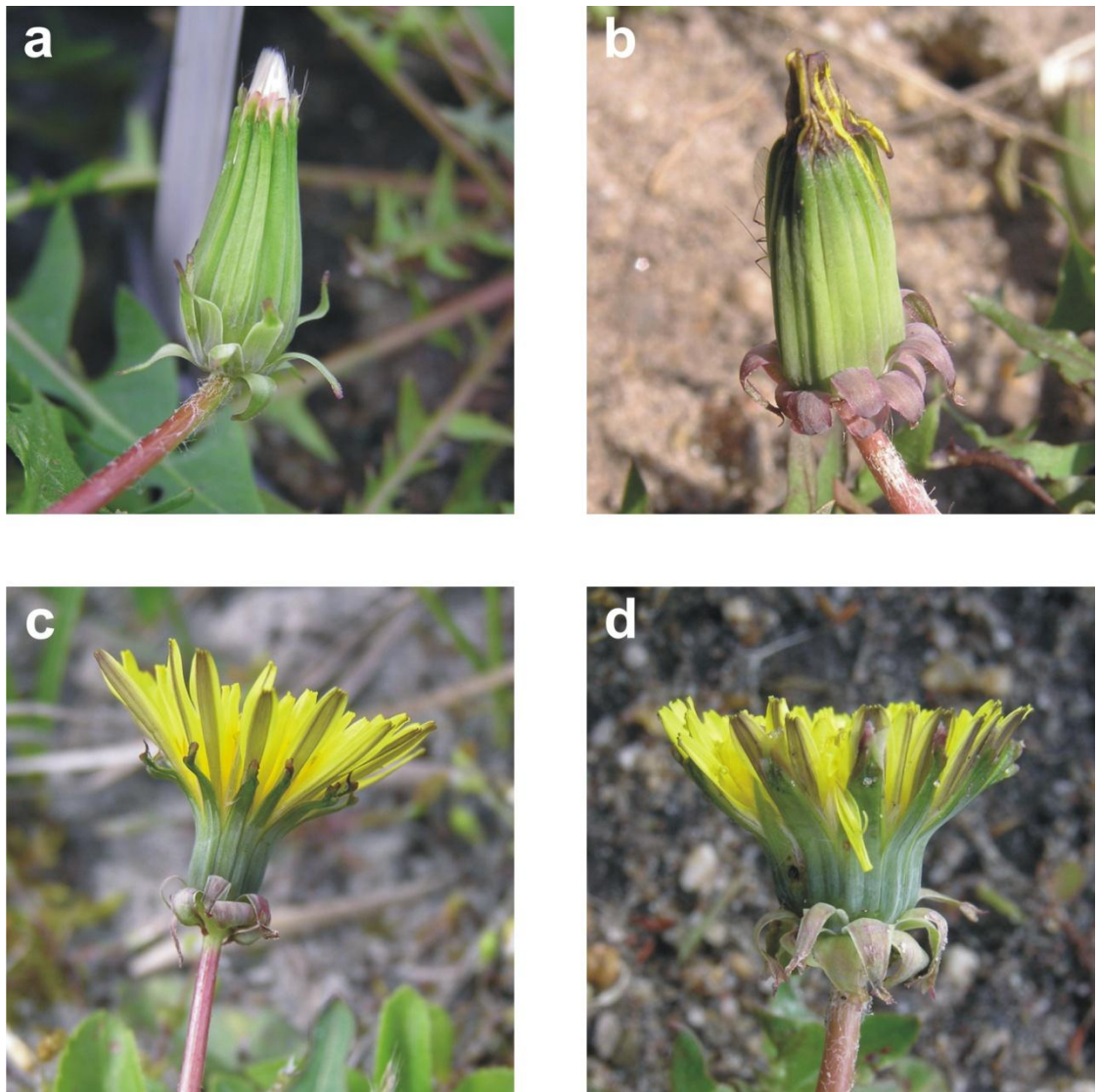
Figure 3. Capitulum of *T. pudicum* and allied species. Capitulum of *T. pudicum* is one of the most distinct determination characters of the species. Capitulum of (a) *T. pudicum* (Czechia, Kravsko-Bojanovice, type locality) is compared to capitulas of (b) *T. cristatum* (Czechia, Bzenec), (c) *T. discretum* (the Netherlands, Schiermonnikoog) and (d) *T. scanicum* agg. "morphotype III" (Czechia, Bzenec).

Ecology: *Taraxacum pudicum* mostly occurs in semi-ruderal dry habitats, especially on sandy soils (mostly paths in open locust-woods of the *Balloto nigrae–Robinion*). However, it grows also on similar semi-ruderal habitats in pine woods, xerothermic grasslands (*Festucion vallesiacae*, *Koelerio–Phleion phleoidis*) and occasionally in ruderal places, such as abandoned mining areas and road verges.

Distribution: *Taraxacum pudicum* is a Central European species. It occurs in southern part of the Czech Republic (both Bohemia and Moravia), Lower Austria and Slovakia. The species is rather rare and it has scattered frequency within the distribution area. Higher concentration of localities was observed in the region between the Brno and Znojmo city in Moravia (at the foothills of the Czech Massive).

Selected Herbarium Specimens
[FCM, FCSS, CN, GD, cult.]

Table 1. Main differences among *T. cristatum*, *T. discretum* and *T. pudicum*.

Morphological character	*T. cristatum*	*T. discretum*	*T. pudicum*
outer bracts - position	disorderly spreading	recurved	erect
outer bracts - hyaline margin	absent	distinct	narrow, but distinct
colour of outer bracts	pale green, when growing on dry biotopes might become purplish	dark green	pale greyish green, always green
achene colour	brown	red–brown	brown-purple
achene cone	0.7–0.9 (–1.0) mm	1.0 mm	0.7 mm
terminal lobe	longer than broad	longer than broad	as long as broad

Discussion

The newly described species, *T. pudicum*, belongs to the *Erythrosperma* section, which comprises (at least) one sexual diploid species, *T. erythrospermum* Andrz., and ca. 150 apomictic microspecies. Within this section, it belongs to the *Taraxacum scanicum* group that consists of 16 hitherto described species (Doll, 1973; Øllgaard, 1986; Schmid, 2002; Vašut, 2003; Vašut et al., 2005; Marciniuk et al, 2009). Widely distributed species of this group, i.e. *T. prunicolor*, *T. cristatum* and *T. scanicum* s. str., can hybridize with *T. erythrospermum* resulting in local or singular hybrid populations (Majeský et al., unpublished). Although we did not detected hybridisation between *T. pudicum* and *T. erythrospermum*, we however found similar morphotypes to *T. pudicum*, but genetically differing from it. Thus hybridisation between these species seems to be highly probable at sites where these species meet. This has to be taken into consideration when determining *T. pudicum* from localities with co-occurrence of *T. erythrospermum*.

The facts, that *i)* the distribution area of *T. pudicum* highly overlaps with NW limits of the distribution of *T. erythrospermum* in Central Europe, that *ii)* the species has significant plasticity depending on the biotope, and that *iii)* "sibling morphotype" occur on sands of Pannonia, led the first author to question whether it is a true species or just an hybrid of *T. erythrospermum* and some member of *T. scanicum* group (e.g. *T. cristatum*). The genetic

analyses of the microsatellites and AFLPs performed by the second author confirmed that individuals from different biotopes and different parts of its area are genetic clones and that if it is an hybrid then it is hybrid of older evolutionary history that spread over the SE part of Central Europe (see also Majeský et al., unpublished). *Taraxacum pudicum* is one out of 22 unnamed morphotypes mentioned by Vašut (2003) from the region of Moravia. Besides this species, Májeský et al. (unpublished) studied also genetic diversity of another three additional types of *T. scanicum* group out of these 22 unnamed types in order to find the origin of these local taxa. Not only *Taraxacum pudicum*, but also "morphotype III" turned out to be genetically uniform, although putatively hybridize with sexual species. Remaining two unnamed morphotypes (with provisional names *T. "graminicola"*, *T. "Trnava"*) appeared to be morphologically as well as genetically variable and therefore considered as a result of on-going hybridisation between group of apomictic species and sexual *T. erythrospermum* (Majeský et al., unpublished). We are convinced that such hybrids—although having apomictic reproduction—do not deserve a species rank and should be named by the name of the group, i.e. either as *T. scanicum* agg. or *T.* sect. *Erythrosperma* only.

CHAPTER 5

Where is the place for apomictic taxa in taxonomic hierarchy of apomictic genera? Let's take a look on apomictic dandelions

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Radim J. Vašut

Abstract

Apomixis, the seed production developed through omitting a syngamy of female and male gametes, is highly interesting mechanism for researchers in plant biotechnology, genetics, evolutionary biology as well in taxonomy. Apomixis may be seen as rescue system for newly produced hybrids, especially if they suffer from the sterility. It enables conservation of genotypes that could be advantageous in certain environmental conditions. Apomictic plants may easily colonize free ecological niches by only one or few individuals and overcome/overgrow slower outcrossing sexuals. In spite of the indisputable short-term advantages of apomixis, the asexuality was often considered to suffer from accumulation of slightly deleterious mutations leading to definite extinction of apomictic clones. However, apomicts in general can be genetically diverse. This variability comes either from accumulation of mutations, or from the (occasional) hybridisation with sexuals. Facultative apomicts are highly variable in degree of sexual and asexual seed production, which cause the high inter- and intraspecific diversification of apomicts. Even in populations of obligate apomicts gene flow from apomicts to sexuals is possible due to functional male meiosis and production of viable pollen grains by apomicts. Occurrence of apomixis in plant genera, in which hybridisation together with polyploidisation play important role in diversification, cause severe problem associated with taxonomy. It is questionable, which approach should be used for taxonomic treatment of apomictic taxa. Definitely, Biological Species Concept is not suitable, but the variety of evolutionary processes in different genera hampers to adopt one universal concept. On following pages we would like to briefly summarize some characteristcs and problems associated with taxonomy of apomicts. We offer basic review of approaches used in some apomictic genera. In more detail we discuss the approach used in taxonomy of apomictic dandelions. Finally, we argued with present system and put some evidences in favour of this concept.

Introduction

The driving force of the evolution is reproduction. This process evolved in all clades within living nature. The reproduction ensure the persistence of genealogical lineage of genes coming from a common ancestor. It leads to creation of a new individual or creation of an exact copy of parental genotypes/phenotypes. Creation of clonal copies of a maternal genotype attracts attention of scientists because of exceptional specifics of this process (for review sees e.g. Ozias–Akins & van Dijk, 2007) and its consequences (e.g. van Dijk, 2003). Uniparental – clonal reproduction by means of seed production is referred as apomixis (Asker & Jerling, 1992). It has several types and modifications as sporophytic apomixis and gametophytic apomixis. The gametophytic apomixis has two major developmental groups, i.e. apospory and diplospory (Koltunow, 1993). Although there is more then fourty modifications and types of apomixis, the genetic consequence is always the same – production of progeny with the maternal genotype (Asker & Jerling, 1992). Developmental pathway of apomixis requires at least two steps, i) avoidance of meiotically reduced megagametophyte instead formation of unreduced female gametophyte – apomeiosis and ii) omitting of syngamy and thus parthenogenic embryo development (Koltunow, 1993; Ozias-Akins, 2006).

Progeny arosen in such way had been for long time considered to be an evolutionary dead end due to lack of an adaptive variability (Asker & Jerling, 1992). However, a high diversity observed in populations of apomicts has changed the opinion on the doomsday scenario for apomicts (Richards, 2003). Asexual organisms have also other sources of genetic variability than genetic recombination during meiosis. These sources include accumulation of somatic mutations, chromosome rearrangements (Richards, 1996), recombination during restitutional meiosis (van Baarlen et al., 2000), polytopic origin of apomicts from genetically divergent sexual ancestor (*Ranunculus carpaticola* x *cassubicifolius* – Hörandl et al., 2009; *Potentilla argentea* agg. – Paule et al., 2011; *Boechera divaricarpa* – Dobeš et al. 2004) and facultative sexuality. Because apomicts have a functional male meiosis, then even obligate apomicts produce (at low ratio) viable pollen grains. Therefore, apomicts partake in a gene flow (Tas & van Dijk, 1999; van Dijk, 2003; Mártonfiová, 2006) This scenario, i.e. apomicts serve as pollen donors for a sexual ovule, is prevailing among apomicts, however, also bidirectional gene flow is possible (de Wet, 1968; Reno et al., 2001; Schranz et al., 2005). Majority of apomictic taxa preserve facultative sex and obligate apomixis is rather rare among flowering plants (Asker & Jerling, 1992). Especially plants with the aposporic type of apomixis tend to be facultative apomicts, Besides the non-reduced megagametophytes, also reduced develop; further, even within one individual/inflorescence both sexual and apomictic ovules can be found (Asker & Jerling, 1992; Koltunow, 1993).

What cause a complexity of species concepts in apomitcs?

Apomixis as a developmental trait is strongly linked to hybridisation and polyploidisation (Asker & Jerling, 1992; Carman, 1997; Whitton et al., 2008a). Many apomictic taxa are found within few genera with hybridisation and polyploidisation as important process of diversification. These genera belong mainly to the three families, i.e. *Asteraceae*, *Rosaceae* and *Poaceae*, although apomicts are (rarely) known in all major clades of flowering plants (Carman, 1997; Richards, 2003; Whitton et al., 2008a). Apomixis causes

formation of reproductively isolated individual genotypes restricted from genotype homogenization, unlike in sexual reproduction. Clonal reproduction combined with a reproduction barrier may lead to overrepresentation of clonal genotypes in short time. If these apomictic lineages are stable in their morphology over time and space, then they signs of the biological species.

Apomictic taxa have extremely narrow morphological variation, which often fails within broad morphological variation of their sexual relatives. In regions where sexuals and apomicts meet, hybrid swarms are often formed for the both, facultative and obligate apomicts. In general, apomictic groups represent a complex reticular network of sexual species, stable (and widespread) apomictic lineages and their singular hybrids (restricted to place of origin). Clonal copying of individual genotypes maintains morphological uniformity of each clone. Nevertheless, apomicts are not without a variability, as discussed above. However, these sources of variability have only a limited impact on the phenotype. The only important source of variability is hybridisation. Facultative sexuality may greatly enrich apomictic gene pool and lead to high phenotypic variability as shown e.g. for *Ranunculus auricomus* complex (Hörandl et al., 2009); North American *Amelanchier* species (Campbell & Wright, 1996); and *Rubus* subg. *Rubus* (Davis, 1958; Nybom, 1995). Whereas obligate apomicts are often discrete morphological lineages, facultative apomicts forms morphological gradients between putative parental species.

In genera with a frequent occurrence of apomixis, infrageneric groups were created and taxons are treated in informal groups as aggregates, complexes or sections, within which particular clones/lineages with well morphological/genetic characteristics are named as microspecies (Kirschner & Štěpánek, 1996; Hörandl et al., 2009). This approach is not always suitable and sometimes under one name several different unrelated clonal lineages are grouped (e.g. *Ranunculus auricomus* complex – Hörandl et al., 2009) or this approach generates separate names to each apomictic clone differing only a bit from another morphologically similar taxa (Weber, 1996).

Good colonization ability of apomicts may lead to rapid distribution of apomictic clone to remote places and occupying wide geographic area e.g. some clones of *Taraxacum officinale* agg., *Rubus plicatus*, *Sorbus danubialis*, several *Hieracium* species etc.). However, when apomicts freely hybridise with sexuals or other facultatively apomictic taxa, and moreover backcross with them, they form a great number of novel hybridogenous genotypes, which may never become more than locally dispersed (e.g. *Potentilla alpicola* – Paule et al. 2012; *Rubus* – Weber, 1996). Giving names to such apomictic lineages will again lead only to more intricate taxonomy.

Species concepts

Evidence that there is no universal species concept equally applicable to sexual and to asexual species can be seen in numerous scientific papers (Richards et al., 1996; Dickinson, 1998; 1999; Hörandl, 1998; Stace, 1998; Weber, 1996; etc). Separation of exclusively sexual taxa within apomictic complexes and their treatment under *Biological Species Concept* helps to purge complicated taxonomy. Sexual taxa in apomictic genera have different origin and come from different evolutionary processes than apomicts (Richards, 1973; Kirschner & Štěpánek, 1996; Hörandl et al., 2009). This approach is used in all of European and North

American apomictic genera, for instance in *Taraxacum* (Kirschner & Štěpánek, 1996), *Rubus* (Weber, 1996), *Crepis* (Stebbins & Babcock, 1939). However, the biological species concept in sense of Mayr (1942) which define species as "groups of interbreeding natural populations reproductively isolated from other such groups" is denied for asexual groups as useless (Hörandl, 1998; Dickinson, 1999 and references therein), because of lack of gene flow within apomictic species.

The *Phenetic Species Concept* put emphasis on a phenetic cluster as a base for species recognition. The species is considered to represent the smallest homogeneous cluster that can be recognized based on a particular criterion as being distinct from other such cluster (Sneath & Sokal, 1973). To define species not only morphology, but also genetic, karyotypic, ecological or behavioral characters can be used (Mishler & Budd, 1990; Hörandl, 1998). This concept is equally applicable to sexuals and asexuals and partly is used in some apomictic groups – *morphospecies*; e.g. *Taraxacum*, *Crataegus*, etc.. Problems may arise when only few characters are available for species delimitation. Molecular markers cannot substitute absence of other characters, because they often show reticular pattern as a consequence of complex history of apomicts. It also lacks the historical/evolutional connection link, which would make species vivid unit, not just individuals sharing some characters. The facultative apomixis will cause another problems by segregating of lineages from variable taxon. On the other side careless using of morphology may lead to endless describing of asexual lineages as separate species (Mishler & Budd, 1990; Hörandl, 1998).

Unfortunately, the discovery of apomixis as an ideal reproduction barrier led to a massive increase of described taxa in 20th century based on the concept of the *morphospecies*. The genera like *Taraxacum*, *Hieracium*, *Rubus*, *Sorbus* and other are known for the plethora of described species. Some authors—expecting that each single morphotype is reproductionally isoloated by apomixis—described tens or even hundreds of species based on limited material or even single herbarium speciemens. This approach has its roots in Scandinavia where only apomicts (in the absence of sexuals) are known and apomicts usually form discrete morphological units. Adopting such approach in regions with co-occurrence of sexuals and apomicts led to describing unrealistic species. The myriads of described "species", which in fact represent either a singular F_1 hybrid (either apomictic or sexual) or even a distinct but unique morphotype of sexual species, made the taxonomy of apomicts more the philately then the science. Apomictic taxa described from a single plant or a single locality in regions with occurrence of sexuals (e.g. Central or Southern Europe) are controversial and should not be considered as true species.

Wiley (1978) put forward the *Evolutionary Species Concept*, under which a unique role of the evolutionary process leading to a formation and maintenance of the lineage of an ancestor and descendants is considering. The species represents "a single lineage of ancestral descendant populations of organisms, which maintains its identity from other such lineages and which has its own evolutionary tendencies" (Wiley, 1978).

Van Valen (1976) adds the importance of a unique ecological niche occupying by the members of an evolutionary unique lineage as another criterion for defining species under the *Ecological Species Concept*. Species is a lineage occupying ecological niche minimally different from that of any other such lineages (van Valen, 1976).

Mishler & Budd (1990) stressed that under the *Evolutionary* and *Ecological Species Concepts* the breeding system represents main cohesive mechanism in the maintenance of species identity, which may be the weakness of such concepts.

The *Cohesion Species Concept* of Templeton (1989) takes into account an importance of cohesive and evolutionary mechanisms and is applicable to both sexual and asexual organisms. "Species is the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms (Templeton, 1989). Main limitation of this concept may be the focus on the processes and not on patterns (Hörandl, 1998) and threat that it is not ensure that recognized species represent phylogenetic lineage (Mishler & Budd, 1990).

Another often discussed species concept is *Phylogenetic Species Concept*. There are several types of this concept, but we refer to the Cracraft´s (1983) concept. Species is the smallest diagnosable unit of individuals with a genealogy maintained by reproductive cohesion (Cracraft, 1983).

Under the *Genealogical Species Concept* species represent groups of organisms, which "genes unite in a common ancestral gene", thus it requires monophyletic lineages/species (Baum & Shaw, 1995). The condition of monophyly is questionable, which taking strictly in apomicts may be much smaller than anyone would like to be (Mishler & Budd, 1990).

Hörandl (1998) proposed the concept where "a species consist of all organisms of an ancestral-descendent lineage, which are products of the same evolutionary process, which have constancy of progeny upheld by a certain reproductive system and consequently a similarity of phenetic and of ecogeographical unity". Emphasis is taken on internal similarity and constancy of characters/features connecting the organisms under species rank regardless of reproduction mechanism.

Overview of concepts used in some apomictic genera

Family *Poaceae*

Apomixis was recorded in cca. 28 genera of grasses with both major types of gametophytic apomixis—apospory and diplospory (e.g. Kellogg, 1990; Asker & Jerling, 1992; Carman, 1997). Apomixis in grasses is facultative and it allows gene flow among different genotypes and mating systems. Extent of sexually and asexually produced progeny within individuals or populations is highly variable. Moreover, it can be influenced by diverse ecological factors, e.g. in *Dichanthium annulatum* complex – day length influenced the percentage of apomictic embryo formation (Asker & Jerling, 1992). Apomictic taxa of grasses are mainly polyploid cytotypes of morphological species, which has also diploid cytotype represented by sexual outcrosser (e.g.; Müntzing & Müntzing, 1971; Schmelzer, 1997; Ozias-Akins & van Dijk, 2007; Akiyama et al. 2011). Apomixis itself does not mean formation of morphologically discrete units in grasses, but in the presence of gene flow leads to an increased genotypic and morphological variability. Finally, extent of any differences (whether genetic or phenotypic) will depend also on species life history and pattern of geographic distribution of apomictic and sexual cytotypes. Numerous studies tried to find differences in morphological characters between apomictic and sexual cytotypes/populations, but the result was similar in all – great continuous overlap of morphological traits (Kellog, 1990 and references therein; Schmelzer, 1997). This indicates increased variation due to existence of several asexual lineages lying on the edge of morphological/genetical/ecological variability and large amount of intermediates connecting the whole population. Following Kellogg

(1990) suggestion, grasses contain high number of "widespread variable hybrid complexes stabilized by facultative apomixis with local differentiation not sufficient to microspecies recognition". Apomictic cytotypes are not treated as separate microspecies, but rather are bulked in one "agamic complex" treated as a single species (Kellogg, 1990).

Genus *Crataegus* (hawthorns; *Rosaceae*)

Crataegus is the large genus well known for its taxonomic complexity (Talent & Dickinson, 2007). Numerous described taxa within genus are the result of too narrow species concept, which led to uncritical species description in second part of 19. century (Dickinson, 1999). Within the genus there are sexual self-incompatible (diploids) and asexual self-compatible (mostly triploid and tetraploid) reproducers (Dickinson et al., 2008). Apomixis is gametophytic type of apospory with pseudogamous endosperm development (Campbell et al., 1991). The genus is divided into ca. 15 sections and 40 series (Dickinson et al., 2008). Polyploidisation—both, allo- and autopolyploidisation—together with common hybridisation and backcrossing as well as with the apomixis are responsible for the complex intrinsic structure (Lo et al., 2010). Morphology together with geographic distribution was the base for recognition of the species within the genus. Many of morphological species represent mixture of cytotypes (Dickinson et al., 2007). To clear the taxonomy partially relaxed biological species concept is used today. Species are assigned into two species categories. Morphologically well differentiated sexual or partially-sexual diploids or tetraploids (forming the series) are recognized on the base of biological species concept. Facultative apomictic polyploid taxa with supposed hybrid origin are assigned into second group and are treated as microspecies or taxa within agamic species complexes (Dickinson et al., 2007; Dickinson et al., 2008). Such agamic complexes comprise a set of morphologically similar apomictic taxa.

Genus *Amelanchier* (shadbushes; *Rosaceae*)

Representatives of the genus *Amelanchier* are medium-sized shrubs or small trees inhabiting the North Temperate Zone. Taxonomically the most diverse is the genus in the North America (NE USA and SE Canada) (http://sbe.umaine.edu/amelanchier/). Unlike the hawthorns, apomictic shadbushes are less numerous, containing 33 species (Robertson et al., 1991). Apomictic taxa are polyploids – mainly tetraploids with the pseudogamous apospory (Campbell et al., 1991). Apomixis is facultative and apomictic individuals possess high degree of sexuality (Campbell & Wright, 1996). Frequent hybridisation within the North American *Amelanchier* resulted into the complex network of hybrids and hybrid swarms (Campbell & Wright, 1996). The problem with evaluating apomictic and hybrid taxa comes from the lack of enough differentiation between population of apomictic taxa and hybridisation among them (Campbell & Wright, 1996). Diploid/sexual taxa (in the North America there is only one exclusively sexual species *A. bartramiana*; Campbell et al., 1987) represent discrete unit and fulfill the definition of species, while polyploid apomictic taxa are weakly differentiated (some based on unique morphological deviation) with much narrow distribution and are referred as microspecies (Campbell et al., 1997; Campbell & Wright, 1996).

Genus *Sorbus* (whitebeam, rowan, service tree, mountain-ash; *Rosaceae*)

Interspecific hybridisation can be seen as the major speciation force in this genus with pseudogamous apospory (Liljefors, 1953). The genus *Sorbus* has worldwide distribution with prevailing sexual reproduction. In Europe there are four to five primary diploid sexual species with clear morphological characters and wide distribution. These are often treated on the subgenera level (Nelson-Jones et al., 2002), or even should be classified as separated genera (Campbell et al. 2007). Similar approach, i.e. sexual species with broad morphological variability and apomictic taxa with very narrow morphological variation, is also applied on the Asian taxa recently (e.g. *Sorbus hupehensis – glabriuscula* complex; McAllister, 2005). Interspecific hybridisation of primary species leads to a formation of homoploid and polyploid hybrids either facultative apomictic or sexual, which are ranking on microspecific level (Nelson-Jones et al., 2002; Lepší et al., 2008). Hybrid taxa have narrow morphological and genotypic variability and have limited distribution area – often being endemic to small area (e.g. Bernátová & Májovský, 2003; Robertson et al., 2004). Because of a wide distribution of some sexuals and primary hybrids, temporally and spatially isolated hybridisation of the same parental combination creates polyphyletic taxa (Robertson et al, 2010). This causes the problem with treating hybrids as separate species, because they are not monophyletic. Some authors consider products of geographically separate hybridisation of the same parental combination as different taxon (Lepší et al., 2008). To avoid an excessive growth of species names, hybridogenous taxa are treated based on morphology as different species, when the taxa are morphologically homogenous, reproductively isolated, have defined distribution and produce homogenous offspring (Lepší et al., 2008). Most of accepted microspecies are endemic species of just a local distribution. They usually have a parapatric distribution to their sexual parental species. While species described from single locality having just few tens or hundreds individuals are known, limited number of apomictic species have a wide distribution area that is overlapping with a distribution of its sexual parents (e.g. *Sorbus latifolia*, *S. mougeottii*, *S. intermedia*, *S. danubialis* etc.). The variation in reproduction modes of the European apomictic *Sorbus* species is yet unknown. However, it becomes obvious that taxa described from regions where sexual *S. aria* is common requires a critical revision (Vít et al., 2012). Many of taxa described from Southern Moravia, Slovakia or Hungary might only represent either a singular hybrid or a swarm of primary hybrids (M. Lepší, P. Vít, pers. communication).

Genus *Potentilla* (cinquefoil; *Rosaceae*)

The genus *Potentilla* consists of both diploid sexual and polyploid apomictic taxa. Apomixis within the genus is a pseudogamous apospory and formation of reduced and unreduced female gametes within polyploid taxa suggests that apomixis is facultative (Asker & Jerling, 1992). Within the genus at least 15 apomictic species are recognized (Paule et al., 2011 and references therein). Hybridisation, polyploidisation and recurrent introgression are considered as main diversification forces in the genus (Dobeš & Paule, 2010) strengthened by stabilizing effect of apomictic reproduction. Several species comprise species aggregates with several forms of different ploidy levels, which are, by some authors, considered as microspecies, subspecies or variants originated through the hybridisation, polyploidisation and

backcrosses of sexual and facultatively apomictic individuals (Tomasz & Kołodziejek, 2008). Recent molecular studies within *P. argentea* and *P. collina* groups (case study of *P. alpicola*) proved that taxa within the groups originate from multiple repeated hybridisations associated with introgression (Paule et al. 2012). Apomictic lineages are homogenous at intrapopulation level but are more variable at interpopulation level (Paule et al, 2011). This is accompanied by the presence of locally distributed lineages, which reflects polytopic origin of these lineages (or cytotypes) from the different parental combinations (Nyléhn et al., 2003; Paule et al. 2012). Although homogenous lineages can be observed within apomictic taxa the backcrosses and lack of well morphological delimitation of taxa do not allow making of comprehensive taxonomic concept in the genus. Until the detail morphometric studies will not reveal the presence of clear characters for species delimitation (Paule et al., 2011; Paule et al., 2012), polyploid apomictic taxa are treated as apomictic cytotypes within the species groups. However, for local flora some authors recognized polyploid hybridogenous taxa on the microspecies level (Gregor, 2008; Tomasz & Kołodziejek, 2008).

Genus *Rubus* (brambles; *Rosaceae*)

Brambles is another taxonomically complicated genus, within which apomixis affected species evolution. The genus is traditionally classified into several subgenera, but only one—i.e., *R.* subg. *Rubus* —represents group with predominantly asexually reproducing species (Alice & Campbell, 1999). Only three exclusively sexual species occur in mainland Europe (plus few other sexual species on Macaronesian islands and one species in Caucasus) and great majority from cca. 750 species are facultative apomictic polyploids (Kurto et al., 2010). Pseudogamous apospory with highly varying level of facultative sexual development of both reduced and unreduced embryo drives enormous potential for hybridisation and formation of novel apomictic lineages (Šarhanová et al., 2012). Polyploidisation and hybridisation among different cytotypes with different reproductive mechanism gave origin of species series with different composition of cytotypes and varying in the level of facultative sexuality and clonal reproduction (M. Sochor, unpublished results). Patchy distribution of different series and cytotypes cause extensive hybridisation and origination of different parental lineages on the local scale. Hybrid lineages are stabilized by apomictic reproduction but occasional switch to sexuality may enrich the gene pool of local apomictic genotypes. Switching between reproduction modes may cause various ecological factors (Šarhanová et al., 2012) and the intricate network of different hybrids/lineages can arise. However, apomictic reproduction is able to stabilize genotypes that can be homogenous and possess discrete morphological characters. Such genotypes can be also recognized by taxonomists in the field (Davis, 1958). Taxonomic treatment of such apomictic species passed several approaches (for review see Weber, 1996). Recently, Weber (1996) suggested the species concept that seems to be most appropriate for taxonomy of apomictic brambles. As the main criterion for species delimitation he chose the width of geographical distribution. He suggested four categories: 1) wide distributed apomictic biotypes with distribution radius $500 \leq 1000$ km (like *R. plicatus*, *R. nessensis*, etc.); 2) regionally distributed biotypes, radius $50 - 250$ km (like e.g. *R, austromoravicus* or *R. bohemiicola*); 3) locally distributed biotypes, distribution area ≤ 20 km and 4) individual biotypes represented by single bush spread by runners only on very restricted area. The taxa from the first two categories are treated as

taxonomic species in local floras, while taxa from the last two categories are not. This system is suitable to describe evolutionary relevant asexual species and avoiding taxonomic conflicts from supernumerary species description. This concept helped to purge the taxonomy from the enormous number of local species described in 19th and 20th century and reduced the number of true species in Floras to reasonable one.

Genus *Alchemilla* (lady´s mantle; *Rosaceae*)

The genus *Alchemilla* is widespread and the species richest in Eurasia, but many species are native also to other parts of the world (Sepp & Paal, 1998; Gehrke et al., 2008). Since Linnaeus described first three species of lady´s mantles (*A. vulgaris*, *A. alpina*, *A. pentaphylle*a), great numbers of species have been described then. There were different opinions about the taxonomy of the genus, both on the suprageneric and also on the subgeneric level, leading to different classification systems (e.g. Sepp & Paal, 1998; Gehrke et al., 2008; and references therein). Recent phylogenetic work of Gehrke et al. (2008) favours acceptance of the condition of monophyly for genus interpretation in the wide sense. Under this condition the genus consist of 4 separate clades *Eualchemilla*, *Aphanes*, *Lachemilla* and *Afromilla*. Infrageneric classification of "*Eualchemilla*-clade" is based on division into sections, series and groups (Sepp & Paal, 1998; Sepp et al., 2000; Gehrke et al., 2008). Split view on the genus classification comes from complicated taxonomy of the whole genus. This is caused by the presence of apomixis – apospory with autonomous endoperm development (Asker & Jerling, 1992; Gehrke et al., 2008), high polyploidy, lack of diploid sexual taxa and lack of distinctive morphology between taxa with continuum of overlapping characters (Sepp & Paal, 1998; Sepp et al., 2000; Gehrke et al., 2008; Pihu et al., 2009). Many of apomictic taxa do not produce pollen, however pollen fertile species are known too (Gehrke et al., 2008; Pihu et al., 2009; and referencies therein). Opinions on apomixis in lady´s mantles differ among authors: as to be obligate (Asker & Jerling, 1992; Gehrke et al., 2008), or facultative (Sepp et al., 2000). Past (and eventually also recent) hybridisation is proposed to be responsible for the complex patterns of morphological variations (Sepp & Paal, 1998; Gehrke et al., 2008). Many apomictic taxa were described as true species or as agamospecies – microspecies (Sepp & Paal, 1998; Sepp & Paal, 2000). Morphological, molecular and ecological investigations found that many of these microspecies are practically indistinguishable and are polymorphic, while only few possess distinctive, weĺl preserved characters enabling their differentiation (Sepp & Paal, 1998; Sepp & Paal, 2000, Sepp et al., 2000; Pihu et al., 2009). High infraspecific variability makes the proper species classification doubtful, while higher taxonomic ranks (as sections, series or agamospecies groups) are well determined in both morphology and genetics (Sepp & Paal, 1998; Sepp & Paal, 2000; Sepp et al., 2000). Classification of apomictic taxa under macrospecies (groups comprising a set of several morphologically similar apomictic taxa classified as different agamospecies) was suggested by Sepp et al. (2000) to reflect the best the current knowledge of relations within *Alchemilla*.

Genus *Boechera* (rockcress; *Brassicacea*)

The North American genus *Boechera* represent unique occurrence of apomixis within the otherwise sexually reproducing family *Brassicaceae*. Apomixis is of *Taraxacum* type – i.e. diplospory with either autonomous or pseudogamous endosperm development (Ozias-Akins & van Dijk, 2007). *Boechera* represents well documented example of apomixis at the diploid level (Dobeš et al. 2007, Kantama et al., 2007). Except diploids, apomictic are also triploids as well as aneuploids (Kantama et al., 2007); tetraploid and higher ploidy levels are rather infrequent (Dobeš et al. 2007). Intensive research showed that apomictic individuals retain high level of sexuality and hybridize with obligate sexuals (Schranz et al., 2005) establishing new asexual lineages. Asexual mode of reproduction in *Boechera* is associated with hybridisation and is of ancient origin (Sharbel et al., 2009; Sharbel et al., 2010; Beck et al., 2011; Kiefer & Koch, 2012). While diploids are highly homozygous because of prevailing self-compatibility, apomicts show high level of heterozygosity (Song et al., 2006). Evolutionary relationships within the genus are highly complex due to reticular hybridisation and apomixis (Rushworth et al., 2011; Kiefer & Koch, 2012). Interspecific hybridisation is very common and sexual species tend to hybridize whenever they come into contact. Both diploid and triploid apomicts are the result of hybridization of various sexual species. These apomictic hybrids are able to further backcross with sexuals (Windham & Al-Shehbaz 2006; 2007a,b; Beck et al. 2011).

Former taxonomic treatment was complicated and not appropriate. It was evident in mixing together different sexual and asexual species (Windham & Al-Shehbaz 2006). The consequent species were considered to be highly variable in both morphology and reproduction (e.g. case of *B. holboellii*, which has been split into 5 species and the case of hybrid *B.* divaricarpa, see Rushworth et al., 2011 and references therein). Following the taxonomic work of Windham & Al-Shehbaz (2006; 2007a,b) currently 71 diploid sexual species and 38 apomictic hybrids are recognized in the Flora of North America. However, there are much more apomictic hybrid taxa with unique genotypes that could be treated at species level. To avoid misidentification and retain sustainable taxonomy, apomictic hybridogenous taxa are recognized on the basis of their geographic distribution and presence of sympatric parental sexual taxa (Windham & Al-Shehbaz 2006; 2007a,b).

Genus *Ranunculus* (butercup/goldilock; *Ranunculaceae*)

Ranunculus represents cosmopolitan genus whose representatives can be found across the all major biomes, from the tropics through the mediterranean to the arctic regions (Emadzade et al., 2011). Polyploid cytotypes represent evolutionary derived level in phylogeny of buttercups. Apomixis is the facultative pseudogamous apospory and is restricted to polyploids – e.g. 4x, 5x, 6x (Nogler 1984; Hörandl et al. 2009; Cosendai et al., 2011). The most known and studied species complex within which apomixis cause reticulate relationships is *R. auricomus* complex (Hörandl, 1998; Paun et al., 2006b; Hörandl et al., 2009; and references therein). In *R. auricomus* complex great diversity of morphotypes, cytotypes and ecotypes was described (Hörandl & Gutermann, 1998a). For the practical purpose, the complex was divided into the smaller morphological groups: "*R. auricomus*", "*R. cassubicus*", "*R. fallax*" and "*R. monophyllus*" (for details see Hörandl et al., 2009), under which groups of

morphologically distinct populations were described as microspecies (Ericsson, 1992; Hörandl & Gutermann 1998b). The origin of the asexual hexaploid cytotype from the "*R. cassubicus*" morphogroup is in hybridisation among two sexual taxa form the "*R. cassubicus*" group: the tetraploid *R. cassubicifolius* and the diploid *R. carpaticola* (e.g. Paun et al., 2006b). Based on molecular studies, these two sexual taxa are well differentiated and separated from each other and form clump of polyploid apomicts (Paun et al., 2006b; Hörandl et al., 2009). For the classification of the clump of polyploid apomictic taxa, the category of nothotaxa (hybrid derivates of well defined sexual taxa) was suggested by Hörandl et al. (2009). For the polyploid product of hybridisation of this parental combination 2x–*R. carpaticola* × 4x–*cassubicifolius*, the name *R. carpaticola* × *R. cassubicifolius* was proposed (Hörandl et al., 2009). Diverse apomictic polyploid genotypes, results of facultative sexual processes and local hybridisation, are treated under this nothotaxon. This approach is useful for the *Ranunculus* apomicts, because unlike in other apomicts (e.g. *Taraxacum*, see Majeský et al. 2012), *Ranunculus* clones have limited distribution area and correspond to what is in *Rubus* called a locally distributed biotype (Cosendai et al., 2011).

Genus *Crepis* (hawksbeard; *Asteraceae*)

In North American species of *Crepis* autonomous apospory was described by Stebbins & Jenkins (1939). The genus became interesting for developmental, morphological and taxonomic studies (Stebbins & Babcock, 1939). The seven North American species comprise both diploid and polyploid populations, the later being sexual or apomictic. Two other species *C. barbigera* and *C. intermedia* are termed as agamospecies and comprise only polyploid apomictic individuals/populations. Apomictic polyploids of *Crepis* combine morphological characters of two or more sexual species (Whitton et al., 2008a,b). It seems, that the diploid sexual species are interconnected by the complex network of polyploid and apomictic hybrids (Stebbins & Babcock, 1939; Whitton et al., 2008a,b). This fact makes the taxonomic treatment difficult. Some of the polyploid apomictic populations were grouped and recognized as subspecies; e.g. *C. occidentalis* (Bogler, 2006).

Species concept in *Taraxacum* (dandelion; *Asteraceae*)

Dandelions, seems to be the successful genus that colonizes wide range of ecological niches from ruderal, or mineral rich fens through xerothermic or steppe to alpine and arctic biotopes. The success comes from the peculiar system how dandelions combine different life strategies providing their survival. Dandelions combine three different systems of reproduction, two sexual – outcrossing and selfing, and one asexual – autonomous diplospory (Kirschner & Štěpánek, 1994). Modes of reproduction are ploidy specific; diploids and very rarely tetraploids (Kirschner et al., 1994) are sexual, while all polyploids are apomictic (Kirschner & Štěpánek, 1994). In European dandelions, apomictic reproduction is prevailing in the most of the distribution area, while sexual diploids or tetraploids occupied much smaller area. Some exceptions with geographically widely distributed sexuals are known; e.g. *Taraxacum bessarabicum, T. erythrospermum, T. linearisquameum*; (den Nijs et al., 1990; Kirschner et al., 1994, Vašut, 2003). Many of sexual species, which are also considered to

represent evolutionary primitive and thus ancestral taxa (e.g. *T. glaciale*, *T. cylleneum* and *T. arcticum*) have relict distribution (Richards, 1973; Kirschner & Štěpánek, 1996). Evolutionary history of the genus is hazy because of strong reticular evolution (e.g. Witzell, 1999; Kirschner et al. 2003; Záveská et al., 2009). However, phylogenetic analyses based on plastome genes and karyology confirmed few links between ancestral and divergent taxa (Mogie & Richards, 1983; Witzell, 1999; Kirschner et al., 2003), which were suggested based on distributional and morphological data (Doll, 1982).

In contrast to facultative apomicts, dandelions prefer strict clonal reproduction before uncontrolled mating with all counterparts around. Although, there is no doubt that apomictic dandelions participate in the gene flow among the ploidy levels in the regions with sympatric occurrence of apomictic and sexual species. This gene flow is most likely bidirectional – ASEX › SEX, SEX › ASEX (Richards, 1970a; Verduijn et al., 2004, Mártonfiová, 2006.; Majeský et al., unpublished), even the scarce evidence for the second possibility (van Baarlen et al., 2000).

Taxonomy of dandelions underwent own "development" that was associated with increasing knowledge from the morphology, the ecology and the genetics of the species leading to better understanding of microevolution within the genus. Diversity of *Taraxacum* flora early led to the need for infrageneric classification, which would reflects evolutionary relations among diverse species. The base for infrageneric taxonomy comes from Handel-Mazzetti (1907) – the Austrian botanist and Dahlstedt (1921) – the Swedish botanist who independently used infrageneric categories in theirs monographic works. They used infrageneric ranks "*section*" (Handell-Mazzetti, 1907) and "*grupperna*" (Dahlstedt, 1921) to cluster the diverse taxa. Handell-Mazzetti describes also "*series*" and "*subsections*" within the described sections (Handell-Mazzetti, 1907; Richards, 1985; Kirschner & Štěpánek, 1987).

Present taxonomic treatment of the genus is based on the groups of ecologically, morphologicaly, karyologicaly and evolutionary similar taxa which are grouped under the sections. Section is the key-category in sorting the large diversity of *Taraxacum* species. The genus consists of more than 60 sections worldwide (Kirschner & Štěpánek 1997, 2004, 2008, Uhlemann et al. 2004). Sections are evolutionary not equal because they possess different origin (Kirschner & Štěpánek, 1996). Division of sections into three categories reflecting their origin and status help to gain the view into relations on sectional level. Richard (1973) divided sections in to the three groups based on the age of sections. This help to gain the insight into the relations among sections. Those three groups are: i) ancestral sections which represent evolutionary old taxa with primitive morphology, ii) precursore sections which are of evolutionary mid-aged and diverged from ancestral, and iii) advanced sections represent evolutionary young sections originated from repeated hybridisation and possess most derived morphological characters. Section contain one or few diploid sexual taxa and a clump of morphologically distinctive apomictic polyploids. Species grouped under sections have similar ecological requirements (Kirschner & Štěpánek, 1997). However, exceptions are present and several sections contain only sexual or only asexual taxa (Kirschner & Štěpánek, 1996). Sexual taxa are described as broad taxa with large distribution and with large phenotypic and genotypic variability (Kirschner & Štěpánek, 1998b; *Taraxacum gilliesii* – Uhlemann et al., 2004). Polyploid apomictic taxa are recognized based on morphology and are traditionally classified as "*microspecies*".

Handell-Mazzetti´s and Dahlstedt´s genus concepts differ in taxonomic approach how they treated the high number of apomictic taxa in *Taraxacum* flora. Handell-Mazetti (1907)

favoured so called "macrospecies" concept, while Dahlstedt (1921) and followed others taraxacologist (e.g. Railonsala, Marklund, van Soest and many others) established concept based on "microspecies". Difference in opinion of Handell-Mazzetti and Dahlstedt could come from different experience they got during they research. While in Northern Europe only apomictic taxa occur, in Southern and Central Europe both mating systems coexists in sympatry. In Europe there are two partially isolated regions with common occurrence of sexual dandelions (in sections as *T.* sect. *Taraxacum* [syn *T.* sect. *Ruderalia*] and *T.* sect. *Erythrosperma*); south-east and south-west with overhang to Central Europe and only islet occurrence of sexuals northern to this border (e.g. see Kirschner & Štěpánek, 1994; den Nijs & Menken 1994; Uhlemann, 2001).

Macrospecies represent broad morphological groups of many different and unrelated genotypes and thus are highly heterogeneous (and partly polyphyletic) in many aspects, evolutionary history not excluding. Even this concept may be "easier" and is still preferred by several researchers in some territories, it is not appropriate and useful. Modes of speciation and evolutionary processes in dandelions differ among groups, regions or they are often combined (Kirschner & Štěpánek, 1994). Macrospecies concept involves taxa with different ecology, evolutionary histories and overlapping morphology and therefore does not match well to any of above discussed species concepts.

In contrast, microspecies concept refers to morphologically homogenous narrow units with (to some extant) defined distribution and clonal reproduction. Nearly obligate clonal reproduction provides enough isolation from hybridisation "trips" in fully apomictic populations, however accidental sexuality cannot be omitted. It will mainly comes from aneuploids, which may lost one or more chromosomes during unbalanced restitutional meiosis (Sörensen, 1958) and thus may lost some from the genetic elements of apomixis. Other "short-cuts to sex" through occasional production of reduced ovules in otherwise diplosporic individuals are also possible (Richards, 1970b; van Baarlen et al., 2000; 2002). But still, these will be rare processes and may be under several negative selection pressures and are not able to significantly shape the genotypic and phenotypic gene pools of asexual clones.

If the monophyletic origin of apomictic lineage is used as a base for species delimitation of apomictic dandelions, this would be applicable to some (e.g. Majeský et al., 2012) but certainly not to all recognized taxa. It is because the evidences for the monophyletic or polyphyletic nature of dandelion clones are only scarce. Monophyletic origin have also some sections; e.g. *T.* sect. *Hamata*; *T.* sect. *Naevosa* etc. (e.g. see Witzell, 1999), but sections with a wide distribution – advanced sections have rather polyphyletic origin; e.g. *T.* sect. *Taraxacum*, *T.* sect. *Palustria*; *T.* sect. *Erythrosperma* – Kirschner & Štěpánek, 1996; Witzell, 1999, Kirschner et al., 2003). It means that on one hand some sections should be treated as one species, while on the other hand other sections should be split into much smaller units.

Phenetic approach is good for taxa which are homogenous and monoclonal. Such monoclonal units may represent evolutionary young species, with minimum of accumulated changes in their genomes. Several dandelion taxa have such monoclonal structure and are of young origin; e.g. *T. albidum*, *T. obliquum*, *T. hollandicum*, *T. pulchrifolium* (Menken & Morita, 1989; van Oostrum et al., 1985; Battjes et al., 1992; Majeský et al., 2012). Nearly monoclonal structure and thus high homogeneity can be found also in evolutionary old taxa, which lost significant portion of variability by shrinking of their areal and presently occupied only relict area; e.g. *T. alpestre* (Kirschner & Štěpánek, 1994). However, many of apomictic taxa have multiclonal structure (Battjes et al. 1992; Witzell, 1999; Kirschner et al., 2003;

Záveská et al., 2009; Majeský et al., 2012; Majeský et al., unpublished) and are phenotypically more plastic. Minor morphological differences among numerous apomictic taxa may cause the problems to fully accept the phenetic approach.

Šuvada et al. (2012) performed morphometric analyses of several characters, used in determination of apomictic microspecies from *T.* sect. *Erythrosperma*. He measured characters on both sexual diploid and apomictic triploid taxa and the results are in favour of presence of important morphological characters discriminating apomictic microspecies from each other. Modern molecular methods, as fingerprinting by various marker systems have good resolution ability on the lower taxonomic level – clones/microspecies and provide valuable data about species delimitation (Reisch, 2004; Majeský et al., 2012, Majeský et al., unpublished). Nevertheless, application of molecular markers on the higher taxonomic level may not help, because of reticular evolution (e.g. Witzell, 1999; Kirschner et al., 2003; Záveská et al. 2009), or residual ancestral polymorphism (Mes et al. 2002; Záveská et al. 2009).

Defining the apomictic taxa based on bulk of morphological, karyological and (in some cases) molecular-genetic characters has proved to be the proper way for characterization of huge number of apomictic taxa in dandelions. Unique evolutionary process and adaptation to specific ecological niche is also important for species delimitation in *Taraxacum* (Kirschner & Štěpánek, 1994).

We see present taxonomy based on sections and microspecies well suitable for systematic treatment of the genus. Sections represent basic unit of evolution in dandelions (Kirschner & Štěpánek, 1994; Kirschner et al., 2003). Sections are evolutionary and ecologically restricted to a given process of establishment and habitat. Among sections, there are marked differences, which (more or less in particular case) clearly delimitate sections (Kirschner & Štěpánek, 1994). On the other hand, microspecies can be seen as proper rank, which represents phenotypic and ecological unit of microevolutionary processes running in populations with mix mating. The clear view what really represent microspecies in the nature can be gained through investigation of ecological, morphological and molecular characteristics of microspecies. If we are looking on ecological population of microspecies as a single system, we will find there many different entities, which may or may not be evolutionary interconnected. Many of genotypes found within a population will simply miss any relations to each other, because they are of different origin, have different morphology, different micro niche requirements and are apomictic, thus reproductively enough isolated from the other. Such genotypes represent distinct microspecies. Clones – microspecies are genetically and morphologically highly homogenous with the low level of genotypic diversity and are significantly different from each other (Reisch, 2004; Majeský et al., 2012; Majeský et al., unpublished). For the diversity of clone many processes may be responsible as: transposon activity, somatic recombinations (e.g. see Richards, 1996) and somatic mutations (van der Hulst et al., 2003; Majeský et al., 2012; Majeský et al., unpublished) or also epigenetic changes (Verhoeven et al., 2010a,b). Through existence of a clone these changes will accumulate within the genome pool, which will result in a network consisted from the original clone and the clonal mates with asexually accumulated changes (Mes et al., 2002; Reisch, 2004; Majeský et al., 2012; Majeský et al., unpublished).

In regions where apomicts share populations with sexuals, gene flow among them is possible. Those apomictic clones that produce functional pollen may enter sexual process and thus expand the gene pool of original clone. If the new individual is created form fertilization

of reduced (haploid) ovule of sexual female by unreduced (3x) or partially reduced (e.g. 2x; or any possible aneuploid form) male gamete from asexual, resultant genome will be from the bigger part derived from the apomictic father and only quarter or third will be inherited from the mother. Then the new individual will be intermediate, with higher performance of characters inherited from parent with higher genetic input. However, the performance of particular characters will depend on the interaction between the alleles. The expectation is that, the new individual will genomicaly and phenotypicaly be more similar to the father than to the mother, but not identical. Such individual may be fully apomictic, or may lack one of the three traits of apomixis in *Taraxacum* – diplospory, parthenogeny or autonomous endosperm development (van Dijk et al., 1999). In this way neo-apomictic clones arise in nature.

Except unreduced or partially reduced male gametes, apomicts may produce also reduced gametes with nearly haploid number of chromosomes, which may fertilize the ovule of sexual. Origin of such gametes is very low, because of highly disturbed male meiosis, but some haploid or aneuploid gametes may arise (Tas & van Dijk, 1999). Diploid hybrids were acquired in crossing experiments but only in very low numbers (Tas & van Dijk, 1999) and it is questionable how frequently such hybrids arise in nature and what is their viability. Observed effect of heterogeneous pollen of polyploid apomicts in crosses with diploids is breakdown of self–incompatibility leading to mostly selfed and only few truly hybrid progeny (Tas & van Dijk, 1999; Mártonfiová, 2005; Mártonfiová, 2010).

Neo-apomicitc clone represent new asexual lineage of particular clone, which after recurrent hybridisation and/or geographical/ecological isolation, may develop into a separate clone – microspecies (Majeský et al., unpublished). Neo-apomictic lineage increases genotypic and phenotypic variability of original clone. Thus, in regions with occurrence of sexuals, apomictic clones – microspecies may consist of few genetically similar apomictic lineages and thus showing higher morphological variability than in fully asexual regions (Kirschner & Štěpánek, 1994; Majeský et al., unpublished). We see no contradiction in understanding of apomictic complexes when clonal taxon – microspecies is represented by more genotypes, as it is normal for sexually reproducing species comprising multitude of genotypes. From the regions of origin of neo-apomictic lineages, these may successfully spread across a wide area by long distance seed dispersal (Tackenberg et al., 2003) and sequentially reach the regions without sexuals.

Considering the status of microspecies as taxonomic rank, it seems to be proper unit for classification of asexual dandelions. In majority of cases microspecies are morphologically and genetically well definable with known geographic distribution (at least for some microspecies and in local floras). Microspecies represent vivid microevolutionary unit within the section. As showed our recent works; Majeský et al. (2012; unpublished) it is possible: to finely fingerprint different microspecies from different sections, to track the amount and origin of diversity within or among microspecies and also to gain some elementary imagination about the relations and possible origin of apomictic clones – microspecies in the nature. However, it is necessary (similarly to other European and North American apomictic genera) take into consideration the real value of apomictic morphotypes. Process of indefinite description of new microspecies that is based on limited (or any!) knowledge of its reproduction behaviour, morphological and genetic homogeneity in both, vertical (in the offspring) and horizontal scale (on large distribution range) or its relationship to sexual species, has to be strictly avoided. If the taxonomy of apomictic dandelions has to be

considered a serious science then species described from insufficiently characterized plants should be omitted from the Floras, especially in regions where sexual species co-occur and the probability of neo-apomicts formation is highly probable.

Conclusions

The question of finding the appropriate species concept, equally applicable to all diverse cases present in living nature, is a big challenge especially if we expect from the concept to reflects the basic evolutionary unit within the group. Diversity of apomictic plant groups is enormous and such diverse are also particular cases of species evolution. Concepts used for delimitation of apomictic taxa are mainly based on morphological, ecological and evolutionary characteristics and try to define taxa with respect to specific processes playing important role in diversification of genera. It is impossible to implement an universal approach to all the groups of organisms with known uniparental reproduction. Considering different life histories, different backgrounds and different mechanisms that accompany the existence and evolution of apomictic genera, which are often not known, it is not possible to treat all these diverse instances by one approach. Some genera is best to described by macrospecies concept (e.g. *Chondrilla* of *Asteraceae*), some genera are best described by the microspecies concept (as it is discussed above) and for some genera there is useful some mid-macrospecies concept like in *Ranunculus*. However, in taxonomy of apomicts should be a unifying rule that strictly stress the importance of understanding the biosystematic background of studied apomictic groups. It is impractical—especially in genera like *Hieracium*, *Rubus*, *Sorbus* or *Taraxacum*—to accept plethora of names referring to distinct morphotypes of uknown origin. Maybe, the most appropriate concept would be General Species Concept as described by de Queiroz (1998), which allows using of several different criteria for species delimitation. We see important point in the clear interpretation of evolutionary unit, which is objectively present in apomictic complex and recognizable in the nature. It facilitates the orientation within particular genera and also communication of specialists. On the other hand, for practical purpose it is essential to hold the systematic of apomicts easy to understand to other specialist outside the specialists on particular apomictic group. Therefore, keeping the infrageneric groups; e.g. collective groups, aggregates, sections, nothotaxa, etc might be crucial in general communication of non specialists.

SUMMARY

Summary

Apomixis is type of asexual reproduction via seeds through modification of mating system and leads to production of swarm of identical genotypes. This developmental trait shape the structure of apomictic genera. Narrow morphological variability of over-dominant clonal genotypes contrasts with genotypic and morphological variability of sexually reproducing species. Presence of this two mating systems in dandelions causes that we observe groups of many narrow morphological units (recognized as microspecies) reproducing clonally and few broad sexually reproducing species with high diversity. Because many asexual microspecies produce pollen grains, they may break reproduction barrier of apomixis and enter the sexual reproduction. This may consequently leads to the formation of new asexual genotypes, which increase diversity of maternal apomictic clone and establish the source for formation of new microspecies.

Analyzed clones of apomictic microspecies from group of *T*. sect. *Taraxacum* revealed to be highly homogeneous in genotyped loci, although they were not always uniclonal. Over-dominance of clonal genotypes in all investigated taxa could be explained as a long-lasted clonal reproduction accompanied by successful spread of those genotypes. High heterozygosity observed for AFLP and SSR markers is the result of fixed heterozygosity in apomicts and reflects a hybrid origin of apomicts. High homogeneity within particular taxa and high significant differences among taxa were evident from clustering methods. Investigated individuals made clusters, which corresponded to morphological identification. Very complex network of groups of genotypes was observed and several levels of relationships within this network may be suggested. Each taxon had one core characteristic clonal (abundant) genotype and several variants of this genotype, with little difference in studied loci. These variable genotypes were generated by the accumulation of small somatic mutations. Moreover, the clonal network is completed by diverse genotypes with bigger differences, which are results of hybridisation process. Two groups of investigated taxa have different origin – they do not share the same genetic pool. Taxa from the first group (consisted of three microspecies, denoted in Chapter 2 as *"OSP"* group) have probably a different and independent origin from each other. While taxa from the second group (group of six microspecies, denoted in Chapter 2 as *"AMP"* group) come rather from "one" diversification process. The close relation of "*AMP*" taxa is supported by allelic similarity of their genotypes as well as by sequencing data of cpDNA haplotypes. Within the "*AMP*" group, taxa share one core haplotype with two variants, which differ only by single-point mutations (deletion and substitution).

Similarities with above mentioned results were observed also in the group of lesser dandelions – *T*. sect. *Erythrosperma*. Because the sampling idea differs a bit from the previous study, it allows the observation of hybridisation process in more detail than in the first study. Characters of molecular diversity expressed by the number of genotypes, genotype diversity, heterozygosity, polymorphism and other population-genetic indices, were similar with indices gained for apomictic taxa from *T*. sect. *Taraxacum*, resembling the asexual life of investigated taxa. Network generated by apomictic taxa, was more complex due to presence of hybridogenous taxa with derived genotypes. Signs for hybridisation origin of these genotypes were evident on genotypic and also on morphological level. Genotypes of sexually derived taxa differed from the maternal clonal genotype in allelic profile and cpDNA haplotype. Moreover, it was possible to detect two groups of hybrid taxa. The first group represents

product of one hybridisation event – cross of maternal apomictic taxa/clone with sexual counterpart, and the second group represents most probably the product of recurrent hybridisation – cross of hybrid genotype with sexual mate. Individuals from both sexually derived groups differed in morphology comparing to the presumed maternal apomictic taxa. Individuals from the first group differed in a few characters – shape of terminal lobe and presence of tooth on distal margin of lateral lobes etc. Individuals from the second group were generally more different than individuals from the first group. Diploid sexual taxa, which were included to this study, showed highest variability for all markers. Almost whole range of allelic and haplotypic diversity observed within apomictic taxa was present within diploids.

Results of genotyping of apomictic taxa (denoted in Chapter 3 as *"PUD"* morphotype) contribute to decision for scientific description of this morphotype as a new microspecies. *Taraxacum pudicum* is a new apomictic microspecies from the section *T.* sect. *Erythrosperma,* native to Central Europe. Years of field observation and cultivation allow recognizing the morphology, chorology and ecology of this apomictic morphotype. Genetic data support the internal homogeneity of this morphotype and its distinctiveness from other similar microspecies.

Results from genotyping of apomictic and sexual taxa from two different groups of dandelions are consistent. They bring new knowledges and extend some already known assumptions about the relations and microevolutionary processes ongoing on "species" level within populations of dandelions. Clones of investigated apomictic taxa are homogeneous and majority of observed diversity may be interpreted as the result of accumulation of small somatic mutations within asexual genotypes. Low genotypic diversity of apomicts is continually increased by hybridisation with sexual taxa in regions of their sympatric occurrence. Such hybridisation mean for apomicts source of new alleles, thus variability, but also a diversification process. New genotypes may gain new traits, which may by positively selected and represent evolutional benefits. This may help to newly arising apomictic clones to spread over large areas – become evolutionary successful. Considering the structure of apomictic clones, their narrow genetic and morphological variability, which is real and possible to detect, it is appropriate to describe well characterized genotypes on the microspecies level. Description of microspecies among apomictic dandelions seems to be adequate method for cataloguing the great diversity of this genus and to make the systematic more plausible.

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SUPPORTING INFORMATION

Supporting information for Chapter 2.

The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts

Table S1. – List of apomictic *Taraxacum* accessions used in this study with sampling details.
Country abbreviations: CZ – Czechia; SK – Slovakia; collector abbreviations: BT – Bohumil Trávniček, RJV – Radim J. Vašut, LM – Ľuboš Majeský. The columns: F/H refers to plant material used for DNA extraction, F – fresh leaves, H – herbarium voucher, FB – flower buds; FCSS/EM refers to method used for examination of reproduction type, FCSS – Flow Cytometry Seed Screen, EM – emasculation, apo – apomictic seed formation; for *T. linearisquameum* FCM is showed where 2x = diploid sexual; SSR/AFLP/cpDNA shows which plants were used for SSR – microsatellite genotyping, AFLP – genotyping, cpDNA - sequencing of *trnL-trnF* region with observed haplotype + GeneBank accession number; b – double sample. Asterisk (*) indicates accessions recognized by taxonomists as validly described microspecies; absence of asterisk indicates distinct morphological groups but formally undescribed accessions (mentioned under „work names").

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
Amplum agg.					
amp1					
T. amplum Markl.					
a3	CZ	Lýsky village near Přerov town, wet meadows on the right bank of the Strhanec brook 0.5 km E of the village; 210 m a.s.l.; 49°28'49"; 17°27'50"; 3.5.1992; BT	F	apo/-	×/-/-
a5	CZ	Přerov town, roasides of the road towards Prosenice in the Žebračka wood N of the town, 210 m a.s.l.; 49°28'11"N; 17°27'49"E; 3.5.1992; BT	F	apo/-	×/-/-
a840	SK	Slavec village near Rožňava town, small meadow at the SW village margin; 48°34'55"N; 20°27'58"E; 7.5.2006; BT	H	-/-	×/-/-
a844	SK	Hronská Breznica village near Žiar nad Hronom town, meadow at the road towards Kozelník village in the valley of Jasenica brook 2 km NNE from the Rejchard hill; 625 m a.s.l.; 48°32'46"N; 19°00'11"E; 8.5.2006; BT	H	-/-	×/-/-
a859	CZ	Polesí village near Počátky town, meadows at the road towards Běleč village 1.2 km N of the village; 690 m a.s.l.; 49°18'15"N; 15°14'42"E; 13.5.2006; BT	H	-/-	×/-/-
a862	CZ	Obrataň village near Pacov town, meadow at the road towards Nechyba settlement at the S village margin; 600 m a.s.l.; 49°25'10"N; 14°56'40"E; 13.5.2006; BT	H	-/-	×/×/cp*1c*/ JQ696774
a865	CZ	Všechov village near Tábor town, meadow S of the road towards Dražice village near the W village margin; 480 m a.s.l.; 49°25'56"N; 14°36'51"E; 14.5.2006; BT	H	-/-	×/×/-
a911	CZ	Hartmanice village near Veselí nad Lužnicí town, lawns in the village and meadow at the E margin of the village; ca. 475 m a.s.l.; 49°12'27"N; 14°34'00"E; 22.4.2007	H	-/-	×/×/cp*1a*/ JQ696775
a913	CZ	Nasavrky village near Slatiňany town, lawns and meadows at the S margin of the village; 49°50'21"N; 15°48'11"E; 25.4.2007; BT	H	-/-	×/×/-
a916	CZ	Vernýřov village near Uhlířské Janovice town, lawns and roadsides in the village; 49°50'53"N; 15°09'21"E; 25.4.2007; BT	H	-/-	×/×/cp2/ JQ696807
a921	SK	Kolonica village near Snina town, meadows and lawns in SE part of the village; 48°56'57"N; 22°15'51"E; 29.4.2007; BT	F	apo/-	×/×/-
a932	CZ	Radkov village near Telč town, lawns and roadsides in the village; 49°08'42"N; 15°28'31"E; 2.5.2007; BT	F	-/-	×/×/-
a953	CZ	Křenov village near Český Krumlov town, meadow at the road 0.5 km S of the village; 540 m a.s.l.; 48°49'40"N; 14°15'12"E; 10.5.2007; BT	F	apo/-	×/-/-
a976/b	CZ	Jezernice village near Lipník nad Bečvou town, lawns and roadsides in centre of the village; 49°32'39"N; 17°37'34"E; 3.5.2008; BT	F/H	apo/-	×/×/-
a978	CZ	Vepice village near Milevsko town, lawns in the village; ca. 530 a.s.l.; 49°31'34"N; 14°18'00"E; 8.5.2008; BT	F	apo/-	×/-/-
a989	CZ	Křížov village near Vlašim town, lawns and meadows in W part of the village; 49°38'30"N; 14°53'39"E; 11.5.2008; BT	F	-/-	×/-/-
a995	CZ	Ponikev village near Konice town, lawns and roadsides in the village; 49°37'29"N; 16°53'01"E; 14.5.2008; BT	F	-/-	×/-/-
a996	CZ	Studená Loučka village near Mohelnice town, lawns and roadsides in the village; 49°46'07"N; 16°49'04"E; 14.5.2008; BT	F	apo/-	×/-/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
a999	CZ	Koclířov village near Svitavy town, lawns in E part of the village (E of centre of the village); 49°45'49"N; 16°33'06"E; 14.5.2008; BT	F	apo/-	×/-/-
a1000	CZ	Pohledec village near Nové Město na Moravě town, lawns and roadsides in N part of the village; 49°34'43"N; 16°05'59"E; 14.5.2008; BT	F	apo/-	×/×/-
aK07	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2007; BT	F	apo/-	×/×/-
aK08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	-/-	×/-/-

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T. albocarpaticum
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abc834	SK	Pstruša village near Detva town, lawns and roadsides near car park at the main road 1.6 km E of the village; 48°32'43"N; 19°20'20"E; 6.5.2006; BT	H	-/-	×/-/-
abc844	SK	Hronská Breznica village near Žiar nad Hronom town, meadow at the road towards Kozelník village in the valley of Jasenica brook 2 km NNE from the Rejchard hill; ca.. 625 m a.s.l.; 48°32'46"N; 19°00'11"E; 8.5.2006; BT	H	-/-	×/×/-
abc845	SK	Sklené Teplice village near Banská Štiavnica town, small meadow at the turning towards Repište settlement (S of the villae); 48°31'16"N; 18°51'43"E; 8.5.2006; BT	H	-/-	×/×/cp_1b_/ JQ696776
abc847	SK	Horná Ves village near Partizánske town, meadow at the road towards Veĺké Pole village SE of the village; 48°36'01"N; 18°30'11"E; 8.5.2006; BT	H	-/-	×/×/ cp_1b_/ JQ696777
abc852	CZ	Hroznětín village near Ledeč nad Sázavou town, meadow N of the road towards Tunochody village (near farm); 49°45'25"N; 15°20'25"E; 10.5. 2006; BT	H	-/-	×/×/ cp_1b_/ JQ696778
abc853	CZ	Miřátky village, meadows S of the road towards Habry town 1.3 km NW (-WNW) od the village (near Jiříkovský potok brook); 49°44'49"N; 15°30'40"E; 10.5.2006; BT	H	-/-	×/×/-
abc857	CZ	Červená Lhota village near Kardašova Řečice town, lawns at the car park in the village; ca.. 490 m a.s.l.; 49°14'56"N; 14°53'00"E; 13.5.2006; BT	H	-/-	×/-/-
abc864	CZ	Chýnov town, meadow N of the road towards Tábor town village near the SW town margin; ca.. 470 m a.s.l.; 49°24'15"N; 14°48'01"E; 14.5.2006; BT	H	-/-	×/×/-
abc902	CZ	Pístina village near Třeboň town, lawns in the village; ca.. 465 m a.s.l.; 49°03'01"N; 14°54'01"E; 21.4.2007; BT	H	-/-	×/-/-
abc904	CZ	Rapšach village near Třeboň town, lawns and roadsides in centre of the village; ca.. 480 M a.s.l.; 48°52'44"N; 14°55'58"E; 21.4.2007; BT	H	-/-	×/-/-
abc905	CZ	Šalmanovice village near Třeboň town, lawns and roadsides in the village; ca.. 480 m a.s.l.; 48°53'04"N; 14°46'26"E; 21.4.2007; BT	F	apo/-	×/×/-
abc906	CZ	Hrachoviště village near Třeboň town, lawns in the village; ca.. 460 M a.s.l.; 48°55'43"N; 14°46'07"E; 21.4.2007; BT	H	-/-	×/-/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
abc907	CZ	Borovany town, lawns at the railway station of Borovany, ca.. 485 m a.s.l.; 48°53'32"N; 14°38'39"E; 22.4.2007; BT	H	-/-	×/-/-
abc908	CZ	Ledenice village near České Budějovice town, lawns in E part of the village; ca.. 485 m a.s.l.; 48°56'05"N; 14°37'25"E; 22.4.2007; BT	H	-/-	×/-/-
abc909	CZ	Lišov village near České Budějovice town, lawns in S part of the village; ca.. 525 m. a.s.l.; 49°00'54"N; 14°36'43"E; 22.4.2007; BT	H	-/-	×/×/-
abc910	CZ	Neplachov village near Veselí nad Lužnicí town, meadow at the S margin of the village; ca.. 460 m a.s.l.; 49°07'35"N; 14°36'05"E; 22.4.2007; BT	H	-/-	×/-/-
abc911	CZ	Hartmanice village near Veselí nad Lužnicí town, lawns in the village and meadow at the E margin of the village; ca.. 475 m a.s.l.; 49°12'27"N; 14°34'00"E; 22.4.2007; BT	H	-/-	×/×/-
abc936	CZ	Velichov village near Ostrov town, lawns in centre of the village; 50°16'58"N; 13°00'34"E; 5.5.2007; BT	H	-/-	×/-/-
abc945	CZ	Dobrá Voda village near Nové Hrady town, lawns and roadsides in the village; ca.. 693 m a.s.l.; 48°44'27"N; 14°43'26"E; 9.5.2007; BT	H	-/-	×/×/-
abc973	CZ	Biskupice village near Luhačovice town, lawns and roadsides in centre of the village; 49°05'00"N; 17°42'35"E; 3.5.2008; BT	F	-/-	×/-/-
abc974	CZ	Slopné village near Luhačovice town, meadow at the brook at S margin of the Podvesí settlement (SSW of the village); 49°08'55"N; 17°50'43"E; 3.5.2008; BT	F	-/-	×/×/ cp*1b*/ JQ696779
abc975	CZ	Lešná village near Valašské Meziříčí town, lawns in park and in centre of the village; 49°31'06"N; 17°55'51"E; 3.5.2008; BT	H	-/-	×/-/-
cfabc846	SK	Žarnovická Huta village near Žarnovica town, meadow N of the road towards Horné Háme village; 48°29'41"N; 18°40'52"E; 8.5.2006; BT	H	-/-	×/-/-
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ad821/b	CZ	Předměřice nad Jizerou village near Benátky nad Jizerou town, small meadow N of the road towards Tuřice village; 50°15'10"N; 14°°46'32"E; 1.5.2006; BT	H	-/-	×/×/cp*1a*/ JQ696780
ad824	CZ	Mrzky village near Český Brod town, lawns and roadsides at the S village margin; 50°02'34"N; 14°48'26"E; 1.5.2006; BT	H	-/-	×/×/-
ad836	SK	Korytárky settlement near Detva town, wet meadow between road and Slatina brook; 48°32'43"N; 19°27'45"E; 6.5.2006; BT	H	-/-	×/-/-
ad846/b	SK	Žarnovická Huta village near Žarnovica town, meadow N of the road towards Horné Háme village; 48°29'41"N; 18°40'52"E; 8.5.2006; BT	H	-/-	×/×/-
ad860/b	CZ	Pelec village near Kamenice nad Lipou town, lawns at the small pond in the village centre; 660 m a.s.l.; 49°19'09"N; 15°08'25"E; 3.5.2006; BT	H	-/-	×/×/-
ad945	CZ	Dobrá Voda village near Nové Hrady town, lawns and roadsides in the village; 693 m a.s.l.; 48°44'27"N; 14°43'26"E; 9.5.2007; BT	H	-/-	×/-/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
ad972	CZ	Kostelec u Holešova village near Holešov town, lawns and roadsides at E margin of the village (at the road towards Roštění village); 49°22'21"N; 17°30'58"E; 3.5.2008; BT	H	-/-	×/×/cp*1a*/ JQ696781
ad995	CZ	Ponikev village near Konice town, lawns and roadsides in the village; 49°37'29"N; 16°53'01"E; 14.5.2008; BT	F	apo/-	×/×/cp*1a*/ JQ696782
ad997	CZ	Dlouhá Ves village near Zábřeh town, lawns and roadsides in the village; 49°49'44"N; 16°47'47"E; 14.5.2008; BT	F	-/-	×/×/-
ad999	CZ	Koclířov village near Svitavy town, lawns in E part of the village (E of centre of the village); 49°45'49"N; 16°33'06"E; 14.5.2008; BT	F	-/-	×/×/-
adK07/b	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2007; BT	F/H	-/apo	×/×/cp*1a*/ JQ696783
adK08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	-/apo	×/×/-
adKZ08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	apo/-	×/×/-
cfad857	CZ	Červená Lhota village near Kardašova Řečice town, lawns at the car park in the village; 490 m a.s.l.; 49°14'56"N; 14°53'00"E; 13.5.2006; BT	H	-/-	×/-/-

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*T. jugiferum
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j835	SK	Hriňová village near Detva town, meadows at road 1 km SSE from the Javorinka hill (918 m a.s.l.) NNW of the village (in the Horná Riečka settlement); 48°36'11"N; 19°31'17"E; 6.5.2006; BT	H	-/-	×/×/-
j904	CZ	Rapšach village near Třeboň town, lawns and roadsides in centre of the village; ca. 480 m a.s.l.; 48°52'44"N; 14°55'58"E; 21.4.2007; BT	H	-/-	×/×/cp*1a*/ JQ696784
j908	CZ	Ledenice village near České Budějovice town, lawns in E part of the village; ca. 485 m a.s.l.; 48°56'05"N; 14°37'25"E; 22.4.2007; BT	H	-/-	×/×/-
j911	CZ	Hartmanice village near Veselí nad Lužnicí town, lawns in the village and meadow at the E margin of the village; ca. 475 m a.s.l.; 49°12'27"N; 14°34'00"E; 22.4.2007; BT	H	-/-	×/-/-
j915	CZ	Damírov village near Golčův Jeníkov town, lawns and roadsides in the village; 49°48'42"N; 15°19'18"E; 25.4. 2007; BT	H	-/-	×/×/cp*1a*/ JQ696785
j916	CZ	Vernýřov village near Uhlířské Janovice town, lawns and roadsides in the village; 49°50'53"N; 15°09'21"E; 25.4.2007; BT	F	apo/apo	×/×/-
j972	CZ	Kostelec u Holešova village near Holešov town, lawns and roadsides at E margin of the village (at the road towards Roštění village); 49°22'21"N; 17°30'58"E; 3.5.2008; BT	F	-/-	×/×/-
j988	CZ	Nové Práchňany near Vlašim town, lawns in the village and meadow at the W margin of the village; 49°36'10"N; 15°01'00"E; 11.5.2008; BT	F	-/-	×/×/-
j989	CZ	Křížov village near Vlašim town, lawns and meadows in W part of the village; 49°38'30"N; 14°53'39"E; 11.5.2008; BT	F	apo/-	×/×/cp*1a*/ JQ696786

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
j993	CZ	Trhový Štěpánov village near Vlašim town, lawns and medaow near the railway station; 49°42'29"N; 15°00'31"E; 11.5.2008; BT	F	apo/-	×/×/cp*1a*/ JQ696787
j995	CZ	Ponikev village near Konice town, lawns and roadsides in the village; 49°37'29"N; 16°53'01"E; 14.5.2008; BT	F	apo/-	×/×/-
jKZ08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	apo/-	×/×/-
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cfsu424	CZ	Držková village near Fryšták town, small meadows between the road and the brook near the water reservoir at the NE margin of the village; 49°19'23"N; 17°47'41"E; 9.5.1999; BT	H	-/-	×/-/-
cfsu466	CZ	Tasov village near Veselí nad Moravou town, lawns along the brook in the W part of the village; 48°54'24"N; 17°25'34"E; 25.4.2000; BT	H	-/-	×/-/-
cfsu616	CZ	Morávka village near Frýdlant nad Ostravicí town, meadow at the road in the Morávka valley near the Mituří settlement; 49°32'48"N; 18°33'02"E; 16.5.2002; BT	H	-/-	×/-/-
su469	CZ	Nivnice village near Uherský Brod town, lawns in small park on the bank of Nivnička brook in SW part of the village; 48°58'34"N; 17°38'28"E; 25.4.2000; BT	H	-/-	×/×/cp*1a*/ JQ696788
su471	CZ	Prusy village near Přerov town, meadow between the road Přerov - Dřevohostice and the fishpond 0.7 km NNW of the village; 49°25'57"N; 17°30'46"E; 27.4.2000; BT	H	-/-	×/×/-
su474	CZ	Špičky village near Hranice na Moravě town, meadows between the railway and the road 1.3 km W (WSW) from the Špičky railway station; 49°32'06"N; 17°47'33"E; 27.4.2000; BT	H	-/-	×/×/-
su524	CZ	Nítkovice village near Kroměříž town, lawns at the crossroad in the village; 49°12'14"N; 17°09'58"E; 1.5.2001; BT	H	-/-	×/×/cp*1a*/ JQ696789
su650	SK	Blatnica village near Martin town, lawns in the S part of the village; 48°56'26"N; 18°55'36"E; 8.5.2003; BT	H	-/-	×/×/-
su735	SK	Maslovenka settlement N of the Turzovka town, meadow at the brook S of the road towards Hrubý Buk settlement 1 km E from the church in the Hrubý Buk settlement; 49°28'01"N; 18°38'29"E; 14.5.2004; BT & RJV	H	-/-	×/×/-
su776	SK	Korňa village near Turzovka town, lawns near brook 0.5 km E of the church in the village; 49°24'50"N; 18°33'07"E; 10.5.2005; BT	H	-/-	×/×/-
su783	SK	Dolní Lhota village, W of Ostrava town, small meadow at the brook near crossroad in the S part of the village; 49°50'12"N; 18°05'16"E; 11.5.2005;BT	H	-/-	×/×/cp*1a*/ JQ696790
su846	SK	Žarnovická Huta village near Žarnovica town, meadow N of the road towards Horné Háme village; 48°29'41"N; 18°40'52"E; 8.5.2006; BT	H	-/-	×/×/cp*1a*/ JQ696791
su847	SK	Horná Ves village near Partizánske town, meadow at the road towards Veľké Pole village SE of the village; 48°36'01"N; 18°30'11"E; 8.5.2006; BT	H	-/-	×/×/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
su973	CZ	Biskupice village near Luhačovice town, lawns and roadsides in centre of the village; 49°05'00"N; 17°42'35"E; 3.5.2008; BT	F	apo/-	×/×/-
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tp813	CZ	Dlouhopolsko village near Městec Králové town, meadow between the road towards Žehuň village and Dlouhopolský rybník fishpond near the SW village margin; 50°10'24"N; 15°18'08"E; 28.4.2006; BT	H	-/-	×/-/-
tp832	CZ	Jarpice village near Slaný town, meadow N of the road towards Horní Kamenice village at the W village margin; 50°19'13"N; 14°04'50"E; 3.5.2006; BT	H	-/-	×/×/-
tp835	SK	Hriňová village near Detva town, meadows at road 1 km SSE from the Javorinka hill (918 m a.s.l.) NNW of the village (in the Horná Riečka settlement); 48°36'11"N; 19°31'17"E; 6.5.2006; BT	H	-/-	×/-/-
tp836	SK	Korytárky settlement near Detva town, wet meadow between road and Slatina brook; 48°32'43"N; 19°27'45"E; 6.5.2006; BT	H	-/-	×/-/-
tp842	SK	Muráň village near Revúca town, meadow near SW village margin; 48°44'20"N; 20°02'21"E; 7.5.2006; BT	H	-/-	×/×/-
tp846	SK	Žarnovická Huta village near Žarnovica town, meadow N of the road towards Horné Hámre village; 48°29'41"N; 18°40'52"E;8.5.2006; BT	H	-/-	×/-/-
tp847	SK	Horná Ves village near Partizánske town, meadow at the road towards Veľké Pole village SE of the village; 48°36'01"N; 18°30'11"E; 8.5.2006; BT	H	-/-	×/-/-
tp849	CZ	Babice village near Světlá nad Sázavou town, meadows along Sázava river at the E village margin; 49°37'59"N; 15°28'38"E; 10.5.2006; BT	H	-/-	×/-/-
tp851	CZ	Hostkovice village near Ledeč nad Sázavou town, lawns in the village; 49°46'12"N; 15°17'29"E; 10.5.2006; BT;	H	-/-	×/×/-
tp860	CZ	Pelec village near Kamenice nad Lipou town, lawns at the small pond in the village centre, 660 m a.s.l.; 49°19'09"N; 15°08'25"E; 13.5.2006; BT	H	-/-	×/×/-
tp864	CZ	Chýnov town, meadow N of the road towards Tábor town village near the SW town margin, 470 m a.s.l.; 49°24'15"N; 14°48'01"E; 14.5.2006; BT	H	-/-	×/-/-
tp865	CZ	Všechov village near Tábor town, meadow S of the road towards Dražice village near the W village margin; 480 m a.s.l.; 49°25'56"N; 14°36'51"E; 14. 5.2006; BT	H	-/-	×/×/-
tp869	CZ	Chotěvice village near Hostinné town, lawns and small meadows at the road towards Pilníkov village 0.7 km ENE from the turning towards Čermná village; 50°31'30"N; 15°47'12"E; 17.5.2006; BT	H	-/-	×/-/-
tp891	CZ	Žiněves village near Mladá Boleslav town, meadow at the SE margin of the village; 50°24'42"N; 15°00'05"E; 15.4.2007; BT	H	-/-	×/×/cp*1a*/ JQ696792
tp893	CZ	Strašov village near Přelouč town, meadows at the Strašovský rybník pond 1.1 km N of the village; 50°05'48"N; 15°31'26"E; 18.4.2007; BT	H	-/-	×/-/-
tp897	CZ	Černilov village near Hradec Králové town, lawns and roadsides in NW part of the village; 50°16'11"N; 15°54'22"E; 18.4.2007; BT	H	-/-	×/-/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
tp903	CZ	Lutová village near Třeboň town, lawns in the village, ca 460 m a.s.l.; 48°59'23"N; 14°54'32"E; 21.4.2007; BT	H	-/-	×/×/-
tp906	CZ	Hrachoviště village near Třeboň town, lawns in the village, ca 460 M a.s.l.; 48°55'43"N; 14°46'07"E; 21.4.2007; BT	H	-/-	×/-/-
tp910	CZ	Neplachov village near Veselí nad Lužnicí town, meadow at the S margin of the village, ca 460 m a.s.l.; 49°07'35"N; 14°36'05"E; 22.4.2007; BT	H	-/-	×/×/-
tp966	CZ	Lužice village near Most town, lawns in N part of the village, ca 260 m a.s.l.; 50°29'36"N; 13°45'09"E; 19.4.2008; BT	H	-/-	×/-/-
tp972	CZ	Kostelec u Holešova village near Holešov town, lawns and roadsides at E margin of the village (at the road towards Roštění village); 49°22'21"N; 17°30'58"E; 3.5.2008; BT	H	-/-	×/-/-
tp973	CZ	Biskupice village near Luhačovice town, lawns and roadsides in centre of the village; 49°05'00"N; 17°42'35"E; 3.5.2008; BT	F	apo/-	×/-/-
tp974	CZ	Slopné village near Luhačovice town, meadow at the brook at S margin of the Podvesí settlement (SSW of the village); 49°08'55"N; 17°50'43"E; 3.5.2008; BT	F	apo/apo	×/-/-
tp975	CZ	Lešná village near Valašské Meziříčí town, lawns in park and in centre of the village; 49°31'06"N; 17°55'51"E; 3.5. 2008; BT	F	-/-	×/-/-
tp976	CZ	Jezernice village near Lipník nad Bečvou town, lawns and roadsides in centre of the village; 49°32'39"N; 17°37'34"E; 3.5.2008; BT	H	-/-	×/×/-
tp983	CZ	Dolní Novosedly village near Písek town, lawns and roadsides in the village, ca 485 M a.s.l.; 49°19'44"N; 14°11'48"E; 9.5.2008; BT	F	apo/-	×/-/-
tp985	CZ	Koloděje nad Lužnicí village near Týn nad Vltavou town, lawns along the road in S part of the village (towards Týn nad Vltavou), ca.. 390 m a.s.l.; 49°14'55"N; 14°25'16"E; 9.5.2008; BT	F	-/-	×/×/cp*1a*/ JQ696793
tp988	CZ	Nové Práchňany near Vlašim town, lawns in the village and meadow at the W margin of the village; 49°36'10"N; 15°01'00"E; 11.5.2008; BT	F	-/-	×/×/cp*1a*/ JQ696794
tp989	CZ	Křížov village near Vlašim town, lawns and meadows in W part of the village; 49°38'30"N; 14°53'39"E; 11.5.2008; BT	F	apo/-	×/-/-
tp995	CZ	Ponikev village near Konice town, lawns and roadsides in the village; 49°37'29"N; 16°53'01"E; 14.5.2008; BT	F	-/-	×/-/-
tp995	CZ	Ponikev village near Konice town, lawns and roadsides in the village; 49°37'29"N; 16°53'01"E; 14.5.2008; BT	F	-/-	×/-/-
tp996/b	CZ	Studená Loučka village near Mohelnice town, lawns and roadsides in the village; 49°46'07"N; 16°49'04"E; 14.5.2008; BT	F	apo/-	×/-/-
tp997	CZ	Dlouhá Ves village near Zábřeh town, lawns and roadsides in the village; 49°49'44"N; 16°47'47"E; 14.5.2008	F	-/-	×/-/-
tp999	CZ	Koclířov village near Svitavy town, lawns in E part of the village (E of centre of the village); 49°45'49"N; 16°33'06"E; 14.5.2008	F	apo/-	×/-/-
tpK07	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2007; BT	F	-/-	×/-/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
tpK08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	-/-	×/-/-
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o1	CZ	Lýsky village near Přerov town, small meadow near the railway underpass at the W margin of the village, 210 m a.s.l.; 49°28'52"N; 17°27'15"E; 3.5.1992; BT	H	-/-	×/-/-
o554	CZ	Vilémovice village near Blansko town, meadows in the valley near the crossroad 0.2 km N from the N margin of the village; 49°22'13"N; 16°44'35"E; 15.5.2001; BT	H	-/-	×/×/cp*1a*/ JQ696799
o555	CZ	Ludíkov village near Boskovice town, small meadow at the crossroad N of the village; 49°27'30"N; 16°44'08"E; 15.5.2001;BT	H	-/-	×/-/-
o558	CZ	Úsobrno village near Jevíčko town, lawns in the S part of the village; 49°34'59"N; 16°46'00"E; 15.5.2001; BT	H	-/-	×/×/-
o563	CZ	Nový Rychnov village near Pelhřimov town, meadow near the SE margin of the village; 49°22'45"N; 15°22'18"E; 17.5.2001; BT	H	-/-	×/-/-
o565	CZ	Bílý Kámen village near Jihlava town, meadow at the road near the NE margin of the village; 49°26'13"N; 15°30'38"E; 17.5.2001; BT	H	-/-	×/×/-
o576	CZ	Plučisko settlement near Chropyně towní, small meadow at the wood near the crossroad not far from the settlement; 49°24'40"N; 17°22'17"E; 25.4.2002; BT	H	-/-	×/×/cp*1a*/ JQ696800
o632	CZ	Prušánky village near Hodonín town, lawns near swimming poll in the NE part of the village; 48°50'02"N; 16°58'46"E; 27.4.2003; BT	H	-/-	×/×/-
o632	CZ	Prušánky village near Hodonín town, lawns near swimming poll in the NE part of the village; 48°50'02"N; 16°58'46"E; 27.4.2003; BT	H	-/-	×/×/-
o650	SK	Blatnica village near Martin town, lawns in the S part of the village; 48°56'26"N; 18°55'36"E; 8.5.2003; BT	H	-/-	×/×/cp*1a*/ JQ696801
o681	CZ	Tovačov town, lawns and meadows in the SE part of the town; 49°25'39"N; 17°17'31"E; 26.4.2004; BT	H	-/-	×/-/-
o914	CZ	Horky village near Čáslav town, lawns in the village; 49°52'14"N; 15°26'21"E; 25.4.2007; BT	F	apo/apo	×/-/-
o915/b	CZ	Damírov village near Golčův Jeníkov town, lawns and roadsides in the village; 49°48'42"N; 15°19'18"E; 25.4.2007; BT	F	-/apo	×/×/cp*1a*/ JQ696802
o932/b	CZ	Radkov village near Telč town, lawns and roadsides in the village; 49°08'42"N; 15°28'31"E; 2.5.2007; BT	F	apo/-	×/-/-
o978	CZ	Vepice village near Milevsko town, lawns in the village; ca.. 530 m.a.s.l.; 49°31'34"N; 14°18'00"E; 8.5.2008; BT	F	apo/-	×/-/-
o996	CZ	Studená Loučka village near Mohelnice town, lawns and roadsides in the village; 49°46'07"N; 16°49'04"E; 14.5.2008; BT	F	apo/-	×/-/-
oH	CZ	Hutisko-Solanec village, cultivated meadow in the village centre, ca. 490 m.a.s.l.; 49°25'51.71"N; 18°13'5.19"E; 3.5.2006; RJV	F	apo/-	×/×/-

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oKZ08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	-/-	×/×/-
oVS	CZ	Vašútky settlemet, wet meadow in the settlement, ca. 680 m.a.s.l.; 49°25'0.89"N, 18°21'52.69"E; 3.5.2006; RJV	F	apo/-	×/×/-
oX	CZ	Černotín village, wet meadow behind the village in direction to the Milotice nad Bečvou village, ca. 250 m.a.s.l.; 49°32'20.16"N; 17°48'3.28"E; 5.5.2009; LM	F	-/-	×/-/-

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Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
s818	CZ	Doubravany village near Nymburk town, lawns in the village centre; 50°18'25"N; 15°07'47"E; 1.5.2006; BT	H	-/-	×/×/cp*1a*/ JQ696806
s812	CZ	Chlumec nad Cidlinou town, lawns and roadsides at the road towards Kladruby village near the bridge across Cidlina river; 50°08'54"N; 15°27'52"E; 28.4.2006; BT	H	-/-	×/-/-
s815	CZ	Bobnice village near Nymburk town, lawns and roadsides in the village centre; 50°13'06"N; 15°03'17"E; 28.4.2006; BT	H	-/-	×/-/-
s816	CZ	Dymokury village near Nymburk town, pasture at the road towards Záhornice village near the E village margin; 50°14'48"N; 15°12'32"E; 28.4.2006; BT	H	-/-	×/-/-
s817	CZ	Hlušice village near Nový Bydžov town, lawns and roadsides in the village; 50°15'45"N; 15°24'05"E; 28.4.2006; BT	H	-/-	×/-/-
s837	SK	Lovinobaňa village, small meadow at the road towards Lučenec town near the crossroad at the SE village margin; 48°25'49"N; 19°35'43"E; 6.5.2006; BT	H	-/-	×/-/-
s868	CZ	Radeč village near Úpice town, meadow at the road towards Starý Rokytník village near cemetery 0.5 km N of the village; 50°30'50"N; 15°59'00"E; 17.5.2006; BT	H	-/-	×/-/-
s885	SK	Gerlachov village near Poprad town, lawns and roadsides in NW part of the village; 49°05'50"N; 20°12'27"E; 26.5.2006; BT	H	-/-	×/×/-
s887	CZ	Pučery village near Zásmuky town, lawns in the village; 49°57'54"N; 15°06'22"E; 15.4.2007; BT	H	-/-	×/×/cp*1a*/ JQ696804
s891	CZ	Židněves village near Mladá Boleslav town, meadow at the SE margin of the village; 50°24'42"N; 15°00'05"E; 15.4.2007; BT	H	-/-	×/×/-
s894	CZ	Žehušice village near Kutná Hora town, lawns and roadsides in SE part of the village; 49°58'02"N; 15°24'38"E; 18.4.2007; BT	F	-/-	×/-/-
s895	CZ	Chotělice village near Nový Bydžov town, meadow and roadsides at E margin of the village; 50°18'19"N; 15°28'04"E; 18.4.2007; BT	H	-/-	×/×/-
s896	CZ	Jeřice village near Hořice town, meadow at NW margin of the village; 50°20'44"N; 15°40'28"E; 18.4.2007; BT	H	-/-	×/-/-
s897	CZ	Černilov village near Hradec Králové town, lawns and roadsides in NW part of the village; 50°16'11"N; 15°54'22"E; 18.4.2007; BT	H	-/-	×/×/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
s905	CZ	Šalmanovice village near Třeboň town, lawns and roadsides in the village; ca.. 480 m a.s.l.; 48°53'04"N; 14°46'26"E; 21.4.2007; BT	H	-/-	×/-/-
s910	CZ	Neplachov village near Veselí nad Lužnicí town, meadow at the S margin of the village; ca.. 460 m a.s.l.; 49°07'35"N; 14°36'05"E; 22.4.2007; BT	H	-/-	×/-/-
s911	CZ	Hartmanice village near Veselí nad Lužnicí town, lawns in the village and meadow at the E margin of the village; ca.. 475 m a.s.l.; 49°12'27"N; 14°34'00"E; 22.4.2007; BT	H	-/-	×/×/-
s912	CZ	Osík village near Litomyšl town, lawns and roadsides in NE part of the village; 49°51'18"N; 16°17'37"E; 25.4.2007; BT	F	apo/-	×/-/-
s915	CZ	Damírov village near Golčův Jeníkov town, lawns and roadsides in the village; 49°48'42"N; 15°19'18"E; 25.4.2007; BT	H	-/-	×/×/cp*1a*/ JQ696805
s933	CZ	Bílkov village near Dačice town, lawns and roadsides in the village; 49°05'16"N; 15°28'31"E; 2.5.2007; BT	F	apo/-	×/×/cp*1a*/ JQ696803
s982	CZ	Zlivice village near Písek town, roadsides and lawns at the pond in the village, ca.. 425 m a.s.l.; 49°21'26"N; 14°06'16"E; 9.5.2008; BT	F	apo/-	×/-/-
s983	CZ	Dolní Novosedly village near Písek town, lawns and roadsides in the village; ca.. 485 m a.s.l.; 49°19'44"N; 14°11'48"E; 9.5.2008; BT	F	apo/apo	×/×/-
s995	CZ	Ponikev village near Konice town, lawns and roadsides in the village; 49°37'29"N; 16°53'01"E; 14.5.2008; BT	F	apo/-	×/-/-

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Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
pul555	CZ	Ludíkov village near Boskovice town, small meadow at the crossroad N of the village; 49°27'30"N; 16°44'08"E; 15.5.2001; BT	H	-/-	×/×/-
pul559	CZ	Brněnec village near Svitavy town, lawns at the road in the W part of the village, 1.5 km W from the Březová nad Svitavou railway station; 49°38'02"N; 16°29'55"E; 15.5.2001; BT	H	-/-	×/×/cp*3*/ JQ696808
pul560	CZ	Malé Hradisko village near Prostějov town, meadow at the road bend near the SE margin of the village; 49°29'30"N; 16°52'55"E; 17.5.2001; BT	H	-/-	×/-/-
pul570	CZ	Svratka village near Hlinsko town, meadow near the wood at the road towards Křižánky 1.4 km ESE from the main crossroad in the village; 49°42'19"N; 16°03'13"E; 20.5.2001; BT	H	-/-	×/×/-
pul573	CZ	Horní Lomná village near Jablunkov town, lawns and small meadows at the road near the Přelač settlement; 49°30'43"N; 18°39'01"E; 23.5.2001; BT	H	-/-	×/×/-
pul651	SK	Oščadnica village near Čadca town, meadow above the road 1.5 km SSE of the Liesková hill (850 m) NNE of the village; 49°28'48"N; 18°55'39"E; 8.5.2003; BT	H	-/-	×/×/cp*3*/ JQ696809
pul681	CZ	Tovačov town, lawns and meadows in the SE part of the town; 49°25'39"N; 17°17'31"E; 26.4.2004; BT	H	-/-	×/×/-
pul739	CZ	Vrchlabí town, wet meadow S from the road towards Valteřice village N from the pond; 50°37'08"N; 15°36'14"E; 17.5.2004; BT	H	-/-	×/×/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCSS/EM	SSR/AFLP/cpDNA
pul750	CZ	Bolelouc village near Tovačov town, lawns and roadsides at the E margin of the village; 49°29'32"N; 17°16'31"E; 27.4.2005; BT	H	-/-	×/×/-
pul912	CZ	Osík village near Litomyšl town, lawns and roadsides in NE part of the village; 49°51'18"N; 16°17'37"E; 25.4.2007; BT	H	-/-	×/-/-
pul943/b	CZ	Zadní Chodov village near Planá town, lawns and roadsides in W part of the village (near church); 49°53'28"N; 12°39'12"E; 6.5.2007; BT	F	apo/-	×/×/cp*3*/ JQ696810
pul996	CZ	Studená Loučka village near Mohelnice town, lawns and roadsides in the village; 49°46'07"N; 16°49'04"E; 14.5.2008; BT	F	-/-	×/-/-
pulH	CZ	Hutisko-Solanec village, cultivated meadow in the village centre, ca. 490 m.a.s.l.; 49°25'51.71"N; 18°13'5.19"E; 3.5.2006; RJV	F	-/-	×/-/-
pulKZ08	CZ	Kojetín town near Kroměříž town, grassy places in gardens near the eastern town margin; 49°20'54"N; 17°18'31"E; 2008; BT	F	-/-	×/-/-
pulTRE	SK	Trebichava village near Bánovce nad Bebravou town, wet meadow behind the village in the SE direction; 48°49'33"N; 18°18'24"E; 28.4.2009; LM & RJV	F	-/-	×/×/-
pulX	CZ	Černotín village, wet meadow behind the village in direction to the Milotice nad Bečvou village, ca. 250 m a.s.l.; 49°32'15.97"N; 17°47'49.29"E; 5.5.2009; LM	F	-/-	×/-
T. linearisquameum Soest				FCM	
R1	CZ	Klentnice village, ruderal grasses and wood pathway verges in vicinity of the castle ruins Sirotčí hrádek; ca. 400 m a. s. l.; 48°50'38.12"N; 16°38'9.75"E; 25.4.2004; RJV	FB	2x	×/×/ cp*1a*/ JQ696795
R3	CZ	Klentnice village, ruderal grasses and wood pathway verges in vicinity of the castle ruins Sirotčí hrádek; ca. 400 m a. s. l.; 48°50'38.12"N; 16°38'9.75"E; 25.4.2004; RJV	FB	2x	×/×/ cp*1a*/ JQ696796
R5	CZ	Klentnice village, ruderal grasses and wood pathway verges in vicinity of the castle ruins Sirotčí hrádek; ca. 400 m a. s. l.; 48°50'38.12"N; 16°38'9.75"E; 25.4.2004; RJV	FB	2x	×/×/ cp*1a*/ JQ696797
R6	CZ	Klentnice village, ruderal grasses and wood pathway verges in vicinity of the castle ruins Sirotčí hrádek; ca. 400 m a. s. l.; 48°50'38.12"N; 16°38'9.75"E; 25.4.2004; RJV	FB	2x	×/×/ cp*1a*/ JQ696798

Supporting information for Chapter 3.

Genotypic variability of obligate apomicts is enriched by the gene pool of sexuals in contact zones between sexual-apomictic dandelions (*Taraxacum* sect. *Erythrosperma*)

Additional file 1. List of *Taraxacum* sect. *Erythrosperma* accessions used in the present study with sampling details.
Country abbreviations: CZ: Czech Republic; SK: Slovakia; HU: Hungary; AT: Austria; PL: Poland; NL: Netherlands; FI: Finland. Collector abbreviations: LM: Ľuboš Majeský; RJV: Radim J. Vašut; MV: Martina Vašutová; HH: Honza Havránek; VŽ: Vojtěch Žíla; MD: Martin Duchoslav; AC: Aneta Czarna; AP: Aleš Pečinka; CES: Carl E. Sonck; JŠ: Jan Štěpánek. The columns: F/H refers to plant material used for DNA extraction; F: fresh leaves; H: herbarium voucher; FCM/FCSS refers to measurement of the relative ploidy level by Flow Cytometry (FCM) (2x: diploid; 3x: triploid) and examination of reproduction type by Flow Cytometry Seed Screen (FCSS) (apo: apomictic seed formation; sex: sexual seed formation); SSR/AFLP/cpDNA shows which plants were used for microsatellite (SSR) genotyping, AFLP genotyping, and sequencing of the *trnL-trnF* region (cpDNA) with the observed haplotype + GenBank accession number; b: double sample. Asterisk (*) indicates accessions recognised by taxonomists as validly described microspecies; absence of an asterisk indicates distinct morphological groups but formally undescribed accessions (mentioned under "work names").

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
CRI - *Taraxacum cristatum* Kirschner, Štěpánek et Vašut					
C_Hra	CZ	Hradčany – Kobeřice town, in stone-pit; N 49°22'9.33" E 17°6'28.46"; 230 m. a.s.l.; 30.4.1999; RJV & MV	H	-/-	+/-/-
C_Roh	CZ	Rohatec village, along the railway; N 48°53'13.35" E 17°11'6.61"; 180 m a.s.l.; 25.4.1998; RJV	H	-/-	+/-/-
C_E1	CZ	Bzenec town, military training area; N 48°57'36.60" E 17°17'17.24"; 193 m a.s.l.; 14.4.1999; RJV	F	-/-	+/+/-
C_52	CZ	Trnava village, lane along field; N 49°15'34" E 15°55'; cca 450 m a.s.l.; 21. 4. 2008, LM & RJV	F	3x/apo	+/+/-
C_34	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/apo	+/+/-
C_27	CZ	Ratíškovice, xerothermous grass along railway on sandy soil in Pine forest; N 48°55'42" E 17°09'32"; 220 m a.s.l.; LM & RJV	F	3x/apo	+/+/cp*1a* KC119515
C_H4	CZ	Výrovice village, xerothermous slopes; N 48°55'36.41" E 16°7'6.25"; 295 m a.s.l.; 1.5.2000; RJV	H	-/-	+/-/-
C_H5	CZ	Hodonín town, edge of the road; N 48°52'33.58" E 17°7'5.92"; 180 m a.s.l.; 26.4.1998; RJV	H	-/-	+/+/-
C_H6	CZ	Krhovice village, abandoned stone-pit; N 48°49'13.34" E 16°9'11.96"; 200 m a.s.l.; 26.4.2000; RJV	H	-/-	+/+/-
C_VŽ	CZ	Horažďovice twon, pagus Krejnice; N 49°14'9.2"; E 13°43'20.1"; 556 m a.s.l.; 8. 5. 2002; VŽ	H	-/-	+/
C_MH1	SK	Malý Horeš village, meadow with steppe vegetation, N 48°24'23.88" E 21°57'5.23"; 118 m a.s.l.; 04. 2010; LM & RJV	F	-/-	+/+/cp*1a* KC119517
C_MH2	SK	Malý Horeš village, meadow with steppe vegetation, N 48°24'23.88" E 21°57'5.23"; 118 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/-
C_KAS2	SK	Ladmovce village; xerothermic vegetation near the village; N 48°25'13.40" E 21°46'34.18"; 149 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/cp*1a* KC119516
C_KAS3	SK	Ladmovce village; xerothermic vegetation near the village; N 48°25'13.40" E 21°46'34.18"; 149 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/-
C_KAS4	SK	Ladmovce village; xerothermic vegetation near the village; N 48°25'13.40" E 21°46'34.18"; 149 m a.s.l.; 04. 2010; LM & RJV	F	-/-	+/-/-
C_KO3	SK	Hrhov village, dimesion Okrúhle, around field lane in vegetaion with Medicago sp.; N 48°36'23.25" E 20°46'49.39"; 244 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-
C_KO6	SK	Hrhov village, dimesion Okrúhle, around field lane in vegetaion with Medicago sp.; N 48°36'23.25" E 20°46'49.39"; 244 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
C_TL3	SK	Lúka village, Považský Inovec Hills, xerotermic slopes; N 48°39'45.52" E 17°53'48.25"; 222 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/cp*1a* KC119518

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
C_TL4	SK	Lúka village, Považský Inovec Hills, xerotermic slopes; N 48°39'45.52" E 17°53'48.25"; 222 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-
C_CEN1	SK	Čenkov village, sandy lane on the margin of the Nature reservation Čenkovská step steppe; N 47°46'8.22" E 18°31'34.80"; 109 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
C_CEN6	SK	Čenkov village, sandy lane on the margin of the Nature reservation Čenkovská step steppe; N 47°46'8.22" E 18°31'34.80"; 109 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-
C_Šaš	SK	Sandy edge of a pine forest, in between Šaštín-Stráže and Borský Mikuláš towns; N 48°37'47.88" E 17°10'50.72"; 180 m a.s.l.; 2001; RJV	H	-/-	+/-/-
C_Stud	SK	Studienka village, on the sandy road in pinewood; N 48°30'57.65" E 17°12'17.33"; 197 m a.s.l.; 2008; LM & RJV	H	-/-	+/-/-
C_51	SK	Plavecký Štvrtok town, edge of a vineyard near the town; N 48°22'36.79" E 17°0'29.01"; 309 m a.s.l.; 2008; LM & RJV	F	3x/apo	+/-/-
C_H3	SK	Borský Svätý Jur village, sandy edge of a pine forest; N 48°37'16.82" E 17°4'10.06"; cca 168 m a.s.l.; 25.4.2001, RJV	H	-/-	+/+/-
C_WITT	SK	Sandy edge of a pine forest, in between Šaštín-Stráže and Borský Mikuláš towns; N 48°3746.70" E 17°10'51.02"; 180 m a.s.l.; 2001; RJV	H	-/-	+/+/-
C_H1	AT	Strasshof an der Nordbahn village, sandy lawn in pine forest; N 48°19'8.41" E 16°37'16.56"; 30.4.2003; RJV	H	-/-	+/+/-
C_GA4	AT	Gänserndorf, sandy lane in pinewood on northern edge of the village; N 48°20'47.23" E 16°43'7.66"; 158 m a.s.l.; 2009; LM & RJV & HH	F	3x/apo	+/+/-
C_GA5	AT	Gänserndorf, sandy lane in pinewood on northern edge of the village; N 48°20'47.23" E 16°43'7.66"; 158 m a.s.l.; 2009; LM & RJV & HH	F	-/apo	+/+/cp*1a* KC119514
C_GA12	AT	Gänserndorf, sandy lane in pinewood on northern edge of the village; N 48°20'47.23" E 16°43'7.66"; 158 m a.s.l.; 2009; LM & RJV & HH	F	-/-	+/-/-
C_Pul	AT	Pulkau: dry grasses along pathways in the wood; N 48°42'52.89" E 15°50'21.77"; 380 m a.s.l.; 26. 4. 2001; RJV	H	-/-	+/-/-
C_CB1	HU	Csákbereny village, limestone fields before the village; N 47°20'25.37" E 18°21'18.46"; 189 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/-
C_CB4	HU	Csákbereny village, limestone fields before the village; N 47°20'25.37" E 18°21'18.46"; 189 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/-

SC - *Taraxacum scanicum* Dahlst. s.str.

SC_TsM	NL	Wageningen, road verges along cycling path to Ede; N 51°59'32.35" E 5°42'28.27"; 40 m a.s.l.; 3.4.2002; RJV	F	-/apo	+/+/cp*2b* KC119528

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
SC_TsE75	PL	Wielkopolska region; Książ Wielkopolska village; forest near site "Torficy"; N 52°3'50.93" E 17°14'49.81"; 90 m a.s.l.2003; AC	F	-/apo	+/+/-
SC_H4	PL	Wielkopolska region; Nowe Miasto nad Wartą, pine wood on sands; N 52°4'58.77" E 17°23'40.30"; 75 m a.s.l.; 2003; AC	H	-/-	+/+/-
SC_H5	PL	Myszkówek, gm. Zagórów, roadside; N 52°9'48.02" E 17°52'31.59"; 16.05.2005; AC	H	-/-	+/-/-
SC_H6	PL	Kliczkow: dry meadows in the river Kwisa valley; N 51°20'0.67" E 15°25'47.73"; 180 m a.s.l.; 29.4.2004; MD	H	-/-	+/+/cp2b KC119529
SC_VŽ	CZ	Bavorov, settlement Bavorovské Svobodné Hory, pastures; N 49°06'43.4"; E 14°06"28.6"; 500m; 7. 5. 2005; VŽ	H	-/-	+/+/cp2b KC119530
SCs - _Taraxacum cf. scanicum_ (morphotypes similar to _T. scanicum_ s.str.)					
SCs_GA10	AT	Gänserndorf, sandy line in a pinewood on northern edge of the village; N 48°20'47.23" E 16°43'7.66"; 2009; LM & RJV & HH	F	-/apo	+/+/cp2b KC119531
SCs _HOR1A	HU	Hortobágyi town, steppe meadows near the town; N 47°35'14.08" E 21°8'37.50"; 85 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/cp2b KC119532
SCs _KO10	SK	Hrhov village, dimesion Okrúhle, around field line in vegetaion with Medicago sp.; N 48°36'23.25" E 20°46'49.39"; 244 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-
SCs_15	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/-	+/+/-
SCs_19	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/apo	+/+/cp1a KC119519
SCs_46	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/-	+/-/-
SCs_50	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/-	+/-/-
MOR_1 - _Taraxacum scanicum_ agg. - unclassified morfotype I.					
M1_KO7	SK	Hrhov village, dimesion Okrúhle, around field line in vegetaion with Medicago sp.; N 48°36'23.25" E 20°46'49.39"; 244 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
M1_KO9	SK	Hrhov village, dimesion Okrúhle, around field line in vegetaion with Medicago sp.; N 48°36'23.25" E 20°46'49.39"; 244 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-
M1_TUR7	SK	Turňa nad Bodvou village, xerothermic limestone slopes of Turniansky hradný vrch hill; N 48°36'41.07" E 20°52'24.13"; 341 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/cp2b KC119533

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
M1_TUR1	SK	Turňa nad Bodvou village, xerothermic limestone slopes of Turniansky hradný vrch hill; N 48°36'41.07" E 20°52'24.13"; 341 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
M1_CHL1	SK	Kováčov village, southern slopes of Kováčovské kopce hills; N 47°49'26.90" E 18°46'12.78"; 230 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
M1_CHL4	SK	Kováčov village, southern slopes of Kováčovské kopce hills; N 47°49'26.90" E 18°46'12.78"; 230 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-

MOR_2 - *Taraxacum scanicum* agg. - unclassifed morfotype II.

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
M2_KH2	SK	Krásnohorské podhradie village, xerothermic slopes around Kásna Hôrka castle; N 48°39'28.61" E 20°36'5.25"; 468 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/cp*1a* KC119520
M2_KH3	SK	Krásnohorské podhradie village, xerothermic slopes around Kásna Hôrka castle; N 48°39'28.61" E 20°36'5.25"; 468 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
M2_BE2	SK	Beckov village, xerothermic slopes around ruins of Beckov castle; N 48°47'25.40" E 17°53'56.12"; 215 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
M2_BE1	SK	Beckov village, xerothermic slopes around ruins of Beckov castle; N 48°47'25.40" E 17°53'56.12"; 215 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-

PU - *Taraxacum pudicum* Vašut et Majeský ined.

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
PU_24	CZ	Budišov village, lanes in pine forest on Kněžský kopec hill; N 49°16'22" E 16°02'02"; cca ca. 490 m a.s.l.; 21.4.2008; RJV & LM	F	3x/apo	+/+/cp*2b* KC119534
PU_H1	CZ	Kravsko village, forest lanes; N 48°54'54.47" E 15°58'40.99"; 370 m a.s. l.; 30.4.2000; RJV	H	-/-	+/+/cp*2b* KC119535
PU_H2	CZ	Tišnov – Květnice village, stony slopes and lanes, south slope; N 49°22'24.11" E 16°26'35.30"; 380 m a.s.l.; 6.5.1999; RJV	H	-/-	+/-/cp*2b* KC119536
PU_H3	CZ	Synalov – Kopaniny village: along the lanes on Sýkoř hill, south slope; N 49°26'39.87" E 16°24'8.92"; 620 m a.s.l.; 6.5.1999; RJV	H	-/-	+/+/-
PU_H4	CZ	Malhostovice village, xerothermic slopes near the village; N 49°19'33" E 16°29'42"; 330 m a.s.l.; 6.5.1999; RJV	H	-/-	+/-/-

ARA - *Taraxacum scanicum* agg. (unclassified morphotype III., provisionally named *T. "arachnitis"*)

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
ARA_H2_1	CZ	Bzenec town, military training area; N 48°57'36.60" E 17°17'17.24"; 193 m a.s.l.; 14.4.1999; RJV	H	-/-	+/+/cp*5* KC119539
ARA_H2_2	HU	Fülöpháza, sandy lanes around road; N 46°53'5.40" E 19°25'29.67"; 102 m a.s.l.; 04. 2010; LM & RJV	H	-/-	+/+/cp*2b* KC119537

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
ARA_H2_3	HU	Balázspuszta, on sandy lanes in a pine forest; N 46°54'2.12" E 19°22'26.51"; 100 m a.s.l.; 04. 2010; LM & RJV	H	-/-	+/+/-
ARA_H2_4	SK	Chotín village, sandy lanes along road, near Natural Reservation Chotínské piesky sands; N 47°48'42.96" E 18°12'50.99"; 114 m a.s.l.; 1.5.2003; RJV	H	-/-	+/-/-
ARA_H6	AT	Pillersdorf: dry grasses in front of the church; N 48°43'2.22" E 15°55'32.10"; 301 m a.s.l.; 2003; RJV & MV	H	-/-	+/+/-
ERY - *Taraxacum erythrospermum Andrz. ex Besser (diploid sexual species)					
ERY_SB1	SK	Spišské Podhradie village, Sivá Brada hill, around baroque chapel on the top; N 49°0'23.28" E 20°43'22.28"; 2010; LM & RJV	F	-/sex	+/+/cp*1a* KC119526
ERY_SH1	SK	Slanec village, xerothermic slopes of Slanec castle hill; N 48°38'13.97" E 21°28'14.19"; 468 m a.s.l.; 04. 2010; LM & RJV	F	2x/sex	+/+/cp4 KC119540
ERY55	CZ	Jamné village, xerothermous slopes near protected area Svídovec; N 49°22'55" E 16°28'12"; 440 m a.s.l.; 21.4.2008; LM & RJV	F	2x/-	+/+/-
ERY59	CZ	Malhostovice village, xerothermic slopes near the village; N 49°19'33" E 16°29'42"; 330 m a.s.l.; 21.4.2008; LM & RJV	F	2x/-	+/+/-
ERY58	CZ	Malhostovice village, xerothermic slopes near the village; N 49°19'33" E 16°29'42"; 330 m a.s.l.; 21.4.2008; LM & RJV	F	2x/-	+/+/-
ERY61	CZ	Jamné village, xerothermous slopes near protected area Svídovec; N 49°22'55" E 16°28'12"; 440 m a.s.l.; 21.4.2008; LM & RJV	F	2x/-	+/+/-
ERY57	CZ	Malhostovice village, xerothermic slopes near the village; N 49°19'33" E 16°29'42"; 330 m a.s.l.; 21.4.2008; LM & RJV	F	2x/sex	+/+/-
ERY33	CZ	Jamné village, xerothermous slopes near protected area Svídovec; N 49°22'55" E 16°28'12"; 440 m a.s.l.; 21.4.2008; LM & RJV	F	2x/-	+/+/cp*1a* KC119527
ERY16	CZ	Moravský Krumlov town,: pěšiny v okolí poutního kostela sv. Floriána; N 49°02'52" E 16°19'11"; 300 m n. m.; 21. 4. 2008; LM & RJV	F	2x/sex	+/+/cp*2b* KC119538
PRU - *T. prunicolor M. Schmid, R.J. Vašut et P. Oosterveld (apomictic triploid microspecies)					
P_H1	SK	Kláštor pod Znievom town, near the church and stations of the Cross; N 48°58'9.33" E 18°47'48.39"; 550 m a.s.l.; 2003; RJV	H	-/-	+/+/-
P_KAS1	SK	Ladmovce village; xerothermic vegetation near the village; N 48°25'13.40" E 21°46'34.18"; 149 m a.s.l.; 04. 2010; LM & RJV	F	-/apo	+/+/-
P_KO8	SK	Hrhov village, dimesion Okrúhle, along field lane in vegetaion with Medicago sp.; N 48°36'23.25" E 20°46'49.39"; 244 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/cp*2a* KC119542

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
P_SAS4	SK	Šášovské Podhradie village, slopes of Šášov castle hill; N 48°34'44.19" E 18°54'0.10"; 332 m a.s.l.; 2009; LM & RJV	F	3x/-	+/+/-
P_CEN3	SK	Čenkov village, sandy lane on the margin of the Nature reservation Čenkovská step steppe; N 47°46'8.22" E 18°31'34.80"; 109 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/cp2a KC119541
P_CEN4	SK	Čenkov village, sandy lane on the margin of the Nature reservation Čenkovská step steppe; N 47°46'8.22" E 18°31'34.80"; 109 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/-/-
P_CEN5	SK	Čenkov village, sandy lane on the margin of the Nature reservation Čenkovská step steppe; N 47°46'8.22" E 18°31'34.80"; 109 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
P_DR1	SK	Drienčany village, xerothermic meadows in Drienčanský kras karst; N 48°29'46.49" E 20° 3'28.27"; 9.5.2008; LM	F	-/apo	+/+/-
P_GA11	AT	Gänserndorf, sandy lane in pinewood on northern edge of the village; N 48°20'47.23" E 16°43'7.66"; 158 m a.s.l.; 2009; LM & RJV & HH	F	-/apo	+/+/-
P_ROH_b	CZ	Rohatec village, along the railway; 180 m a.s.l.; 25.4.1998; RJV	H	-/-	+/-/-
P_Stud	SK	Studienka village, edge of pine forest, on sandy soil; N 48°31'6.64" E 17°9'4.45"; 2008; LM & RJV	H	-/-	+/-/-
P_PŠ_b	SK	Plavecký Štvrtok town, on the margin of vneyard near the town; N 48°22'36.79" E 17°0'29.01"; 309 m a.s.l.; 2008; LM & RJV	H	-/-	+/+/-
P_10	SK	Čachtice village, xerothermic slopes around ruins of Čachtický hrad castle; N 48°43'28.72" E 17°45'41.07"; 362 m a.s.l.; 2008; LM & RJV	F	3x/apo	+/+/-
P_8	CZ	Ratíškovice village, xerothermic vegetation along railway and lanes in pine forest on sand soil; N 48°55'42", E 17°09'32"; 220 m a.s.l.; LM & RJV	F	3x/apo	+/-/-
P_6	SK	Studienka village, xerotermic vegetation along forest lanes, pine forest on sands; N 48°31'10" E 17°07'54"; 200 m a.s.l.; 25.4.2008; LM & RJV	F	3x/apo	+/-/-
P_5	SK	Borský Mikuláš town, xerotermic vegetation along forest lanes, pine forest on sand, near the hill Ruženica; N 48°36'22" E 17°12'19"; 250 m a.s.l.; 25. 4. 2008; LM & RJV	F	3x/apo	+/-/-
P_4	SK	Lakšarská Nová Ves village, xerotermic vegetation along forest lanes, pine forest on sands; N 48°35'32" E 17°11'11"; 220 m a.s.l.; 25.4.2008; LM & RJV	F	3x/apo	+/-/-
P_3	SK	Plavecký Štvrtok town, xerotermic vegetation along forest lanes, pine forest on sands; N 48°21'58" E 17°00'58"; 160 m a.s.l.; 25.4.2008; LM & RJV	F	3x/apo	+/-/-
P_H4	CZ	Rohatec village, settlement Soboňky, xerothermic subruderal grasses on sand; N 48°54'0.81" E 17°12'53.32"; 170 m a.s.l.; 25.4.1998; RJV	H	-/-	+/-/-
P_H6	CZ	Hoštejn village, around castle ruins; N 49°52'36.51" E 16°46'36.07", 346 m a.s.l.; 29.4.1999; RJV & AP	H	-/-	+/-/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
P_H7	PL	Murzynowo Leśne, gm. Krzykosy, Robinia forest 2 km. S from the village; N 52°8'13.52" E 17°21'20.40"; 77 m a.s.l.; 10.05.2003; AC	H	-/-	+/-/-
P_H8	CZ	Tábor, rocky slopes near castle ruins Příběnice; N 49°23'34.455" E 14°33'41.346"; 400 m a.s.l.; 2007; MD	H	-/-	+/-/-
BEL - *T. bellicum C.E. Sonck (apomictic triploid microspecies described and known from single locality only)					
P_BELL	FI	Inari, Kyrkobyn, Miesniemi, along road to Kankiniemi, near former WWII military camp; N 68°52'21.79" E 27°20'36.39"; 130 m a.s.l.; 7. 7. 1982; CES; cultivated in the Institute of Botany ASCR, Průhonice, CZ (provided by JŠ).	H	-/-	+/+/-
pru_sex - *Taraxacum prunicolor* × *erythrospermum* (?)					
Ps_H2	AT	Waitzendorf village, road verges between villages Waitzendorf and Untermixnitz, closer to Waitzendorf; N 48°44'29.99" E 15°52'05.91"; 417 m a.s.l.; 4.5.2003; RJV & MV	H	-/-	+/+/-
Ps_H3	AT	Oberfladnitz village, road verges near the village; N 48°46'15.88" E 15°52'6.79"; 400 m a.s.l.; 4.5.2003; RJV & MV	H	-/-	+/+/cp*1a* KC119521
Ps_EUR	AT	Untermixnitz village, lanes around Europawarte lookout; N 48°44'50.07" E 15°51'59.78"; 420 m a.s.l.; 4.5.2003; RJV & MV	H	-/-	+/+/cp*1a* KC119522
Ps_TL2	SK	Lúka village, Považský Inovec Hills, xerotermic slopes; N 48°39'45.52" E 17°53'48.25"; 222 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
Ps_TO1	SK	Topoľčianske Podhradie village, xerothermic slopes around ruin of castle; N 48°39'28.47" E 18°3'4.12"; 457 m a.s.l.; 2009; LM & RJV	F	3x/apo	+/+/-
PRU_derived - *Taraxacum scanicum* agg. (unclassified morphotype IV, similar to *T. prunicolor*)					
Pd_PTA	CZ	Ptáčov village: xerothermic lawn near the village; N 49°14'2.458" E 15°55'52.309"; 420 m a.s.l.; 1.5.2000; RJV	H	-/-	+/+/-
Pd_18	CZ	Trnava village, lane along field; N 49°15'34" E 15°55'; cca 450 m a.s.l.; 21. 4. 2008, LM & RJV	F	3x/apo	+/+/cp*1a* KC119523
Pd_17	CZ	Moravský Krumlov town, around the church St. Florian; N 49°02'52" E 16°19'11"; 300 m a.s.l.; 21.4.2008; LM & RJV	F	3x/apo	+/+/cp*1a* KC119524
Pd_44	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/apo	+/+/cp*1a* KC119525
Pd_48	SK	Plavecký Štvrtok town, on the margin of vneyard near the town; N 48°22'36.79" E 17°0'29.01"; 309 m a.s.l.; 2008; LM & RJV	F	3x/apo	+/-/-
Pd_49	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/-	+/+/-

Group/Taxon/Code	Country	Locality, GPS, Date, Collector	F/H	FCM/FCSS	SSR/AFLP/cpDNA
Pd_45	CZ	Trnava village, xerothermous slope on Kobylinec hill; N 49°14'59" E 15°56'11"; cca 450 m a.s.l.; 21. 4. 2008; LM & RJV	F	3x/-	+/+/-
Pd_H1	CZ	Kramolín village; Green Hill, around Babylon lookout; N 49°7'39.46"N, E 16°8'52.01"E; 491 m a.s.l.; 22.4.1999; RJV	H	-/-	+/-/-

Genotypic variability of obligate apomicts is enriched by the gene pool of sexuals in contact zones between sexual-apomictic dandelions (*Taraxacum* sect. *Erythrosperma*)

Additional file 2. A comparison of cpDNA haplotypes (*trnL-trnF*) observed within apomictic *Taraxacum* sect. *Erythrosperma* accessions.
Numbers refer to variable position observed in final alignment; · - represent match with cp*1a* haplpotype; * - represent insertion/deletion. For reference of cp haplotype to particular individual see Additional file 1and 3.

haplotype	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	accession presence
	9	9	9	0	0	0	0	0	0	6	8	6	6	7	9	0	0	0	1	2	3	3	4	5	5	6	6	6	7	7	
	7	8	9	0	1	2	3	4	5	0	5	0	1	6	3	0	2	5	0	3	7	8	9	3	5	0	2	4	0	7	
cp*1a*	*	*	*	*	*	*	*	*	*	G	C	C	C	C	C	C	T	T	G	A	T	G	T	C	T	G	G	A	A	G	C	*CRI, pru-sex, PRU_d, SCs, MOR_2, ERY*
cp*2a*	*	*	*	*	*	*	*	*	*	T	·	·	·	·	·	·	·	·	·	·	·	·	C	C	T	C	·	·	G	G	C	T	*PRU*
cp*2b*	*	*	*	*	*	*	*	*	*	T	·	·	·	·	·	·	·	·	·	·	·	·	C	C	T	C	*	·	G	G	C	T	*PU, ARA, MOR_1, SC, SCs, ERY*
cp*4*	*	*	*	*	*	*	*	*	*	T	·	·	·	·	·	·	·	·	·	·	·	·	C	C	·	·	·	A	·	G	·	T	*ERY*
cp*5*	A	T	T	A	C	A	A	A	T	T	T	G	T	T	G	C	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*ARA*

Supporting information for Chapter 3.

Genotypic variability of obligate apomicts is enriched by the gene pool of sexuals in contact zones between sexual-apomictic dandelions (*Taraxacum* sect. *Erythrosperma*)

Additional file 3. Allelic profile of 109 apomictic individuals of *Taraxacum* sect. *Erythrosperma* genotyped for six microsatellite loci.
Fixed alleles observed within this study (202_{MSTA53}/165_{MSTA78}) are in bold, and the proposed taxonomic-specific allele ($156_{MSTA131}$) in bold and underlined. The chloroplast haplotype observed for particular individuals is shown in the last column. Missing data are denoted as "miss".

group	sample	Locus																				cpDNA haplotype
		MSTA44B			MSTA53			MSTA78			MSTA93			MSTA131			MSTA133					
"CRI"	C_Hra	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_Roh	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_Šaš	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_Stud	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_E1	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_51	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	300	288	268			n.a.
	C_52	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	310	288	269			n.a.
	C_34	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	306	289	268			n.a.
	C_27	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	310	288	268			cp1a
	C_H1	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_H3	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	312	288	268			n.a.
	C_H4	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_H5	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_H6	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_WITT	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_VŽ	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_MH1	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			cp1a
	C_MH2	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_KAS2	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	292	268			cp1a
	C_KAS3	178	178	178	235	235	202	176	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_KAS4	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_CB1	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_KO3	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_KO6	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_CEN6	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_TL3	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			cp1a
	C_TL4	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_GA4	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_GA5	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			cp1a
	C_GA12	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_Pul	178	178	178	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.
	C_CB4	180	180	180	235	235	202	178	169	165	278	278	278	163	163	156	292	288	268			n.a.
	C_CEN1	180	180	180	235	235	202	178	169	165	278	278	278	163	163	156	308	288	268			n.a.

group	sample	MSTA44B			MSTA53			MSTA78			MSTA93			MSTA131			MSTA133			cpDNA haplotype
„SC"	SC_TsM	184	184	184	240	207	**202**	177	**165**	185	288	282	272	**156**	**156**	**156**	274	270	256	cp2b
	SC_TsE75	184	184	184	240	207	**202**	177	**165**	185	288	282	272	**156**	**156**	**156**	274	270	256	n.a.
	SC_H4	184	184	184	240	207	**202**	177	**165**	158	288	282	272	**156**	**156**	**156**	274	270	256	n.a.
	SC_H5	184	184	184	242	207	**202**	177	**165**	158	288	282	272	**156**	**156**	**156**	274	270	256	n.a.
	SC_H6	184	184	184	240	207	**202**	177	**165**	158	288	282	272	**156**	**156**	**156**	274	270	256	cp2b
	SC_VŽ	184	184	184	240	207	**202**	178	**165**	158	289	277	277	**156**	**156**	**156**	274	256	256	cp2b
„SC_s"	SCs_GA10	188	184	184	242	214	**202**	190	167	**165**	288	276	276	191	191	**156**	276	270	258	cp2b
	SCs_HOR1A	170	170	170	240	205	205	172	170	**165**	294	282	282	171	171	171	275	270	270	cp2b
	SCs_KO10	170	170	170	237	237	**202**	172	169	**165**	294	280	280	171	171	**156**	276	274	270	n.a.
	SCs_15	181	181	181	248	222	205	168	**165**	158	294	288	288	188	186	**156**	288	280	264	n.a.
	SCs_19	181	181	181	248	222	205	168	**165**	158	294	288	288	188	186	**156**	288	280	264	cp1a
	SCs_46	180	180	180	250	225	205	168	**165**	158	292	289	289	188	186	**156**	miss	miss	miss	n.a.
	SCs_50	180	180	180	250	225	205	168	**165**	158	292	289	289	188	186	**156**	290	282	267	n.a.
„MOR_1"	M1_KO7	169	169	169	240	240	**202**	172	169	**165**	294	280	280	171	171	**156**	278	266	266	n.a.
	M1_KO9	169	169	169	240	240	**202**	172	169	**165**	294	280	280	171	171	**156**	278	266	266	n.a.
	M1_TUR7	169	169	169	240	240	**202**	172	169	**165**	294	280	280	171	171	**156**	278	266	266	cp2b
	M1_TUR1	169	169	169	240	240	**202**	172	169	**165**	294	280	280	171	171	**156**	278	266	266	n.a.
	M1_CHL1	169	169	169	240	240	**202**	172	169	**165**	294	280	280	171	171	**156**	278	266	266	n.a.
	M1_CHL4	169	169	169	240	240	**202**	172	169	**165**	294	280	280	171	171	**156**	278	266	266	n.a.
„MOR_2"	M2_KH2	184	178	173	250	242	**202**	180	**165**	158	310	310	310	180	180	**156**	290	266	254	cp1a
	M2_KH3	184	178	173	250	242	**202**	180	**165**	158	310	310	310	180	180	**156**	290	266	254	n.a.
	M2_BE2	184	177	173	250	240	**202**	180	**165**	158	308	308	308	180	180	**156**	289	267	251	n.a.
	M2_BE1	173	173	173	243	237	**202**	186	167	**165**	290	274	274	187	171	163	188	286	255	n.a.

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group	sample	MSTA44B			MSTA53			MSTA78			MSTA93			MSTA131			MSTA133			cpDNA haplotype
																				Locus
„PRU"	P_H1	173	173	173	244	234	221	172	167	**165**	286	285	280	190	180	**156**	306	288	280	n.a.
	P_KAS1	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	266	n.a.
	P_KO8	171	171	171	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	cp*2a*
	P_SAS4	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_CEN3	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	cp*2a*
	P_CEN4	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_CEN5	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_DR1	173	173	173	250	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_GA11	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_ROH_b	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_Stud	173	173	173	246	206	196	186	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_PŠ_b	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_10	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	279	276	276	n.a.
	P_8	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_6	173	173	173	246	206	196	186	167	**165**	288	286	286	180	180	**156**	280	276	276	n.a.
	P_5	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_4	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_3	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_H4	188	173	173	247	206	196	182	167	**165**	289	285	285	190	180	163	288	280	276	n.a.
	P_H6	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
	P_H7	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	270	270	n.a.
	P_H8	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	283	276	276	n.a.
	P_BE	173	173	173	246	206	196	182	167	**165**	288	286	282	180	180	**156**	280	276	276	n.a.
„PRU_sex"	Ps_H2	190	173	173	246	207	204	182	170	**165**	288	286	282	188	180	**156**	288	280	280	n.a.
	Ps_H3	190	173	173	246	207	204	182	170	**165**	288	286	282	188	180	**156**	288	280	280	cp*1a*
	Ps_EUR	190	173	173	246	207	203	182	170	**165**	289	286	286	188	180	**156**	286	280	280	cp*1a*
	Ps_TL2	184	173	173	250	212	205	182	176	**165**	282	280	280	180	180	**156**	276	266	266	n.a.
	Ps_TO1	184	173	173	250	214	206	182	176	**165**	286	282	282	180	180	**156**	276	266	266	n.a.

group	sample	MSTA44B			MSTA53			MSTA78			MSTA93			MSTA131			MSTA133			cpDNA haplotype
																	Locus			
„PRU_d"	Pd_PTA	173	173	173	246	230	207	182	180	**165**	290	282	282	184	184	**156**	292	274	274	n.a.
	Pd_18	173	173	173	248	228	207	182	180	**165**	290	282	282	184	184	**156**	290	274	274	cp*1a*
	Pd_17	173	173	173	246	230	207	180	182	**165**	290	282	282	184	184	**156**	292	274	274	cp*1a*
	Pd_44	173	173	173	248	235	207	183	180	**165**	290	286	284	184	184	**156**	294	274	274	cp*1a*
	Pd_48	173	173	173	250	230	207	183	180	**165**	292	286	284	184	184	**156**	298	274	274	n.a.
	Pd_49	173	173	173	248	235	207	183	180	**165**	290	286	284	184	184	**156**	294	274	274	n.a.
	Pd_45	191	182	173	215	211	209	170	165	**165**	290	278	278	194	186	186	300	280	266	n.a.
	Pd_H1	165	165	165	250	215	207	182	174	**165**	290	290	290	186	164	**156**	280	270	270	n.a.

group	sample	MSTA44B			MSTA53			MSTA78			MSTA93			MSTA131			MSTA133			cpDNA haplotypes
																	Locus			
„PU"	PU_24	189	183	183	248	213	206	170	154	154	290	290	290	188	180	**156**	302	292	256	cp*2b*
	PU_H1	189	183	183	246	215	206	170	154	154	290	290	290	190	180	**156**	302	292	256	cp*2b*
	PU_H2	189	183	183	246	215	206	170	154	154	290	290	290	190	180	**156**	302	292	256	cp*2b*
	PU_H3	miss	miss	miss	240	212	204	181	168	160	290	276	276	180	180	180	302	254	254	n.a.
	PU_H4	miss	miss	miss	246	212	206	170	154	154	290	290	290	miss	miss	miss	301	294	256	n.a.

group	sample	MSTA44B			MSTA53			MSTA78			MSTA93			MSTA131			MSTA133			cpDNA haplotypes
																	Locus			
„ARA"	ARA_H2_1	186	181	181	246	240	216	180	**165**	158	286	268	268	188	188	**156**	278	260	256	cp*5*
	ARA_H2_2	186	181	181	242	240	218	182	**165**	158	286	286	286	188	188	**156**	278	260	256	cp*2b*
	ARA_H2_3	186	181	181	242	240	216	178	**165**	158	286	286	286	188	188	**156**	278	260	256	n.a.
	ARA_H2_4	miss	miss	miss	248	240	216	180	**165**	158	286	286	286	miss	miss	miss	278	260	256	n.a.
	ARA_H6	193	183	183	242	213	205	170	**165**	164	292	280	280	190	187	164	304	258	258	n.a.

group	sample	locus												cpDNA haplotypes
		MSTA44B		MSTA53		MSTA78		MSTA93		MSTA131		MSTA133		
„SEX"	ERY_SB1	181	181	246	206	158	158	294	280	172	172	268	256	cp*1a*
	ERY_SH1	178	164	248	240	180	168	292	276	**156**	**156**	298	256	cp*4*
	ERY55	170	170	242	203	190	170	316	286	171	**156**	270	256	n.a.
	ERY59	191	178	238	204	177	177	288	276	164	**156**	280	256	n.a.
	ERY58	191	191	238	238	180	180	274	274	**156**	**156**	280	256	n.a.
	ERY61	173	173	256	247	188	176	292	274	171	**156**	300	298	n.a.
	ERY57	192	192	250	248	188	155	312	298	164	**156**	292	254	n.a.
	ERY33	172	172	244	211	176	174	297	296	**156**	**156**	270	256	cp*1a*
	ERY16	181	181	242	215	175	162	294	294	185	171	290	268	cp*2b*

Supporting information for Chapter 4.

Taraxacum pudicum, **a new apomictic microspecies of the section *Erythrosperma* from Central Europe**

Appendix 1 – List of examined herbarium specimens of *Taraxacum pudicum*

Czechia – Bohemia: Obory (Příbram): along the field path near the locality Hromádky (441.6 m), 2–2.5 km W (R. Hlaváček 31. 5. 1995 herb. R. Hlaváček, #C–7633). – Chrást u Kovářova, margin of forest on right bank of the Orlická přehrada dam, opposite the Orlík castle. 365 m a. s. l. (M. Soukup, V. Chán & V. Žíla 2003 herb. Žíla) [*very typical plants!*]

Moravia: Budišov village, paths in pine forest on Kněžský kopec hill, 1.5 km towards E from the centre of village, 490 m a.s.l., 49°16'22"N 16°02'02"E (R. J. Vašut & Ľ. Majeský 2008 OL) [FC, GD, cult.]. – Havraníky: heath 1.2 km towards W fom the village (P. Bureš et V. Grulich 1991 BRNU). – Ketkovice: castle ruins Levnov, 3.0 km towards SW from the centre of village, 330 m a. s. l. (R. J. Vašut 1999 OL). – Kravsko: road verges and paths, 1.5 km towards N from the centre of the village, 350 m n. m (R. J. Vašut 2000 OL). – Kuřimská Nová Ves: xerothermic slopes on S outskirts of the village, 0.5 km towards NW from the centre of village, 480 m a. s. l. (R. J. Vašut 2000 OL). – Malhostovice: Malhostovická Pecka hill, 1.0 km towards SSW from the centre of village, 330 m s. s. l., 49°19'33"N+16°29'42"E (R. J. Vašut 1999 OL). [FC, FCSS, GD, cult.] – Olbramkostel: castle ruins Šimperk, 3.0 km towards WWN from the centre of the village, 390 m a. s. l. (R. J. Vašut 2000 OL). – Pocoucov: xerothermic hill near the road to the Trnavy village, 1.5 km towards NE from the centre of village, 460 m a. s. l. (R. J. Vašut 2000 OL). – Synalov – Kopaniny: pathways on S slope of the hill Sýkoř (702 m), 1.0 km towards S from its summit, 620 m a. s. l. (R. J. Vašut 1999 OL). [FC, GD, cult.] – Tišnov – Květnice hill (470 m): pathway and S rocky slopes, 0.8 km towards SW from its summit, 380 m a. s. l. (R. Vašut 1999 OL). [FC, GD, cult.] – Trnava u Třebíče, dry pasture, ca. 0.8 km towards NNW from the church in village, 460 m a. s. l., N49°15'33,9", E015°55'32,1" (L. Ekrt 2012 Ekrt herb.) [*T*. cf. *pudicum*].

Czechia – Lower Austria: Baden, paths along castle ruins Rauhenstein, ca. 300 m a. s. l., 48°00'48"N 16°12'18"E (R. J. Vašut & Ľ. Majeský 2009 OL) [*T*. cf. *pudicum*].

Poland: occurrence of species is yet unknown, however, plants extreme in their morphology (grown either in shadow or in high grass) that resemble T. pudicum in several characters (outer bracts, achenes, the design of leaf-blade morphology) were collected in vicinity of Żuchlów in the Wielkopolska region. This occurrence has to be confirmed by observing well developed plants.