universität wien

DISSERTATION/DOCTORAL THESIS

Titel der Dissertation / Title of the Doctoral Thesis

Identification of processes governing co-occurrence of sexual and apomictic polyploids in *Potentilla puberula*

verfasst von / submitted by

Lic. Henar Alonso-Marcos, MU.

angestreber akademischer Grad / in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD)

Wien, 2019 / Vienna, 2019

Studienkennzahl lt. Studienblatt / degree programme code:	A 794 685 437
Dissertationsgebiet lt. Studienblatt / field of study as it appears on the student record sheet:	Biologie/Biology
Betreut von / Supervisor:	Mag. Dr. Karl Hülber

ACKNOWLEDGMENTS

I would like to express my gratitude to the following people and institutions. First, I would like to thank my supervisor Priv.-Doz. Mag. Dr. Karl Hülber, who provided me guidance and advice for the completion of this thesis. I also acknowledge Dr. Christoph Dobeš for his experience and knowledge as head of the project. I want to thank my colleague MSc. Flavia Domizia Nardi, for the time spent in the Alps together and for being a talented researcher from whom I learnt every day.

Many thanks to all the friendly members and colleagues of the Austrian Research Centre for Forests (BFW), to Dr. Berthold Heinze, Dr. Michael Mengl and Alexander Schmid for their support in the lab, and to Ing. Raffaela Wettl for her work with soil analysis. I very much appreciate DI. Karl-Manfred Schweinzer, whose data skills are in keeping to his great kindness, DI Ambros Berger and Cristina Bouissou Maturana for her friendship.

I also would like to thank Christof Endl and Dr. Johannes Saukel; Mag. Susanne Scheffknecht for her generous advice and all her valuable previous hard work; Dr. Johannes Wessely, for his mathematical knowledge and always-cheerful attitude; and Dr. Andreas Tribsch for his contributions from Salzburg.

I am deeply grateful to each of the master students who worked with me during this project, especially in the field and the experimental garden. Thanks to Julian Ananda Haider, Simon Stiftler, Moritz Falch, Iason Pifeas, Sandra Meglic, Patricia Pérez Rodíguez and Tuuli Myllynen, for their commitment, enthusiasm and great dedication. I also want to acknowledge the FWF (Austrian Science Foundation) for financial support (Project P27688, granted to Dr. Christoph Dobeš), the anonymous reviwers of the three manuscripts who helped me to improve, the Life Sciences StudyServiceCentre of my faculty, and the two external examiners who kindly agreed to review this thesis.

My gratitude goes to my fabulous friends in Vienna and Barcelona, especially to Joaquim Moreno Cáceres, Marta Doménech Cabré, Silvia Trillo Daura, Josep Serrat i Orri, and Javier Luque Martínez, whose friendship is priceless to me. Finally, I would like to thank my beloved parents Felipe and Ángeles, and my brother Felipe Alonso-Marcos: they are my heart, my home and guide. Words are not enough to thank my life partner and one-of-a-kind Raúl Jiménez-Heredia, for his unconditional and true love, and to whom I dedicate this thesis.

TABLE OF CONTENTS

ABSTRACT

Polyploidization can be associated with reproductive changes like the breakdown of self-incompatibility and the evolution of apomixis (i.e. asexual reproduction via seeds). Apomicts and sexual individuals might occur in cytologically mixed populations, but they frequently show a spatial structure: apomicts tend to have larger ranges in ecologically more extreme environments than their sexual progenitors. Furthermore, reproductive interactions can suppress or promote cytotypes. Apart from the minority cytotype exclusion, sexuals might have reduced reproductive success due to cross-fertilization with apomicts in mixed-ploidy populations, or may even be transformed due to introgression of apomixis genes. Therefore, ploidy and reproductive mode might affect the distribution of cytotypes, although it is still less known which one is the most decisive factor.

In *Potentilla puberula* (Rosaceae) the presence of sexual tetraploids and penta- to octoploid apomicts is negatively associated. We related occurrence patterns of sexual and apomictic cytotypes in 238 sympatric populations from the Eastern European Alps to bioclimatic, soil and topographic data. We also estimated genetic relatedness and structure between and within cytotypes using AFLPs and plastid DNA sequencing. Moreover, we also tested the role of pollen precedence as a barrier against cross-fertilization with controlled hand-pollinated crosses, and inferred embryo's paternity through DNA ploidy estimation by means of flow cytometry seed screen (FCSS).

Our results showed that sexuals preferred primary habitats at drier, steeper, south-oriented slopes, while apomicts lived mostly in wetter human-disturbed habitats. In contrast, no relevant ecological differentiation among apomictic higher ploids was found. We observed a genetic clustering between sexual tetraploids and apomictic higher ploids individuals, being the apomictic ones of autopolyploid origin. Regarding their reproductive interaction, the obtained seed yield was significantly lower in hetero- than in homoploid crosses, and there was evidence for homoploid pollen precedence. Even if some of the fertilized embryos experimented a change in ploidy in the mixed-ploidy crossings, pollen precedence can operate as a reproductive barrier.

Therefore, we determine that the observed ecological differentiation in sympatric conditions between sexual and apomictic individuals, the intraspecific origin of apomictic genotypes as well as the evidence of pollen precedence in mixed-ploidy fertilizations

indicate that is the reproductive mode rather than ploidy the main differential driver that promotes the current co-existence of cytotypes in *P. puberula*.

ZUSAMMENFASSUNG

Polyploidisierungsereignisse können mit Veränderungen des Reproduktionssystems, wie dem Zusammenbruch von Selbstinkompatibilitäten oder der Entwicklung von Apomixis (i.e. asexueller Reproduktion mittels Samen) einhergehen. Apomiktische und sexuelle Pflanzen können in gemischten Populationen gemeinsam vorkommen, meist zeigt sich hingegen eine räumliche Struktur des Vorkommens: Apomikten haben tendenziell größere Verbreitungsgebiete und besiedeln ökologisch extremere Lebensräume als ihre sexuellen Vorfahren. Außerdem können reproduktive Interaktionen einzelne Zytotypen selektiv fördern oder unterdrücken. So kann beispielsweise Fremdbestäubung durch Apomikten zu vermindertem Reproduktionserfolg bei Sexuellen oder sogar zu deren Transformation durch die Introgression von Apomixisgenen führen. Sowohl die Ploidiestufe als auch der Reproduktionsmodus können also die Verteilung der Zytotypen im Raum beeinflussen, es ist aber noch unzureichend bekannt, welches der entscheidende Faktor ist.

Potentilla puberula (Rosaceae) beinhaltet sowohl sexuelle, tetraploide Pflanzen als auch apomiktische Individuen höherer Ploidiestufe (pentaploid bis oktoploid), die einen Trend des gegenseitigen räumlichen Ausschlusses zeigen. Wir untersuchten das Vorkommen dieser Zytotypen hinsichtlich Bodenproben, klimatischer und topographischer Daten in 238 Populationen der östlichen Alpen. Des Weiteren ermittelten wir den Grad der genetischen Verwandtschaft zwischen und innerhalb der verschiedenen Zytotypen mittels AFLP, sowie DNA Sequenzierung des Plastids. Außerdem quantifizierten wir das Ausmaß der Bevorzugung von homoploiden Pollen als Barriere gegen Fremdbestäubung im Rahmen von kontrollierten Bestäubungsexperimenten per Hand und determinierten die Ploidiestufe zur Vaterschaftsbestimmung.

Wir konnten zeigen, dass sexuelle Pflanzen bevorzugt auf primären Standorten wie trockenen, steilen, südorientierten Hängen zu finden sind während Apomikten vermehrt in feuchteren, durch menschlichen Einfluss geprägten Habitaten vorkommen. Es wurden jedoch keine relevanten Unterschiede der ökologischen Nischen der apomiktischen Zytotypen festgestellt.

Wir beobachteten eine genetische Gruppierung zwischen sexuellen Tetraploiden und apomiktisch höher ploiden Individuen, wobei die apomiktischen einer autopolyploiden Herkunft entspringen. Der Samenertrag aus homoploiden Kreuzungen war signifikant höher, als jener in heteroploiden und es gab Hinweise auf erhöhten Befruchtungserfolg homoploiden Pollens. Obwohl sich einige der befruchteten Embryos während der Kreuzung mit Samen verschiedener Polyploidiestufen einer Veränderung des Ploidiegrads unterzogen, ist die Bevorzugung von homoploiden Pollen als Barriere gegen Fremdbestäubung gegeben.

Aufgrund der Ergebnisse dieser Studie, kommen wir zu dem Schluss, dass die ökologischen Unterschiede in sympatrischen Konditionen von sexuellen und apomiktischen Individuen, sowie die intraspezifische Herkunft der apomiktischen Genotypen, als auch die Existenz der Bevorzugung von homoploiden Pollen darauf hinweisen, dass die Triebkraft der Koexistenz verschiedener Zytotypen in *P. puberula* bevorzugt auf den Modus der Reproduktion und nicht auf den Grad der Ploidie zurückzuführen ist.

INTRODUCTION

The *abominable mystery* of angiosperm diversification

The evolution of the first angiosperms and the mechanisms through which they diversified in almost all terrestrial habitats have been long-debated questions. In 1879, a surprised **Charles Darwin** already suspected that the angiosperm radiation must have originated from one gymnosperm-like species that dominated the world during the Early Cretaceous period. However, when he coined the 'abominable mystery' expression (Darwin, 1872; Darwin & Seward, 1903; Davies et al., 2004), he did not refer to the huge variety of angiosperm species but to the idea that flowering plants could contradict the very sense of the evolutionary theory, sustained in gradual changes in time (Friedman, 2009). If natural selection could *jump* from one biological key feature to the next one (i.e. saltational evolution), as it seemed with the sudden rise of angiosperms, then these abrupt and rapid changes could validate creationist arguments instead.

The *mystery* appeared under a slightly different light to **Gregor Mendel** while working with *Hieracium* (Koltunow et al, 2011; Bicknell et al, 2016). He wanted to replicate his previous observations of the inheritance of traits. However, the segregation results with hawkweed seeds did not support those obtained in the sexually reproducing *Pisum sativum*: this was because many *Hieracium* species formed seeds through an asexual mechanism, about which Mendel was not aware. Again, angiosperms would allegedly contradict this now well-known hypothesis, since some of their peculiar features were at that time still not fully grasped.

If the asexual pathways were not properly understood, a similar situation occurred during the study of the nucleotides containing the genetic information. In the 1920s, it was thought that animals had different nuclear acids from higher plants and yeasts. The animal thymonycleic acid received its name from the thymus of calves used during the isolation, while the plant nucleic acid was derived mostly from wheat and rye germ (Kasten, 2003). The sugars associated to both acids were erroneously thought to be different: a pentose for the plants and a hexose for animals. This perspective changed when Feulgen & Rossenbeck (1924) devised a selective cytochemical detection in cell nuclei of hydrolized thymonucleic acid, named the **Feulgen reaction** (Chieco & Derenzini, 1999). It allowed to identify chromosomal material in a given cell through a staining technique and showed that both animals and plants contained the same nuclear substance: deoxyribonucleic acid

or DNA. That was the starting point for the study of DNA content, genome size, ploidy identification and further developments of densiometry techniques, flow cytometry and laser scanning cytometry (Doležel et al., 2007; Greihulber, 2008; Pozarowski et al., 2013).

The polyploid condition

The high angiosperm diversity has often been attributed to **diffuse coevolution** with pollinators and herbivores (Bakker, 1978; Crepet, 1984; Sussman, 1991; Barret & Willis, 2001; Hu et al., 2008; Van der Niet et al., 2014). Other arguments that may explain angiosperm high competitiveness are the novel morphological features – like the flower or fleshy fruit – that allowed them to outcompete formerly dominant ferns and gymnosperms. Examples include a more efficient gas exchange activity, major photosynthetic capacity, changes in the nerve architecture and genome downsizing (Berendse & Scheffer, 2009; Brodribb & Field, 2010; de Boer et al., 2012; Simonin & Roddy, 2018). However, although these characteristics can be true for most species, one must notice that gymnosperms (especially conifers) are still more abundant than angiosperms in particular geographic regions and habitats like boreal forests (but see Fragnière et al., 2015).

The acquisition of evolutionary novelties that lead to the angiosperm diversification may have been promoted by **whole genome duplication** events (WGD), in which the whole genome of a cell is doubled, resulting in additional sets of chromosomes (Soltis et al., 2009; Moriyama & Koshiba-Takeuchi, 2018). The seed plants have undergone two distinct rounds of ancestral WGD. The first round, circa ~310 Mya, predated the origin of the seed plants, while the second one, around ~160 Mya, has been associated with the origin of the early angiosperms related to the ANITA grade (Jiao et al, 2011), a basal group including *Amborella*, Nymphaeales, Illiciales and Triminaceae-Austrobaileya (Qiu et al., 1999; *Amborella* Genome Project, 2013). The condition of possessing more than two sets of chromosomes, also called polyploidy, originated several times in all major clades of the eukaryote phylogeny, being more frequent in the plant kingdom. Duplication events thus continued to occur ubiquitously among angiosperms, including repeated rounds of polyploidization. Between 30% and 70% of them are thought to be of a recent polyploid origin (Otto & Whitton, 2000; Soltis et al., 2009; Van der Peer, 2009; Vanneste et al., 2014; Clark & Donoghue, 2018), with a high incidence in the Poaceae, Asteraceae

and Rosaceae families (Stebbins, 1950; Grant, 1981; Vamosi & Dickinson, 2006). Besides duplication events, polyspermy, i.e. the fertilization by more than one sperm cell, can also be a rare route towards polyploidy, at least as observed in *Arabidopsis* (Nakel et al., 2017), broadening the bi-organismal concept of parentage.

Polyploidy, as a major driver of plant speciation, entails important ecological, morphological and evolutionary effects, including genomic re-arrangements, gene silencing, epigenetic instability and/or even niche shifts (Ramsey & Schemske, 2002; Comai, 2005; Adams & Wendel, 2015). The two main types are autopolyploidy, which emerges from within a species (i.e. intraspecifically); and allopolyploidy, involving the merger of genomes of different species or diverged evolutionary lineages (i.e. via hybridization) (te Beest et al., 2012). Autopolyploids theoretically have a genome made of multiple copies of homologous chromosomes (e.g. $A_1A_2A_3A_4$), which show polysomic inheritance. Contrariwise, the chromosomes contributed by different species to allopolyploids are referred to as homoeologous (e.g. $A_1A_2B_1B_2$, in a strict sense they are non-homologous), leading mostly to a disomic inheritance (Stift et al., 2008). The duplicated genes may experience different fates: they either may be pseudogenized, lost, silenced or diverged from each other by means of adaptative processes (Moriyama & Koshiba-Tageuchi, 2018). In that latter case, the "extra" copy can evolve another specific function (i.e. subfunctionalization) or develop an entirely novel function (i.e. neofunctionalization). Hence, following the 'Radiation Lag-Time' model, WGD events are followed by diversification shifts after a certain lag period (Schranz et al., 2012; Tank et al., 2015). Several rounds of polyploidization (the "wondrous cycle of polyploidy", Wendel, 2015) occur during long evolutionary timescales until reaching the point of polyploidy *drop* in which organisms head towards diploidization again (Mandáková & Lysak, 2018). The stochasticity of the polyploid drop after the WGD event is actually considered what promotes the subsequent *abominable* radiation (Baduel et al., 2018).

A factor that greatly contributes to polyploidization is the formation of unreduced gametes (Otto & Whitton, 2000; Spoelhof et al., 2017). Irregularities during the meiotic division is one of the several challenges that newly formed polyploids have to overcome. Thus, not all the modifications or novelties are straightforward. Polyploids usually originate within a diploid population where their chances to succeed depend on the interactions of the neopolyploids with the surrounding ancestral individuals in time and space. According to Levin's **minority cytotype exclusion principle** (1975), rare

cytotypes (i.e. individuals with a ploidy level different from of the majority of plants in the population) may be supressed due to reduced female fertility in mixed-ploidy populations under random mating conditions. In fact, a difference in ploidy among mating partners commonly results into a disrupted development or even the abortion of the zygote (Coyne & Orr, 1998; Kultunow & Grossniklaus, 2003). Moreover, the existence of direct competition with the parental diploids enhances the pressure that new polyploids undergo.

In order to avoid these threats, polyploids can be assumed to possess characteristics reducing the competitive pressure with the parental individuals and, hence, allow them to increase their rates of establishment and persistence. This purpose can be achieved if polyploids and diploids show differences in ecological requirements. **Niche divergence,** for instance, has been found in natural populations of *Achillea borealis* (Ramsey, 2011). Using field transplant experiments, the author reported that neohexaploids adapted better to dune habitats than their tetraploids counterparts did. In *Arabidopsis thaliana*, the autotetraploids displayed a higher salt tolerance than diploids (Chao et al., 2013), supporting the idea that polyploidization can alter physiological properties and confer instant advantages to a novel environment.

Apomixis and the role of sex

Another strategy to mitigate the costs of rarity is to reduce the probability of disadvantageous matings with another ploidy level individual (i.e. heteroploid fertilization), and at the same time, increase the chances to reproduce with an individual of the same cytotype (i.e. homoploid fertilization). **Reproductive isolation** is therefore fundamental to maintain the co-occurrence of cytotypes in a given habitat. The suite of mechanisms that restrict gene flow as reproductive barriers are divided into pre- and post-zygotic barriers. Pre-zygotic barriers are the ones that operate against the formation of the zygote, before the fertilization takes place. They can act through spatial isolation (Hülber et al., 2009; Sonnleitner et al., 2010), temporal isolation via asynchronous flowering time (Petit et al., 1997; Lamont et al., 2003; Botes et al., 2008), differential flower architecture (Grant, 1994; Kay, 2006) leading to assortative pollinator behaviour (Husband & Schemske, 2000; Lowry et al., 2008), selfing – including recently discovered anther rubbing (Abdelaziz et al., 2019) –, and pollen precedence (Howard, 1999). Conversely, post-zygotic barriers minimize the reproductive potential of the population through the abortion of the zygote/seed or reduced viability of the progeny (Muller, 1932;

Dobzhansky, 1936). Male-female gametophyte interactions after pollination (i.e. pollen-pistil) can also cause pollen tube incompatibility, slower pollen tube growth and/or delayed generative mitosis (Williams et al., 1999; Laninken & Skogsmyr, 2002; Swanson et al, 2016).

Another approach to avoid mixed-ploidy reproductive costs is shifting to asexual reproduction, through either vegetative reproduction or **apomixis** (i.e. asexual reproduction via seeds, Asker & Jerling, 1992; Hörandl et al., 2008). Polyploidy is often associated with the breakdown of self-incompatibility, both in apomictic (Hörandl, 2010) and in sexual polyploids (Barret, 2002; Robertson et al., 2011). Reproductive assurance, i.e. the ability to self-pollinate, has been proposed to confer asexual polyploids greater colonization abilities in remote areas without potential partners and lack of pollinators, resulting into larger distributions according to the geographical parthenogenesis hypothesis (Vandel, 1928; Bierzychudek, 1985; van Dijk, 2003; Hörandl & Paun, 2007; Hörandl et al., 2008; Vrijenhoek & Parker, 2009; Cosendai et al., 2013; Karunarathne et al., 2018).

Apomixis was first reported as a reproductive anomaly in *Alchornea ilicifolia* (Smith, 1841*),* years before Mendel stumbled with the aforementioned *mystery* with *Hieracium*, a genus comprising mainly apomictic lineages (Nogler, 2006; Bicknell et al., 2016). However, nowadays it is well known that apomixis is taxonomically widespread (Hojsgaard et al., 2014). For the raise of apomixis from a sexual background, at least two mutations are needed to operate together: one for **apomeiosis**, to develop the female gametophyte without a reductional division (via diplospory or apospory), and one for **parthenogenesis**, to develop the embryo only with maternal genetic material. During the female sexual meiosis, one out of the four megaspores undergoes three rounds of mitotic divisions giving rise to an 8-nucleate female gametophyte (also called embryo sac) (Van Dijk, 2009). It contains two synergids that signalize the way for the pollen tube; one egg cell (n) which fuses with one sperm cell (n) to form the zygote and finally the embryo (2n); two polar nuclei (n + n) that fuse with the other sperm cell (n) to form the endosperm (3n); and three antipodal cells which eventually degenerate. Sexuality in the angiosperms is therefore characterized by a double fertilization and a meiotically reduced female gametophyte. In contrast, in gametophytic apomixis (Nogler, 1984) female meiosis does not occur. This causes an imbalance of the 2:1 *normal* maternal-paternal genomic ratio of the endosperm, either to a 4:1 with only unreduced female but reduced male

contribution, or to a 4:2 ratio, with both unreduced male and female gametes. However, apomicts have developed adjustments to maintain this ratio, like formation of unreduced pollen grains or a four-nucleated female gametophyte with only one polar nucleus (Van Dijk, 2009).

Generally, diploids (or low polyploids) reproduce sexually whereas polyploids (or high-ploid individuals) are apomictic (Bayer, 1997; Hojsgaard et al., 2008; Cosendai et al., 2011). Interestingly, in some apomicts, a fertilization event is still necessary to trigger the development of the endosperm (i.e. **pseudogamy**) and thereby functionality of the seed (Rutishauser, 1969). Moreover, apomicts may retain the male capacity and be potentially able to fertilize their sexual counterparts, which can lead to a reproductive asymmetry in reproductively mixed populations (Van Dijk & Vijverberg, 2005; Hersch et al, 2016; Dobeš et al., 2018), affecting sexual offspring. Furthermore, apomixis is considered an inheritable trait (Ozias-Akins & Van Dijk, 2007) and, at least theoretically, can introgress, and transform sexual populations (Joshi & Moodie, 1998). Several models have been proposed to explain and predict the dynamics of reproductively mixed-populations, accounting for reproductive transformation and suppression in case of panmixis (Joshi & Moddy, 1995) and considering space via a diffusion function (Britton and Mogie, 2001). However, the assumption of equal probability of pollen transfer and fertilization to all individuals regardless of their cytotype is far from realistic expectations under natural conditions, since cytotypes tend to cluster resulting in non-random mating (Keeler, 1992; Husband & Schemske, 2000; Baack, 2005; Duchoslav et al., 2010).

The need of sex and the advantages of being apomictic seem to be the two faces of the same reproductive coin, and the discussion continues. For Albertini et al. (2019), neither sex evolved from apomixis nor apomixis evolved from sex. Instead, the authors defy sexuality as the default condition and propose that both reproductive modes are polyphenisms of each other. In this way, when facing different environmental conditions, plants will switch on/off certain genes. Regarding the evolution of sex, Mirzaghaderi & Hörandl (2016) suggested that the primary function of meiosis is DNA restoration rather than recombination. Recent studies also explore the relationship of polyploidy with global change. According to Cai et al. (2019), WGD in Malpighiales coincided with the Eocene global climatic upheaval, and a similar parallelism with the current situation can be drawn. Even if in the near future around 33% of plant species may disappear, new ones will still form, specially auto- and allopolyploids, and to a lesser extent, through

homoploid hybrid speciation and chromosomal rearrangements (Vallejo-Marín & Hiscock, 2016; Levin, 2019). Polyploidy can be seen as a "*Las Vegas*" strategy (Sessa, 2019), in which WGD events are like bets in the evolutionary casino: perhaps most of them end up with a loss, but the ones that *win*, may experience strong diversification.

The model system

In this thesis, the rosaceous species ***Potentilla puberula*** Krašan (*Potentilla pusilla* Host; Soják, 2010) is used as a suitable model for studying the drivers of the eco-geographic distribution of cytotypes and the interactions between sexual and apomictic individuals. It is an herbaceous perennial plant, growing preferably in xeric grasslands of the Eastern European Alps and Western Carpathians (Kurtto et al., 2004). The flowers are yellow, pentamerous and insect-pollinated. The fruitlets, brownish and one-seeded, measure on average about 1 mm in length and have no adaptations to long-distance dispersal.

P. puberula exhibits a series of reproductively differentiated ploidy levels. Five cytotypes are reported: tetra- (x= 7; 2n = 28), penta- (2n = 35), hexa- (2n = 42), hepta- (2n = 49), and octoploids (2n = 56; Dobeš, 1999). Tetraploid individuals reproduce almost

exclusively sexually and are self-incompatible, while higher ploids are predominantly self-compatible gametophytic apomicts and pseudogamous, because they require fertilization for the development of the endosperm and seed formation (Dobeš et al., 2013). In our model system, tetraploid individuals act as functional diploids, in a similar way Mráz et al. (2008) suggested for *Pilosella officinarum*.

Mixed-ploidy populations occur in sympatry in the Eastern Alps (Dobeš et al., 2013) and although cytotypes do not differ morphologically from each other (Bigl et al., 2019), their reproductive modes tend to be ecologically differentiated. Tetraploid individuals usually inhabit pristine sites, some of which in areas which served as glacial *refugia* during the last glacial maximum (LGM, Tribsch & Schönswetter, 2003), while higher polyploids tend to occupy secondary and more anthropogenically disturbed habitats at higher elevations. However, it is still unknown whether the ecological differences among cytotypes are due to polyploidy or apomixis-mediated effects. Besides, the fact that sexual and apomictic individuals can reproductively interact with each other may influence the demographic dynamics of the species.

Although *P. puberula* is considered an allopolyploid species (Wolf, 1908; Ehrendorfer, 1970; Soják, 2010), it is unclear how the apomictic cytotypes originated. Paule et al. (2012) suggested that they could have evolved via autopolyploidization, namely intraspecifically without hybridization events, since molecular differentiation between sexual and apomictic cytotypes was slight.

Aims of the thesis

The aims of this PhD thesis were defined to answer three **main questions**:

1. To maintain the co-occurrence of cytotypes of the complex amphi-apomictic system that *P. puberula* is, which is the role of ploidy level and reproductive mode on ecological parthenogenesis?

2. Considering the genetic relatedness with sexual tetraploids, are the apomictic cytotypes of *P. puberula* of allopolyploid (i.e. hybridization) or autopolyploid origin (i.e. intraspecific)? Does the formation of new apomicts arise *de novo* from sexual parents?

3. In mixed-ploidy populations, what is the role of pollen precedence in reproductive interactions between sexual and apomictic individuals of *P. puberula*? How can this post-pollination pre-fertilization mechanism operate and what could be its potential effects on the co-occurrence of cytotypes?

The first chapter, corresponding to the first question, is entitled "Difference in reproductive mode rather than ploidy explains niche differentiation in sympatric sexual and apomictic populations of *Potentilla puberula*". I studied the regional distribution of the model species in terms of **ecological parthenogenesis**. Since the phenomenon of apomixis is closely related to polyploidy, the causes of niche divergence among cytotypes are usually intermingled and cannot be unambiguously assigned to one of these two processes.

To clarify whether ploidy level or reproductive mode is the major driver of differentiation in sympatric populations of *P. puberula*, I compared the ecological requirements of sexual tetraploids with those of the apomictic higher ploids, correlating bioclimatic, soil and topographic data to their occurrence. In addition, I compared the ecological requirements among the three apomictic cytotypes. A survey of other published studies on ecological parthenogenesis was done, indicating for each case the difficulties to disentangle reproductive, ploidy and hybridization effects in the ecological differentiation. Due to the sympatric conditions of the cytotypes of *P. puberula*, the differential reproductive mode between tetraploids and high ploids, and the existence of more than one apomictic ploidy level, we could dismiss confounding hybridization or migration effects in our results.

Although sexual and apomictic individuals are ecologically differentiated, the distribution of the cytotypes is considered sympatric. Therefore, I was interested in the **genetic distances** between sexual and apomicts and their **reproductive interactions,** and how these interactions may affect the population structure. Thus, chapter 2, "Sexual intraspecific recombination but not *de novo* origin governs the genesis of new apomictic genotypes in *Potentilla puberula* (Rosaceae)", focus on estimating genetic relatedness and structure by means of molecular methods with Amplified Fragment Length Polymorphisms (AFLPs) and plastid DNA (cpDNA) sequencing. First introduced by Vos et al. (1995), AFLP fingerprinting allows comparing sexual and apomictic genotypes of *P. puberula*, scoring the polymorphisms at the individual level in a presence/absence

matrix (Paun & Schönswetter, 2012). In addition, six closely related species (*P. argentea*, *P. aurea*, *P. brauneana*, *P. crantzii*, *P. frigida* and *P. grandiflora*) were introduced in the analyses to test whether a third taxa was involved in the **origin of apomictic genotypes**. The observed genetic clustering between sexual tetraploids and apomictic higher ploids lead us to investigate how frequent heteroploid fertilization occurs in the formation of new cytotypes.

Hence, in chapter 3, entitled "Pollen precedence in sexual *Potentilla puberula* Krašan and its role as a protective reproductive barrier against apomictic cytotypes", I analysed the role of **pollen precedence** in heteroploid fertilizations of sexuals with apomictic pollen. Ex-situ crossing experiments have already shown a reduction of the seed set in sexual tetraploids in heteroploid crosses with apomictic cytotypes (Dobeš et al., 2018). This suggests, together with the viability of mainly meiotically reduced pollen of the apomicts, that reproductive interactions between sexuality and apomixis are potentially important drivers of the (fine-scale) distribution of the cytotypes in mixed-ploidy populations. We therefore explored sexual-apomictic reproductive interactions by means of experimental crossings and the paternity of the obtained seeds through flow cytometric seed screen analyses (FCSS), a reliable and efficient technique even in rare hemisexual species (Kolarčic et al., 2018). Knowing to which extend homo-, hetero and mixed-ploidy fertilizations succeed and the resultant paternity of the seeds allows us to understand the reproductive mechanisms in which cytotypes can co-occur.

REFERENCES OF THE INTRODUCTION

Abdelaziz, M., Bakkali, M., Gómez, J.M., Olivieri E. & Perfectti E. 2019. Anther rubbing, a new mechanism that actively promotes selfing in plants. *Amer. Naturalist*. 193, 140–147. https://doi.org/10.1086/700875

Adams, K.L. & Wendel, J.F. 2005. Polyploidy and genome evolution in plants. *Curr. Opin. Plant Biol.* 8: 135–141. https://doi. org/10.1016/J.PBI.2005.01.001

Albertini, E., Barcaccia, G., Carman, J.G. & Pupilli, F. 2019. Did apomixis evolve from sex or was it the other way around? *J. Exp. Bot*. 70: 2951–2964. https://doi.org/10.1093/jxb/erz109

***Amborella* Genome Project.** 2013. The *Amborella* genome and the evolution of flowering plants. *Science*. 342: 1241089.

Asker S.E. & Jerling, L. 1992. *Apomixis in plants*. Boca Raton: CRC Press.

Baack, E.J. 2005. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): minority cytotype exclusion and barriers to triploid formation. *Am. J. Bot.* 92: 1827–1832. https://doi.org/10.3732/ajb.92.11.1827

Baduel, P., Bray, S., Vallejo-Marín, M., Kolář, F. & Yant, L. 2018. The "polyploid hop": Shifting challenges and opportunities over the evolutionary lifespan of genome duplications. *Front. Ecol. Evol*. 6: 117. https://doi.org/10.3389/fevo.2018.00117

Bakker, R.T. 1978. Dinosaur feeding behaviour and the origin of flowering plants. *Nature* 274: 661–663.

Barrett, P.M. & Willis, K.J. 2001. Did dinosaurs invent flowers? Dinosaur–angiosperm coevolution revisited. *Biol. Rev. Camb. Philos. Soc.* 76: 411–447.

Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nat. Rev. Genet*. 3: 274–284. https://doi.org/10.1038/nrg776

Bayer, R.J. 1997. *Antennaria rosea* (Asteraceae) – A model group for the study of the evolution of polyploid agamic complexes. *Opera Bot.* 132: 53–65.

Berendse, F. & Scheffer, M. 2009. The angiosperm radiation revisited: an ecological explanation for Darwin's 'abominable mystery'. *Ecol. Lett*. 12: 865–872. https://doi.org/10.1111/j.1461-0248.2009.01342.x

Bicknell, R., Catanach, A., Hand, M. & Koltunow, A. 2016. Seeds of doubt: Mendel's choice of *Hieracium* to study inheritance, a case or right plant, wrong trait. *Theor. Appl. Genet.* 129: 2253–2266. https://doi.org/10.1007/s00122-016-2788-x

Bierzychudek, P. 1985. Patterns in plant parthenogenesis. *Experientia* 41: 1255–1264. https://doi.org/10.1007/BF01952068

Bigl, K., Paule, J. & Dobeš, C. 2019. The morphometrics of autopolyploidy: insignificant differentiation among sexual-apomictic cytotypes. *AoB PLANTS*. 11(3). https://doi.org. 10.1093/aobpla/plz028

Botes, C., Johnson, S.D. & Cowling, R.M. 2008. Coexistence of succulent tree aloes: Partitioning of bird pollinators by floral traits and flowering phenology. *Oikos* 117: 875–882. https://doi.org/10.1111/j.0030-1299.2008.16391.x

Britton, N.F. & Mogie, M. 2001. Poor male function favors coexistence of sexual and asexual relatives. *Ecol. Lett*. 4: 116–121. https://doi.org/10.1046/j.1461-0248.2001.00201.x

Brodribb, T.J. & Field, T.S. 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecol. Lett*. 13: 175–183. https://doi.org/10.1111/j.1461-0248.2009.01410.x

Cai, L., Xi, Z., Amorim, A.M., Sugumaran, M., Rest, J.S., Liu, L. & Davis, C.C. 2019. Widespread ancient whole-genome duplications in Malpighiales coincide with Eocene global climatic upheaval. *New Phytol.* 221: 565–576. https://doi.org/10.1111/nph.15357

Chao, D.Y., Dilkes, D., Luo, H., Douglas, A., Yakubova, E., Lahner, B. & Salt, D.E. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis. *Science* 341: 658–659. https://doi.org/10.1126/science.1240561

Chieco, P. & Derenzini, M. 1999. The Feulgen reaction: 75 years on. *Histochem. Cell Biol*. 111: 345–358. https://doi.org/10.1007/s004180050367

Clark, J.W. & Donoghue, P.C.J. 2018. Whole-genome duplication and plant macroevolution. *Trends Plant Sci.* 23: 933–945. https://doi.org/10.1016/j.tplants.2018.07.006

Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6: 836–846. https://doi.org/10.1038/nrg1711

Cosendai, A.-C., Rodewald, J. & Hörandl, E. 2011. Origin and distribution of autopolyploids via apomixis in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Taxon* 60: 355–364.

Cosendai, A.-C., Wagner, J., Ladinig, U., Rosche, C. & Hörandl, E. 2013. Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Heredity* 110: 560–569. https://doi.org/10.1038/hdy.2013.1

Coyne, J.A. & Orr, H.A. 1998. The evolutionary genetics of speciation. *Philos. Trans., Ser. B.*353: 287–305. https://doi.org/10.1098/rstb.1998.0210

Crepet, W.L. 1984. Advanced (constant) insect pollination mechanisms: pattern of evolution and implication vis-à-vis angiosperm diversity. *Ann. Missouri Bot. Gard*. 71: 607. https://doi.org/10.2307/2399041

Darwin, C. 1872. *The origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: John Murray.

Darwin, F., & A. C. Seward (Eds.). 1903. *More letters of Charles Darwin*. London, UK: John Murray.

Davies, T.J., Barraclough, T.G., Chase, M.W., Soltis, P.S., Soltis, D.E. & Savolainen, V. 2004. Darwin's abominable mystery: Insights from a supertree of the angiosperms. *Proc. Natl. Acad. Sci. U.S.A.* 101: 1904–1909. https://doi.org/10.1073/pnas.0308127100

De Boer, H.J., Eppinga, M.B., Wassen, M.J. & Dekker, S.C. 2012. A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. *Nat. Commun*. 3: 1221. https://doi.org/10.1038/ncomms2217

Dobeš, C. 1999**.** Die Karyogeographie des *Potentilla verna* agg. (Rosaceae) in Österreich – mit ergänzenden Angaben aus Slowenien, Kroatien, der Slowakei und Tschechien. *Ann. Naturhist. Mus. Wien*, B. 101: 599–629.

Dobeš, C., Milosevic, A., Prohaska, D., Scheffknecht, S., Sharbel, T.F. & Hülber, K. 2013. Reproductive differentiation into sexual and apomictic polyploid cytotypes in *Potentilla puberula* (*Potentilleae*, Rosaceae). *Ann. Bot*. 112: 1159–1168. https://doi.org/10.1093/aob/mct167

Dobeš, C., Scheffknecht, S., Fenko, Y., Prohaska, D., Sykora, C. & Hülber, K. 2018. Asymmetric reproductive interference: The consequences of pollination on reproductive success in sexual – apomictic populations of *Potentilla puberula* (Rosaceae). *Ecol. Evol.* 00: 1–17. https://doi.org/10.1002/ece3.3684

Dobzhansky, T. 1936. Position effects on genes. *Biol. Rev.* 11: 364–384. https://doi.org/10.1111/j.1469-185X.1936.tb00911.x

Doležel, J., Greilhuber, J. & Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nat. Protoc.* 2: 2233–2244. https://doi.org/10.1038/nprot.2007.310

Duchoslav, M., Šafářová, L. & Krahulec, F. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Ann. Bot.* 105: 719–735. https://doi.org/10.1093/aob/mcq035

Ehrendorfer, F. 1970. Mediterran-mitteleuropäische Florenbeziehungen im Lichte cytyotaxomischer Befunde. *Feddes Repert*. 81: 3–32. https://doi.org/10.1002/fedr.19700810103

Feulgen R. & Rossenbeck, H. 1924. Mikroskopisch-chemischer Nachweis einer Nucleinsäure von Typus der Thymonucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in Mikroskopischer Präparaten. Hoppe Seyler's Z. *Physiol Chem*. 135: 203–248.

Fragnière, Y., Bétrisey, S., Cardinaux, L., Stoffel, M. & Kozlowski, G. 2015. Fighting their last stand? A global analysis of the distribution and conservation status of gymnosperms. *J. Biogeogr.* 42: 809–820. https://doi.org/10.1111/jbi.12480

Friedman, W. E. 2009. The meaning of Darwin's "abominable mystery." *Am. J. Bot*. 96: 5–21. https://doi.org/10.3732/ajb.0800150

Grant, V. 1981. *Plant speciation*. New York: Columbia University Press.

Grant, V. 1994. Modes and origins of mechanical and ethological isolation in Angiosperms. *Proc. Natl. Acad. Sci. U.S.A.* 91: 3–2.

Greilhuber, J. 2008. Cytochemistry and C-values: The less-well-known world of Nuclear DNA Amounts. *Ann. Bot*. 101: 791–804. http://doi.org/10.1093/aob/mcm250

Hersh, E., Grimm, J. & Whitton, J. 2016. Attack of the clones: reproductive interference between sexuals and asexuals in the *Crepis* agamic complex. *Ecol. Evol.* 6: 6473–6483. https://doi.org/10.1002/ece3.2353

Hojsgaard, D., Klatt, S., Baier, R., Carman, J.G. & Hörandl, E. 2014. Taxonomy and Biogeography of Apomixis in Angiosperms and Associated Biodiversity Characteristics. *Crit. Rev. Plant Sci*. 33: 414–42. https://doi.org/10.1080/07352689.2014.898488

Hojsgaard, D., Schegg, E., Valls, J.F.M., Martínez, E.J. & Quarin, C.L. 2008. Sexuality, apomixis, ploidy levels, and genomic relationships among four *Paspalum* species of the subgenus *Anachyris* (Poaceae). *Flora Morphol. Distrib.*

Funct. Ecol. Plants. 203: 535–547. https://doi.org/10.1016/j.flora.2007.09.005

Hörandl, E. 2010. The evolution of self-fertility in apomictic plants. *Sex. Plant Reprod*. 23: 73–86. https://doi.org/10.1007/s00497-009-0122-3

Hörandl, E., Cosendai, A.-C. & Temsch & E.M. 2008. Understanding the geographic distributions of apomictic plants: A case for a pluralistic approach. *Plant Ecol. Divers.* 1: 309–320. https://doi.org/10.1080/17550870802351175

Hörandl, E. & Paun, O. 2007. Patterns and sources of genetic diversity in apomictic plants: Implications for evolutionary potentials. Pp.169–194 in: Hörandl, E., Van Dijk, P.J., Grossniklaus, U. & Sharbel, T.F. (Eds.), *Apomixis: Evolution, mechanisms and perspectives*. Ruggell, Liechtenstein: Gantner.

Howard, D.J. 1999. Conspecific sperm and pollen precedence and speciation. *Annual Rev. Ecol. Evol. Syst.* 30: 109–132.

Hu, S., Dilcher, D.L., Jarzen, D.M. & Taylor, D.W. 2008. Early steps of angiosperm-pollinator coevolution. *Proc. Natl. Acad. Sci. U.S.A.* 105: 240–245. https://doi.org/10.1073/pnas.0707989105

Hülber, K., Sonnleitner, M., Flatscher, R., Berger, A., Dobrovsky, R., Niesser, S., Nigl, T., Schneeweiss, G.M., Kubešová, M., Rauchová, J., Suda, J. & Schönswetter, P. 2009. Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. *Preslia* 81: 309–319.

Husband, B.C. & Schemske, D.W. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *J. Ecol.* 88: 689–701. https://doi.org/10.1046/j.1365-2745.2000.00481.x

Jiao, Y., Wickett, N.J., Ayyampalayam, S., Chanderbali, A.S., Landherr, L., Ralph, P.E., Tomsho, L.P., Hu, Y., Liang, H., Soltis, P.S., Soltis, D.E., Clifton, S.W., Schlarbaum, S.E., Schuster, S.C., Ma, H., Leebens-Mack, J. & de Pamphilis, C.W. 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature* 473: 97–100. https://doi.org/10.1038/nature09916

Joshi A. & Moody M.E. 1995. Male gamete output of asexuals and the dynamics of

populations polymorphic for reproductive mode. *J. Theor. Biol.* 174: 189–197.

Joshi A. & Moody M.E. 1998. The cost of sex revisited: effects of male gamete output of hermaphrodites that are asexual in their female capacity. *J. Theor. Biol.* 195: 533–542.

Karunarathne, P., Schedler, M., Martínez, E.J., Honfi, A.I., Novichkova, A., Hojsgaard, D. 2018. Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. *Ann. Bot.* 121: 1183–1196. https://doi.org/10.1093/aob/mcy004

Kasten, F.H. 2003. Robert Feulgen and his histochemical reaction for DNA. *Biotech. Histochem.* 78: 45–49. https://doi.org/10.1080/10520290312120009

Kay, K.M. 2006. Reproductive isolation between two closely related hummingbird-pollinated neotropical gingers. *Evolution* 60: 538–552.

Keeler, K.H. 1992. Local polyploid variation in the native prairie grass *Andropogon gerardii*. *Am. J. Bot.* 79: 1229–1232. https://doi.org/10.2307/2445049

Kolarčik, V., Kocová, V. & Vašková, D. 2018. Flow cytometric seed screen data are consistent with models of chromosome inheritance in asymmetrically compensating allopolyploids. *Cytometry* 93: 737–748. https://doi.org/10.1002/cyto.a.23511

Koltunow, A.M. & Grossniklaus, U. 2003. Apomixis: a developmental perspective. *Annual Rev. Pl. Biol.* 54: 547–574. https://doi.org/10.1146/annurev.arplant.54.110901.160842

Koltunow, A.M., Johnson, S.D., Rogrigues, J.C., Okada, T., Hu, Y., Tsuchiya, T., Wilson, S., Flechter, P., Ito, K., Suzuki, G., Mukai, Y., Fehrer, J. & Bicknell, R.A. 2011. Sexual reproduction is the default mode in apomictic *Hieracium* subgenus *Pilosella*, in which two dominant loci function to enable apomixis. *Plant J.* 66: 890–902. https://doi.org/10.1111/j.1365-313X.2011.04556.x

Kurtto, A., Lampinen, R. & Junikka, L. 2004. *Atlas Florae Europaeae 13. Distribution of vascular plants in Europe*, vol 13, *Rosaceae (*Spiraea *to* Fragaria, *excl.* Rubus).

The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki.

Lamont, B.B., He, T., Enright, N.J., Krauss, S.L. & Miller, B.P. 2003. Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. *J. Evol. Biol.* 16: 551–557. https://doi.org/10.1046/j.1420-9101.2003.00548.x

Laninken, Å. & Skogsmyr, I. 2002. Pollen competitive ability: the effect of proportion in two-donor crosses. *Evol. Eco. Res*. 4: 687–700.

Levin, D.A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43. https://doi.org/10.2307/1218997

Levin, D.A. 2019. Plant Speciation in the Age of Climate Change. *Ann. Bot.* mcz108. https://doi.org/10.1093/aob/mcz108

Lowry, D.B., Modliszewski, J.L., Wright, K.M., Wu, C.A. & Willis, J.H. 2008. The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philos. Trans., Ser. B.* 363: 3009–3021. https://doi.org/10.1098/rstb.2008.0064

Mandáková, T., & Lysak, M. 2018. Post-polyploid diploidization and diversification through dysploid changes. *Curr. Opin. Plant Biol.* 42: 55–65 https://doi.org/10.1016/j.pbi.2018.03.001

Mirzaghaderi, G. & Hörandl, E. 2016. The evolution of meiotic sex and its alternatives. *Proc. R. Soc. B.* 283: 20161221. http://doi.org/10.1098/rspb.2016.1221

Moriyama, Y. & Koshiba-Takeuchi, K. 2018. Significance of whole-genome duplications on the emergence of evolutionary novelties. *Brief. Funct. Genomics*. 17: 329–338. https://doi.org/10.1093/bfgp/ely007

Mráz, P., Šingliarová, B., Urfus, T. & Krahulec, F. 2008. Cytogeography of *Pilosella officinarum* (Compositae): altitudinal and longitudinal differences in ploidy levels distribution in the Czech Republic and Slovakia and the general pattern in Europe. *Ann. Bot.* 101: 59–71. https://doi.org/10.1093/aob/mcm282

Muller, H.J. 1932. Further studies on the nature and causes of gene mutations. Pp. 213–255 in: Jones, D.F. (ed.), *Proceedings of the Sixth International Congress of Genetics, Ithaca, New York, 1932*, vol. 1. Menasha, Wisconsin: Brooklyn Botanical Garden. : 213–255

Nakel., T., Tekleyohans, D.G., Mao, Y., Fuchert, G., Vo, D. & Groß-Hardt, R. 2017. Triparental plants provide direct evidence for polyspermy induced polyploidy. *Nat. Commun*. 8: 1033. https://doi.org/10.1038/s41467-017-01044-y

Nogler, G.A. 1984. Gametophytic apomixis. Pp. 475–518 in: Johri, B.M. (Ed.), *Embriology of Angiosperms*. Berlin: Springer-Verlag. https://doi.org/10.1007/978-3-642-69302-1_10

Nogler, G.A. 2006. The Lesser-known Mendel: His experiments on Hieracium. *Genetics* 172: 1–6.

Otto, S.P. & Whitton, J. 2000. Polyploid incidence and evolution. *Annu. Rev. Genet.* 34: 401–437. https://doi.org/10.1146/annurev.genet.34.1.401

Ozias-Akins, P. & Van Dijk, P.J. 2007. Mendelian genetics of apomixis in plants. *Annual Rev. Genet*. 41: 509–537. https://doi.org/10.1146/annurev.genet.40.110405.090511

Paule, J., Scherbatin, A. & Dobeš, C. 2012. Implications of hybridisation and cytotypic differentiation in speciation assessed by AFLP and plastid haplotypes: A case study of *Potentilla alpicola* La Soie. *B.M.C. Evol. Biol*. 12:132. https://doi.org/10.1186/1471-2148-12-132

Paun, O. & Schönswetter, P. 2012. Amplified fragment length polymorphism: an invaluable fingerprinting technique for genomic, transcriptomic, and epigenetic studies. *Methods Mol. Biol.* 862: 75–87. https://doi.org/10.1007/978-1-61779-609-8_7

Petit, C., Lesbros, P., Ge, X. & Thompson, J.D. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79: 31–40.

Pozarowski, P., Holden, E. & Darzynkiewicz, Z. 2013. Laser Scanning Cytometry: Principles and Applications–An Update. *Methods Mol. Biol.* 931: 187–212. https://doi.org/10.1007/978-1-62703-056-4_11

Qui, Y-L., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Zimmer, E.A., Chen, Z., Savolainen, V. & Chase, M.W. 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402: 404–407.

Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proc. Natl. Acad. Sci. U.S.A.* 108: 7096–7101. https://doi.org/10.1073/pnas.1016631108

Ramsey, J. & Schemske, D.W. 2002. Neopolyploidy in flowering plants. *Annu. Rev. Ecol. Syst.* 33: 589–639. https://doi.org/10.1146/annurev.ecolsys.33.010802.150437

Robertson, K., Goldberg, E.E. & Igić, B. 2011. Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. *Evolution* 65: 139–155. https://doi.org/10.1111/j.1558-5646.2010.01099.x

Rutishauser, A. 1969. *Embryologie und Fortplfanznugbiologie der Angiosperme*. Springer: Wien.

Schranz, M.E., Mohammadin, S. & Edger, P.P. 2012. Ancient whole genome duplications, novelty and diversification: The WGD Radiation Lag-Time Model. *Curr. Opin Plant Biol.* 15: 147–153. https://doi.org/10.1016/j.pbi.2012.03.011

Sessa, E. B. 2019. Polyploidy as a mechanism for surviving global change. *New Phytol*. 221: 5–6. https://doi. org/10.1111/nph.15513

Simonin, K.A. & Roddy, A.B. 2018. Genome downsizing, physiological novelty, and the global dominance of flowering plants. *PLoS Biol*. 16(1): e2003706 https://doi.org/10.1371/journal.pbio.2003706

Smith, J. 1841. XXXII. Notice of a Plant which produces perfect Seeds without any apparent Action of Pollen. *Trans. Linn. Soc. Lond.* https://doi.org/10.1111/j.1095-8339.1838.tb00200.x

Soják, J. 2010. Origin of *Potentilla crantzii*, *P. verna* and *P. puberula* (Rosaceae) with a note on the nomenclature of *P. pusilla*. *Feddes Repert.* 121: 112–116. https://doi.org/10.1002/fedr.201000010

Soltis, P.S. & Soltis, D.E. 2009. The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.* 60: 561–588. https://doi.org/10.1146/annurev.arplant.043008.092039

Sonnleitner, M., Flatscher, R., Garcia, P.E., Rauchová, J., Suda, J., Schneeweiss, G.M., Hülber, K. & Schönswetter, P. 2010. Distribution and habitat segregation on different spatial scales among diploid, tetraplod and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Ann. Bot. (Oxford)*. 106: 967–977. https://doi.org/10.1093/aob/mcq192

Spoelhof, J.P., Soltis, P.S & Soltis, D.E. 2017. Pure polyploidy: Closing the gaps in autopolyploid research. *J. Syst. Evol*. 55: 340–352. https://doi.org/10.1111/jse.12253

Stebbins, G.L. 1950. *Variation and evolution in plants*. London, UK: Oxford University Press,

Stift, M., Berenos, C., Kuperus, P. & van Tienderen, P.H. 2008. Segregation models for disomic, tetrasomic and intermediate inheritance in tetraploids: a general procedure applied to Rorippa (yellow cress) microsatellite data. *Genetics* 179: 2113–2123. https://doi.org/10.1534/genetics.107.085027

Sussman, R.W. 1991. Primate origins and the evolution of angiosperms. *Am. J. Primatol.* 23: 209–223. https://doi.org/10.1002/ajp.1350230402

Swanson, R.J., Hammond, A.T., Carlson, A.L., Gong, H. & Donovan, T.K. 2016. Pollen performance traits reveal prezygotic non-random mating and interference competition in *Arabidopsis thaliana*. *Am. J Bot*. 103: 498–513. https://doi.org/10.3732/ajb.1500172

Tank, D.C., Eastman, J.M., Pennell, M.W., Soltis, P.S, Soltis, D.E., Hinchliff, C.E., Brown, J.W., Sessa, E.B. & Harmon L.J. 2015. Nested radiations and the

pulse of angiosperms diversification: increased diversification rates often follow whole genome duplications. *New Phytol.* 207: 454–467. https://doi.org/10.1111/nph.13491

te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubešová, M. & Pyšek, P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Ann. Bot.* 109: 19–45. https://doi.org/10.1093/aob/mcr277

Tribsch, A. & Schönswetter, P. 2003. Patterns of endemism and comparative phylogeography confirm paleoenvironmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon* 52: 477–497. https://doi.org/10.2307/3647447

Vallejo-Marín, M. & Hiscock, S.J. 2016. Hybridization and hybrid speciation under global change. *New Phytol.* 211: 1170–1187. https://doi.org/10.1111/nph.14004

Vamosi J.C. & Dickinson, T.A. 2006. Polyploidy and diversification: a phylogenetic investigation in Rosaceae. *Int. J. Plant Sci.* 167:349–358.

Van der Niet, T., Peakall, R. & Johnson, S.D. 2014. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Ann. Bot.* 113: 199–211. https://doi.org/10.1093/aob/mct290

Van der Peer, Y., Maere, S. & Meyer, A. 2009. The evolutionary significance of ancient genome duplications. *Nat. Rev. Genet*. 10: 725–732. https://doi.org/10.1038/nrg2600

Van Dijk, P.J. 2003. Ecological and evolutionary opportunities of apomixis: Insights from *Taraxacum* and *Chondrilla*. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 358: 1113–1121. https://doi.org/10.1098/rstb.2003.1302

Van Dijk, P. 2009. Apomixis: Basics for Non-botanists. In: Schön I., Martens K., Dijk P. (Eds.), *Lost Sex*. Dordrecht: Springer. https://doi.org/10.1007/978-90-481-2770-2_3

Van Dijk P.J. & Vijverberg K. 2005. The significance of apomixis in the evolution of the angiosperms: a reappraisal. Pp. 101–116 in: Bakker F.T., Chatrou L.W., Gravendeel B., Pelzer P.B. (Eds.), *Plant Dpecies-Level Dystematics. New*

Perspectives on Pattern and Process, vol. 143. Ruggell, Liechtenstein: Gantner Verlag.

Vandel, A. 1928. La parthénogenese géographique. Contribution à l'étude biologique et cytologique de la parthénogenese naturelle. *Bulletin Biologique de la France et de la Bélgique*. 62: 164–182.

Vanneste, K., Baele, G., Maere, S. & Van der Peer, Y. 2014. Analysis of 41 genomes supports a wave of successful genome duplications in association with the Cretaceous-Paleogene boundary. *Genome Res*. 24: 1334–1347. https://doi.org/10.1101/gr.168997.113

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J. & Kuiper, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*. 23: 4407–4414. https://doi.org/10.1093/nar/23.21.4407

Vrijenhoek, R.C. & Parker, E.D. 2009. *Geographical parthenogenesis: General purpose genotypes and frozen niche variation.* Pp. 99–131 in I. Schön, K. Martens, & P. van Dijk (Eds.), *Lost sex*. Dordrecht, the Netherlands, Heidelberg, Germany, London, UK, New York, NY: Springer.

Wendel, J.F. 2015. The wondrous cycles of polyploidy in plants. *Am. J. Bot.* 102: 1753–1753. https://doi.org/10.3732/ajb.1500320

Williams, J.H., Friedman, W.E. & Arnold, M.L. 1999. Developmental selection within the angiosperm style: Using gamete DNA to visualise interspecific pollen competition. *Proc. Natl. Acad. Sci. U.S.A.* 96: 9201–9206. https://doi.org/10.1073/pnas.96.16.9201

Wolf, T. 1908. Monographie der Gattung *Potentilla*. Stuttgart: E. Schweizerbartsche Verlagsbuchhandlung.

CHAPTER 1

Difference in reproductive mode rather than ploidy explains niche differentiation in sympatric sexual and apomictic populations of *Potentilla puberula*

Henar Alonso-Marcos, Flavia Domizia Nardi, Susanne Scheffknecht,

Andreas Tribsch, Karl Hülber, Christoph Dobeš

Status: Published in *Ecology & Evolution*, March 2019

Contribution: field material and data collection, data analysis and visualization, writing – original draft manuscript, writing – review and editing

Difference in reproductive mode rather than ploidy explains niche differentiation in sympatric sexual and apomictic populations of *Potentilla puberula*

HENAR ALONSO-MARCOS[1, 2*], FLAVIA DOMIZIA NARDI[1, 3*], SUSANNE SCHEFFKNECHT[4], ANDREAS TRIBSCH[3], KARL HÜLBER[2+] AND CHRISTOPH DOBEŠ[1+]

[1]Department of Forest Genetics, Austrian Research Centre for Forests, Seckendorff-Gudent-Weg 8, A-1131, Vienna, Austria

[2]Department of Conservation Biology, Vegetation Ecology and Landscape Ecology, University of Vienna, Rennweg 14, 1030 Vienna, Austria

[3]Department of Biosciences, University of Salzburg, Hellbrunnerstraße 34, 5020, Salzburg, Austria

[4]Institute of Botany, University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33, 1180, Vienna, Austria

[*]These authors contributed equally to this work.

[+] These authors jointly supervised this work.

Running title: Ecological parthenogenesis in *Potentilla puberula*

Corresponding author: Flavia Domizia Nardi flavia.d.nardi@gmail.com

Alonso-Marcos, H., Nardi, F.D., Scheffknecht, S., Tribsch, A., Hülber, K., & Dobeš, C. 2019. Difference in reproductive mode rather than ploidy explains niche differentiation in sympatric sexual and apomictic populations of *Potentilla puberula*. *Ecol. Evol.* 9: 3588–3598. https://doi.org/10.1002/ece3.4992

ABSTRACT

Apomicts tend to have larger geographical distributional ranges and to occur in ecologically more extreme environments than their sexual progenitors. However, the expression of apomixis is typically linked to polyploidy. Thus, it is *a priori* not clear whether intrinsic effects related to the change in the reproductive mode or rather in the ploidy drive ecological differentiation. We used sympatric sexual and apomictic populations of *Potentilla puberula* to test for ecological differentiation. To distinguish the effects of reproductive mode and ploidy on the ecology of cytotypes, we compared the niches (i) of sexuals (tetraploids) and autopolyploid apomicts (penta-, hepta- and octoploids) and (ii) of the three apomictic cytotypes. We based comparisons on a ploidy screen of 238 populations along a latitudinal transect through the Eastern European Alps and associated bioclimatic, soil and topographic data. Sexual tetraploids preferred primary habitats at drier, steeper, more south-oriented slopes, while apomicts mostly occurred in human-made habitats with higher water availability. Contrariwise, we found no or only marginal ecological differentiation among the apomictic higher ploids. Based on the pronounced ecological differences found between sexuals and apomicts, in addition to the lack of niche differentiation among cytotypes of the same reproductive mode, we conclude that reproductive mode rather than ploidy is the main driver of the observed differences. Moreover, we compared our system with others from the literature, to stress the importance of identifying alternative confounding effects (such as hybrid origin). Finally, we underline the relevance of studying ecological parthenogenesis in sympatry, to minimise the effects of differential migration abilities.

Keywords: apomixis, ecological differentiation, European Alps, polyploidy, reproductive mode, sympatry

INTRODUCTION

Asexual plants tend to have larger geographical distribution ranges and to occur at higher latitudes and elevations compared to their sexual progenitors (Bierzychudek, 1985; Hörandl, 2006; Hörandl et al., 2008; van Dijk, 2003), a pattern known as 'geographical parthenogenesis' (Vandel, 1928). Such a geographical parthenogenesis is often linked to ecological differentiation among sexual and asexual populations, a phenomenon defined

as 'ecological parthenogenesis' (Bell, 1982). Specifically, asexuals tend to i) inhabit cooler or drier habitats and ii) have broader ecological ranges (e.g., in *Erigeron,* Noyes et al., 2006; *Paspalum intermedium,* Karunarathne et al., 2018; *Ranunculus auricomus*, Paule et al., 2018), albeit examples showing opposite trends (e.g., asexuals inhabit more humid habitats in *Limonium*, Caperta et al., 2017, and *Ranunculus kuepferi,* Kirchheimer et al., 2016; Schinkel et al., 2016) indicate the idiosyncratic response of taxa.

Opposing hypotheses attempt to explain the differences in ecogeographical ranges of sexual and asexual populations. Following the General-Purpose Genotype model (Lynch, 1984), selection would favour clones with broad ecological tolerance due to environmental heterogeneity over time (Lynch, 1984; Parker et al., 1977; Vrijenhoek and Parker, 2009) indicating asexuals as generalists having broader ecological tolerances and allowing the occupation of wider ranges. In contrast, following the Frozen Niche Variation model (Vrijenhoek, 1984), selection favours newly-formed asexual clonal lineages with ecological niche optima at the margins of the ecological potential of the sexual parents (i.e., specialists), determining a shift in ecological preferences and ultimately promoting colonisation of new geographical areas (Cosendai et al., 2013; de Kovel and de Jong, 2000; Vrijenhoek and Parker, 2009).

Ecological differentiation of sexuals and apomicts (i.e., plants reproducing asexually via seeds; Asker and Jerling, 1992) is often studied in the context of geographical parthenogenesis (e.g., Karunarathne et al., 2018; Lo et al., 2013; Noyes et al., 2006; Paule et al., 2018). However, differences in the geographical distribution may reflect different biogeographical histories or dispersal limitation rather than differences in ecological preferences (Brown et al., 1996; Sexton et al., 2009). Shifts in ecological niches may therefore occur subsequently to colonisation via adaptive processes to the new local environment, rather than arising as a direct effect of polyploidisation or reproductive shift (Kirchheimer et al., 2016). Sympatry of sexuals with apomicts (*sensu* Rivas, 1964) thus, offers the opportunity to unveil the immediate role of ecological differentiation of cytotypes on their distribution.

Despite rare exceptions (Böcher, 1951; Dobeš et al., 2006; Siena et al., 2008), apomicts are polyploid (Asker and Jerling, 1992; Carman, 1997; Comai, 2005; Dickinson et al., 2007) and often form so-called amphi-apomictic systems with sexual diploids (e.g., Asker, 1970; Bayer, 1997; Böcher, 1951; Campbell and Wright, 1996; Hörandl and Gutermann, 1999). As a consequence, geographical and ecological parthenogenesis may

depend on intrinsic effects of polyploidy (i.e., nucleotypic effects, Levin, 1983), rather than those of asexuality itself (Bierzychudek, 1985; Vandel, 1940). Polyploidisation may thereby affect the geographical and ecological distribution of taxa either as a direct result of nucleotypic effects (e.g., changes in gene expression patterns), or by subsequent divergence in gene function (Adams and Wendel, 2005; Blanc and Wolfe, 2004). Thus, it may promote ecological differentiation of cytotypes (Soltis et al., 2004; Visger et al., 2016) and extend ecological (Fawcett et al., 2009) and geographical ranges (Stebbins, 1985).

In this study, we aim to examine the role of ploidy and reproductive mode on ecological parthenogenesis. As our model system, we study sexual and apomictic sympatric populations of *Potentilla puberula* (Rosaceae), a species comprising five ploidy cytotypes well-differentiated in their reproductive mode: sexual tetraploids contrast autopolyploid apomicts with higher ploidy (penta- to octoploids). Based on a ploidy screen of 238 populations in the Eastern European Alps, and climatic variables, soil and topographic data, we i) investigate the existence of ecological parthenogenesis; and we ii) test for ploidy level-mediated ecological differentiation among the apomictic cytotypes. In case ecological parthenogenesis is followed by no or only marginal effects of ploidy, we explain the potential ecological differences among sexuals and apomicts by reproductive mode.

MATERIALS AND METHODS

Study species

The rosaceous species *Potentilla puberula* Krašan is an herbaceous plant growing preferably in xeric grasslands of the Eastern European Alps and Western Carpathians (Kurtto et al., 2004). It exhibits a series of reproductively differentiated ploidy levels comprising tetra- ($x = 7$; $2n = 28$), penta- ($2n = 35$), hexa- ($2n = 42$), hepta- ($2n = 49$) and octoploids ($2n = 56$; Dobeš, 1999). Tetraploid individuals reproduce almost exclusively sexually and are self-incompatible, whereas higher ploids are predominantly self-compatible and pseudogamous apomicts (Dobeš et al., 2018, 2013; Nardi et al., 2018). For hexaploids, both reproductive modes are documented, with apomictic individuals usually clustering with other apomictic cytotypes, and sexual individuals occurring in low frequencies within sexual tetraploid populations (which probably originate frequently *de*

novo as autohexaploids, Nardi et al., 2018). No fine-scale cytogeography has ever been obtained across the whole distributional range (see Kurtto et al., 2004), although data collected by Dobeš (1999) suggest that only the apomictic cytotypes are present in Eastern Austria. On the contrary, sexual and apomictic cytotypes exhibit a sympatric distribution in the Eastern Alps (Fig. 1; Dobeš et al., 2013; Nardi et al., 2018). Apomictic cytotypes are autopolyploid (i.e., being derived by duplication of a single genome; Nardi et al., 2018). Therefore, we could exclude potentially confounding effects of hybridisation (Hörandl, 2006), as observed in allopolyploid model systems (e.g., in *Crataegus*, Coughlan et al., 2014).

Study area and plant material

A total of 238 populations in the Eastern European Alps, between 45.53° – 47.78° N latitude and 10.10° – 13.81° E longitude, spanning an elevational range from 110 to 2,060 m a.s.l. were visited from 1999 to 2015 (Table A1). Per population, we collected an average of 15 individuals at least 2 m apart from each other and we kept them wet in zipped plastic bags for further cultivation in the experimental garden. We used Seradix B as a root-inducing medium on the tips of the roots before potting and watering plants with the fungicide Previcur 0.15%. We drew a map of the study area with QGIS v.2.8.2 (QGIS Development Team, 2018).

DNA ploidy level estimation

We determined DNA ploidy levels (Suda et al., 2006), from now on referred to as ploidy levels, for convenience) of 3,716 individuals of *P. puberula* by flow cytometric analyses of fresh leaf petioles using the Partec Ploidy Analyser PA and Partec CyFlow (Partec, Münster, Germany) following the protocol of Doležel et al., (2007). The ploidy of 1,838 individuals from 123 populations collected in 2015, was already presented and is described by Nardi et al. (2018). We estimated the ploidy of 1,878 additional individuals from 115 populations, collected during previous years (Table A1), using the standard two-step (Otto) protocol described by Doležel et al., (2007) with an internal standard (*Solanum pseudocapsicum*: Temsch et al., 2010; or *Lycopersicon esculentum* cv. Stupické polní tyčkové rané: Doležel and Bartoš, 2005). DAPI (4′-6-diamidino-2-phenylindole) served as DNA-selective stain. We calculated the sample/standard fluorescence ratios from the means of the sample and standard fluorescence histograms. We considered only histograms with coefficients of variation (CVs) < 5% for the G_0/G_1

peak of the analysed sample. We then attributed ploidy levels based upon the correlation of sample/standard fluorescence ratios with those of karyotyped individuals (Ptl4048, 2*n* = 4*x* = 28; Ptl4184, 2*n* = 5*x* = 35; Ptl4133, 2*n* = 7*x* = 49; Paule et al., 2012). In each measurement, we pooled individuals from the same population (usually five to eight).

Ecological variables

We combined climatic descriptors with topographic parameters, a variable representing the land use intensity and an index of vegetation density based on satellite-imaginary. Elevation, inclination and aspect (values of 0°, 180° and 90° indicate facing direction north, south and east/west, respectively) were used as topographic parameters. We assessed land use as primary (oligohemerobic, mostly natural grassland on shallow soils and rocky places) and secondary (mesohemerobic, i.e., anthropogenically disturbed pastures and meadows) habitats (Blume and Sukopp, 1976; Jalas, 1955) based on own field observations. We retrieved NDVI data (normalised difference vegetation index) from NASA through a Moderate Resolution Imaging Spectroradiometer (MODIS; modis.gsfc.nasa.gov) that incorporates a MOD13Q1 sensor (Didan, 2015), and we used it as index of vegetation density. Nine-teen bioclimatic variables were retrieved unprocessed from the CHELSA Climate database (Karger et al., 2017a, 2017b; available at http://chelsa-climate.org/) with a spatial resolution of 30″. We performed Spearman correlation tests to identify highly correlated ($|r|>0.75$) continuous variables (Table A2) and we reduced the set of variables to temperature seasonality (bio04), annual precipitation (bio12), precipitation seasonality (bio15), elevation, inclination, aspect, land use and NDVI. In addition, we collected soil samples from 121 populations to analyse their pH, carbonate content, organic carbon and nitrogen content, and exchangeable cation content (see Appendix 2 and Table A3 for details). All obtained ecological data are available on Dryad (http://dx.doi.org/10.5061/dryad.bj0436h).

Statistical analyses

Given the strong correspondence between ploidy level and reproductive mode, with more than 90% of seeds by cytotype being derived by either reproductive mode (Dobeš et al., 2018, 2013; Nardi et al., 2018), we considered henceforth tetraploid individuals to be sexual and penta-, hepta- and octoploids to be apomicts. According to Nardi et al. (2018), hexaploids comprise sexual autohexaploids (ca. 40% of genotypes) and apomicts (ca. 60%) and thus, cannot be strictly associated to either sexual or apomictic reproductive

mode. Therefore, we excluded hexaploid individuals from the analyses. To test for ecological differentiation, we performed pairwise comparisons of cytotypes separately for each ecological variable. Thereby, the probability of occurrence of a cytotype in a specific population (i.e., 0 or 1 in single-cytotype populations and 0.5 in mixed populations) was related to ecological variables by means of logistic regressions. We used occurrences instead of frequencies (i.e., number of individuals) because of uneven sample sizes and to reduce the effects of possible reproductive interference among cytotypes (Dobeš et al., 2018), which may influence the relative frequencies of sexual and apomictic cytotypes in mixed populations. The Bonferroni correction was applied to the obtained p-values. In addition, we compared sexual tetraploids to the pooled dataset of apomicts (i.e., penta-, hepta- and octoploids) to test for ecological differences between reproductive modes. For all the analyses we used the statistical computing environment R (R Development Core Team, 2018) and we represented the results by means of boxplots with the ggplot2 package (Wickham, 2016).

RESULTS

In total, we found 1,934 (52.0%) tetra-, 1,080 (29.1%) penta-, 196 (5.3%) hexa-, 409 (11.0%) hepta- and 97 (2.6%) octoploid individuals in the 238 sampled populations (Fig. 1). All cytotypes were present across the whole study area, but sexual tetraploids tended to occur in populations geographically separated from the apomictic ones (102, 74.45%), which were – in turn – usually formed by more than one cytotype (102, 76.12%; Fig. 1). Individual flow cytometric measurements of the 115 population not analysed by Nardi et al. (2018) are available on Dryad (http://dx.doi.org/10.5061/dryad.bj0436h). Several ecological variables differed significantly between sexual and apomictic cytotypes (Table 1). First, sexual tetraploids were more (although not exclusively) associated to primary oligohemerobic habitats than the pooled dataset of apomicts (Table 1): 38 (37.25%) pure sexual populations inhabited this particular environment versus 5 (5.05%) pure apomictic and 8 (22.86%) mixed sexual-apomictic populations. This generally applied for all comparisons between tetraploids and the apomictic cytotypes taken singularly, although tetraploids and octoploids significantly differed only in annual precipitation (bio12) after we applied the Bonferroni correction (Table 1).

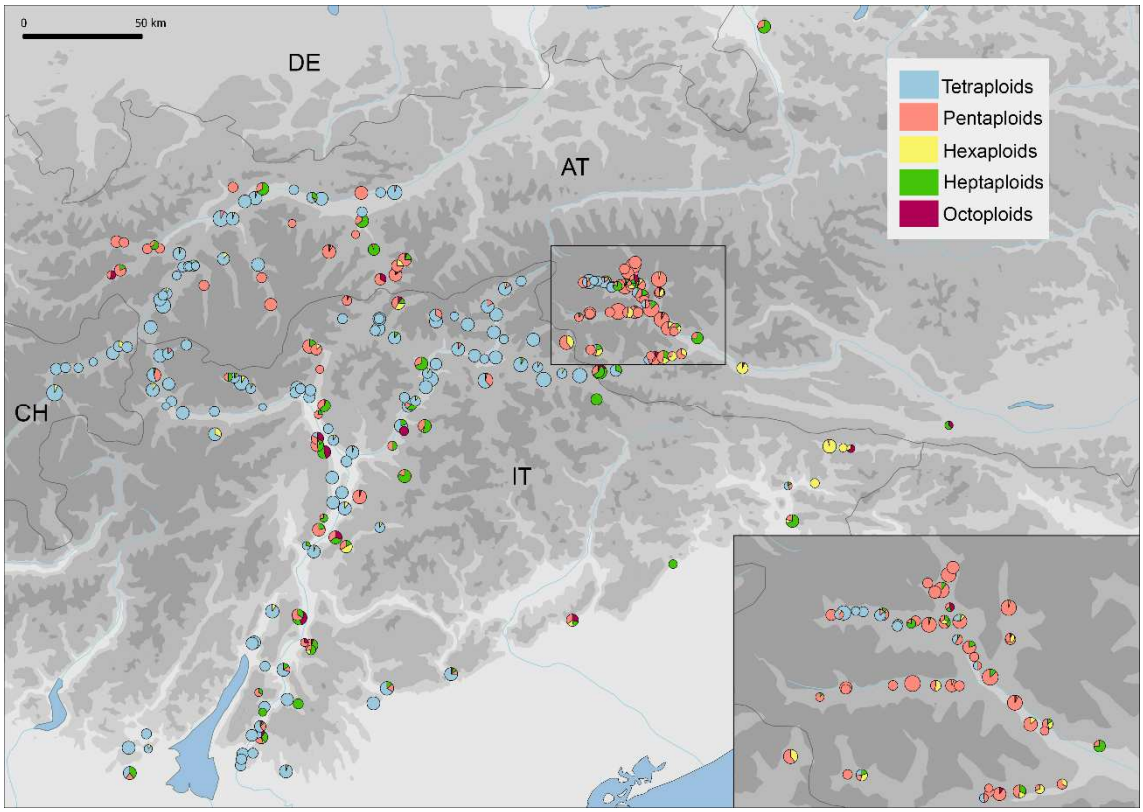


Fig. 1. Cytotype composition at 238 populations (see Table A1) of *Potentilla puberula* in the Eastern European Alps. The pie charts represent the relative frequency of tetra-, penta-, hexa-, hepta- and octoploids and their size is proportional to the number of sampled individuals. AT, CH, DE, and IT: Austria, Switzerland, Germany and Italy, respectively.

In addition, heptaploids and pentaploids inhabited populations with stronger temperature seasonality (bio04) and higher elevations than sexuals, respectively, but the significance of these differences did not persist after the Bonferroni correction. No significant differences among cytotypes arose from any of the studied soil variables (Table A4). Among the apomictic cytotypes, the regression analyses revealed no significant ecological differentiation. The exceptions of pentaploids occurring at populations at lower temperature seasonality (bio04) and at higher elevation compared to heptaploids did not show significant values after the adjustment of probability with the Bonferroni correction (Table 1).

Table 1. Pairwise comparison of ecological variables indicating site conditions among sexual and apomictic cytotypes of *Potentilla puberula*.

	Sexuals vs. Apomicts				Apomicts vs. Apomicts		
Variable	Tetraploids vs. Apomicts	Tetra- vs. Pentaploids	Tetra- vs. Heptaploids	Tetra- vs. Octoploids	Penta- vs. Heptaploids	Penta- vs. Octoploids	Hepta- vs. Octoploids
Elevation	−0.24 ± 0.12	−0.29 ± 0.13*	0.01 ± 0.14	0.05 ± 0.20	0.32 ± 0.15*	0.35 ± 0.19	0.05 ± 0.21
Inclination	**0.50 ± 0.13*** **	**0.49 ± 0.14*** **	**0.56 ± 0.16*** **	0.59 ± 0.22**	0.08 ± 0.15	0.12 ± 0.20	0.04 ± 0.22
Aspect	**0.25 ± 0.12***	0.24 ± 0.13	**0.39 ± 0.14****	0.34 ± 0.17*	0.17 ± 0.14	0.16 ± 0.19	0.00 ± 0.21
Land use	**−1.55 ± 0.34*** **	**−1.59 ± 0.36*** **	**−1.40 ± 0.42*** **	−1.19 ± 0.57*	0.20 ± 0.49	0.40 ± 0.62	0.21 ± 0.66
NDVI	0.02 ± 0.12	−0.04 ± 0.13	−0.04 ± 0.15	−0.07 ± 0.21	0.00 ± 0.16	−0.02 ± 0.22	−0.01 ± 0.23
Bio04	−0.06 ± 0.12	0.02 ± 0.12	−0.30 ± 0.15*	−0.33 ± 0.20	−0.37 ± 0.16*	−0.39 ± 0.21	−0.05 ± 0.22
Bio12	**−0.62 ± 0.15*** **	**−0.59 ± 0.16*** **	**−0.64 ± 0.17*** **	**−0.74 ± 0.20*** **	−0.11 ± 0.14	−0.23 ± 0.20	−0.10 ± 0.22
Bio15	0.18 ± 0.12	0.15 ± 0.13	0.25 ± 0.15	0.37 ± 0.19	0.10 ± 0.14	0.24 ± 0.21	0.13 ± 0.22

Note. Coefficients of regression ± standard errors are given. Positive and negative coefficients indicate higher and lower values for the cytotypes given first in the column headings, respectively. Significance levels are indicated as: *p < 0.05; **p < 0.01; ***p < 0.001; significant comparisons after Bonferroni correction (adjusted p < 0.05) are formatted in bold.bio04: temperature seasonality; bio12: annual precipitation; bio15: precipitation seasonality; NDVI: Normalized difference vegetation index.

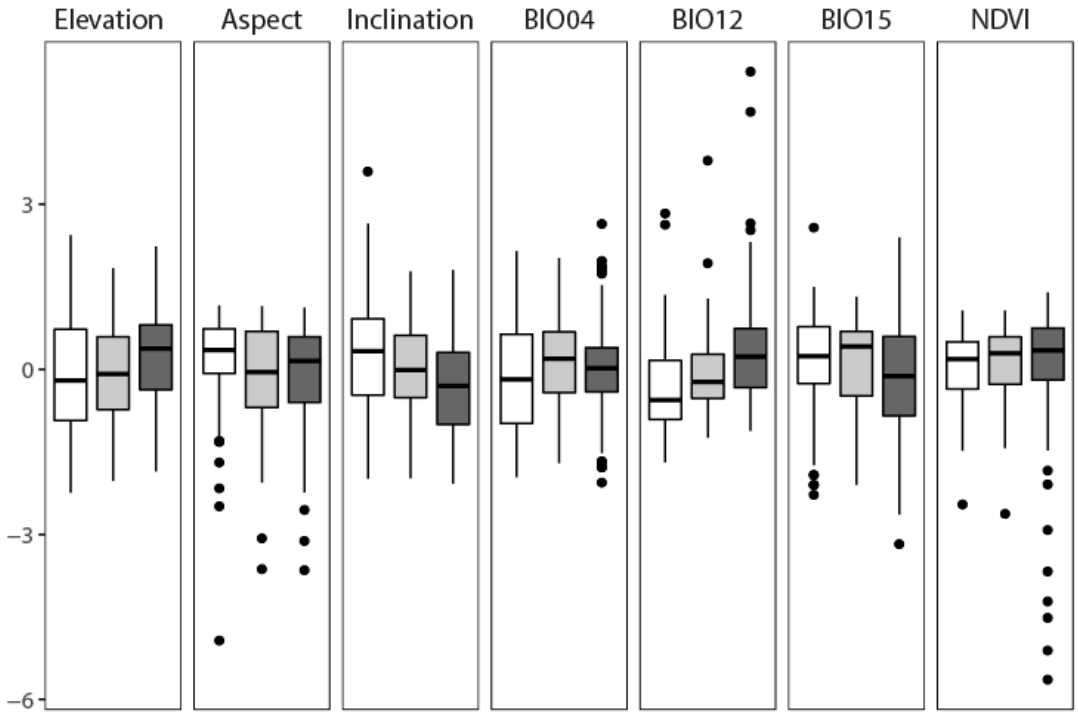


Fig. 2. Boxplots of continuous ecological variables compared among 102 purely sexual (white), 99 purely apomictic (dark grey) and 35 mixed populations (light grey) of *Potentilla puberula*.

Standardized values are reported. The boxes include the interquartile range (delimited by the 25th and the 75th percentiles), with the median reported as a horizontal line. The whiskers include values beyond the quartiles and up to 1.5 times the interquartile range above and below the upper and lower quartiles, respectively. The dots represent outlier values. Abbreviations: temperature seasonality (BIO04), annual precipitation (BIO12), precipitation seasonality (BIO15), normalized difference vegetation index (NDVI).

DISCUSSION

We analysed the ecological preferences of one sexual and three apomictic sympatric cytotypes of *P. puberula* in the Eastern European Alps. Tetraploids inhabited steeper and more south-oriented slopes than the apomictic cytotypes, which, in turn, were associated to man-made habitats with higher annual precipitation. The differences can be explained mainly by reproductive mode, since ecological occurrences were largely independent of the ploidy of the individuals, at least among the apomictic cytotypes.

Effects of reproductive mode versus ploidy and hybridity

Our results indicate that ecological differentiation among cytotypes of *P. puberula* is driven by differences in the reproductive mode rather than by effects of ploidy. Two lines of evidence support this argumentation: i) the apomictic cytotypes hardly differed in their ecological niches; and ii) the niches of the apomictic cytotypes showed a strong differentiation from those of sexual tetraploids and these differences were similar for each of the three apomictic cytotypes.

Generally, niche differentiation among cytotypes can be expected due to the inherent differences in the number of genome copies which might cause differential gene expression through gene dosage effects or epigenetic changes (e.g., Baduel et al., 2018; Comai, 2005; Parisod et al., 2010; te Beest et al., 2012). However, studies on synthetic plant autopolyploids of several taxa indicate differences in expression patterns only for a minority of genes, suggesting that gene expression may change consistently only in allopolyploids (see Parisod et al., 2010 and references herein). In the autopolyploid *P. puberula,* indeed, our results suggest no nucleotypic effects. Since gene dosage effects depend by definition on the number of gene copies, we do not expect that differences in gene expression arise only between tetraploids and penta- to octoploids, but not among the apomictic cytotypes.

Since there is a strong link between ploidy level and reproductive mode, (the low number of exceptional individuals prevent a stringent statistical test) and only one sexual cytotype exists, *a priori* our system does not allow a perfectly conclusive identification of the main driver of the observed ecological differentiation. However, it should be noted that (to our best knowledge), no perfect taxon is available to unambiguously separate the effects of reproductive mode, ploidy level and in case of allopolyploidy, hybridity. Studies dealing with these issues often remained inconclusive, due to limitations inherent to the studied taxa, such as lack of cytotypic variation within reproductive mode or different evolutionary history of sexuals and apomicts (see Table A5 for a review of ecological studies in amphi-apomictic systems). For instance, Schinkel et al., (2016) found apomictic individuals in three pure diploid populations of *Ranunculus kuepferi*, which shared the ecological site conditions of co-occurring sexuals. However, their number was not high enough to prove that polyploidy was a requirement for range expansion. Kao, (2008) found no habitat differentiation between apomictic triploid and tetraploid *Arnica cordifolia*, similar to what we found in *P. puberula* within the apomictic cytotypes, but in her study, the comparison with sexual diploids was missing.

Perhaps the most complete attempt to address simultaneously the role of ploidy and reproductive mode on ecological differentiation of cytotypes is the genus-wide study of Mau et al. (2015), who compared multiple species of *Boechera*, a taxon comprising both sexual and apomictic diploids and triploids. Oppositely to our findings in *P. puberula*, the authors concluded that ploidy, rather than mating system, determines a shift in ecological niches in this genus. Nevertheless, the results were not fully consistent, as niche differentiation among reproductive modes was observed at the diploid but not at the triploid level, and between ploidy levels in sexuals but not in apomicts (Mau et al., 2015). In contrast to *P. puberula*, hybridisation plays an important role in the formation of apomicts in *Boechera* (e.g., Dobeš et al., 2007) and a differential origin of apomictic cytotypes has been proposed: apomictic diploids may originate intraspecifically while triploids derive by interspecific hybridisation (Lovell et al., 2013).

Hybridisation, often involved in the origin of apomixis (Grimanelli et al., 2001; Soltis and Soltis, 2009), may play an additional confounding role when studying the effects of reproductive mode on ecology. Genomic rearrangements, differential gene expression and fixed heterozygosity are possible consequences of hybridisation (Soltis and Soltis, 2000; te Beest et al., 2012; Wendel, 2000) leading to a shift of ecological niche

of the progeny relative to those of the parents. Apomictic allopolyploids such as *Antennaria rosea* (Bayer et al., 1991) and *Crataegus suksdorfii* and *Crataegus douglasii* (Coughlan et al., 2017) occupy ecological niches intermediate to those of the parental species, a hint that ecological niche shifts of those taxa indeed may be owing to their hybrid origin, rather than to apomixis itself. Contrariwise, in *Potentilla puberula* the apomictic cytotypes are autopolyploid and genetically very similar to the sexual tetraploids (Nardi et al., 2018), ruling out strong effects of hybridisation.

Evolutionary consequences of the ecological differentiation between sexuals and apomicts

Tetraploids occurred at drier, steeper, more south-oriented and oligohemerobic sites than apomicts. These primary habitats typically represented open forest (submediterranean deciduous forests and red pine forests in the southern and central parts of the Alps, respectively), natural xeric grasslands and exposed rock vegetation. In contrast, apomicts preferred mesohemerobic habitats with a stronger anthropogenic impact such as mesic meadows and pastures of cattle, sheep and goats on gentle slopes. The rarity of apomictic populations in primary habitats indicates that apomicts may benefit from human disturbance, establishing new populations and maintaining existing ones. Examples describing a stronger association of apomicts to anthropogenic habitat include *Taraxacum* sect. *Ruderalia* (Meirmans et al., 1999), *Ranunculus carpaticola* (Paun et al., 2006), and Asiatic *Eupatorium* species (Watanabe, 1986). According to Eckert et al. (2010), anthropogenic disturbance has a disrupting effect on mating systems in plant populations, which can generate a shift towards increased rates of self-fertilisation in self-compatible taxa (e.g., Luijten et al., 1999). Following this argumentation, self-incompatible taxa such as tetraploid *P. puberula*, might be easily outcompeted by self-compatible (and apomictic) relatives. Moreover, low genetic diversity may be of advantage in moderately disturbed and unstructured habitats like meadows and pastures, where few clonal lineages may effectively exploit a homogeneous environment (Bell, 1982; Hörandl, 2006; Vrijenhoek, 1984). According to the Frozen Niche Variation hypothesis, clonal lineages can establish only when they occupy the margins of the sexuals' niche (Vrijenhoek, 1984). Apomicts may thus establish preferentially in habitats where competition with sexuals is lower than in habitats at the sexuals' niche optimum. Oligohemerobic and drier habitats, favoured by sexual tetraploids, typically occur at the southern edge at the margin of the Alps towards the Po plain of our study area, which

potentially has served as a glacial refugium for the montane to subalpine *P. puberula* (Tribsch and Schönswetter, 2003). The rather long-term persistence of tetraploids at these southern populations, thus, would have promoted adaptation to local ecological conditions.

In fact, recombination is considered an advantage in habitats where either biotic (Red Queen hypothesis, Van Valen, 1973) or resource (Tangled Bank hypothesis, Bell, 1982; Song et al., 2011) competition is high enough to promote high evolvability. Thus, the mating system may be associated with ecological traits related to the Competitive-Stress-tolerant-Ruderal classification (Grime, 1977, 1974), in which asexuals mostly occur in marginal ruderal areas and tend to be less competitive than the outcrossers. Yang et al. (2018) found evidence for this, testing for ecological strategies in four *Capsella* species, finding that selfers were more sensitive to competition than outcrossers.

A trend of apomictic plants to occur under more extreme environmental conditions compared to their sexual relatives has been repeatedly reported. Drought was identified as a factor driving the pattern in *Draba* (Price, 1980), *Antennaria* (Bierzychudek, 1985), *Bidens* (Crowe and Parker, 1981), *Paspalum intermedium* (Karunarathne et al., 2018) and the *Ranunculus auricomus* complex (Paule et al., 2018). The opposite pattern, however, was found in this study: sexual *P. puberula* prevailed at drier sites, a tendency also observed in *Ranunculus kuepferi* (Schinkel et al., 2016). This might be explained by the negative effects of rainy weather on pollinators' behaviour (Puškadija et al., 2007), which avoid flying under the rain or foraging on wet blossoms. Frequent rain affects also flower display and can lead to closed inflorescences, as the case in dandelions (Mártonfiová, 2015). Therefore, sexual individuals, which depend on insect-mediated outcross pollination, are likely to be favoured in dry habitats in regions not affected by strong droughts, where water shortage become the dominant factor. Otherwise, in areas with higher precipitation, apomictic individuals can self-fertilise independently of pollinators and (poor) weather conditions. Moreover, in moister habitats, fungal and microbial activity (Talley et al., 2002) as well as airborne spore abundance and release (see Crandall and Gilbert, 2017) are increased, which might indicate higher resistance of apomicts against pathogens. This would also explain why sexual individuals of *P. puberula* prefer steeper south-exposed sites, which imply shallower and drier soils.

Ecological parthenogenesis in sympatry

The realised geographical range does not necessarily coincide with the potential range, which can be occupied by a taxon based only on its environmental requirements. Such range boundary disequilibria (Gaston, 2003; Sexton et al., 2009) might be due to dispersal limitation, colonisation history and even stochasticity (Sexton et al., 2009). In amphi-apomictic systems, an advantage of apomicts in colonisation given by uniparental reproduction (Baker's law; Baker, 1955; Stebbins, 1957) can be expected (Hao et al., 2011; Rambuda and Johnson, 2004). The break-down of self-incompatibility, common in both apomictic (Hörandl, 2010) and sexual polyploids (Barrett, 2002; Robertson et al., 2011; but see Mable, 2004), provides the possibility of founding new populations and thus expanding the geographical range by a single individual even when pollination is still required (i.e., in pseudogamous apomicts, such as *P. puberula*, or in sexual selfers). Geographical range expansion of outcrossing sexuals, on the contrary, may be hindered by demographic dynamics (e.g., Allee effects, Allee et al., 1949) or pollinator dependence. Apomicts may thus have a priority effect (Levins and Culver, 1971; Shulman et al., 1983) in regions potentially suitable for sexuals and block their establishment by competitive exclusion and asymmetrical reproductive interference (Dobeš et al., 2018; Kyogoku, 2015; Mogie, 1992).

Given the central role that apomixis plays in colonisation and range expansion, most studies on amphi-apomictic systems focus on geographical parthenogenesis, rather than in purely ecological patterns (see Bierzychudek, 1985). Furthermore, in studies investigating the existence of ecological parthenogenesis, the geographical aspect is often neglected and conclusions on ecological differentiation between sexuals and apomicts are drawn without distinguishing between sympatric and allopatric populations (e.g., *Antennaria rosea*, Bayer et al., 1991; *Ranunculus carpaticola*, Paun et al., 2006; *Pilosella officinarum*, Mráz et al., 2008; or *Paspalum intermedium*, Karunarathne et al., 2018; see Table A4). Ecological differentiation of allopatric apomicts may be in fact the result of post-colonisation adaptation, or simply an artefact of range boundary disequilibria along eco-geographical gradients (Sexton et al., 2009).

While studying ecological differentiation in *Ranunculus kuepferi*, a species with clear geographical parthenogenesis, Kirchheimer et al. (2016) found that ecological niche shift and niche breadth was consistently larger in the allopatric apomictic populations, while the sympatric apomicts occupied a narrower niche at the margin of the sexual one.

Therefore, the authors argued that the most relevant component of niche differentiation was derived by local adaptation which followed colonisation of new areas (Kirchheimer et al., 2016).

In consequence, we affirm that sympatry of sexuals with apomicts is the most feasible scenario to separate the effects of immediate ecological differentiation from those arising as a consequence of range expansion. Nevertheless, only few studies used such sympatric systems or corrected for geographical distance (e.g., *Arnica*, Kao and Parker, 2010; *Boechera*, Mau et al., 2015; *Taraxacum* sect. *Ruderalia*, Meirmans et al., 1999; Verduijn et al., 2004; see Table A4). We studied ecological parthenogenesis of *P. puberula* in a transect through the Eastern Alps, where both sexuals and apomicts occur sympatrically in a mosaic-like distribution (Fig. 1). Given the strong association of apomicts with human-disturbed habitats, one may argue that human dispersal may re-introduce an effect of micro-colonisation patterns, even in a sympatric area. However, most sexual populations of *P. puberula* are found in mesohemerobic habitats as well. Therefore, we do not expect a preferential dispersal of apomicts due to human activity, and the effects of colonisation patterns should be minimised in this sympatric region.

Associations between both reproductive mode and ploidy and ecological differentiation would need further experimental comparisons at multiple spatial scales with reciprocal transplant and common garden experiments, in which environmental parameters can be controlled for a clearer distinction between sexual and apomictic responses. Few studies tested for genetically fixed ecological differentiation in these terms, either between ploidy levels (e.g., Bretagnolle and Thompson, 2001; Münzbergová, 2007; Petit and Thompson, 1997) or reproductive modes (e.g., de Kovel and de Jong, 1999). When testing for differences between ploidy levels, the aforementioned authors did not find variation of phenotypic plasticity among cytotypes. The results were comparable to our lack of differences among the apomictic cytotypes of *P. puberula*. In *Taraxacum officinale*, apomictic triploids did not differ from sexual diploids in leaf height and length under lighted conditions, but they showed higher values under a shade treatment, suggesting that their phenotypic plasticity is higher than those of sexuals (de Kovel and de Jong, 1999). However, albeit the authors found significant differences in leaf plasticity between new and established triploids (de Kovel and de Jong, 2000), it is still unclear whether selection may have favoured polyploidy or apomictic-related traits.

In conclusion, we stress the importance of identifying and possibly neutralising intermingled confounding factors (such as ploidy, hybrid origin and spatial patterns) that could overestimate or mask the actual effects of reproductive mode in determining ecological parthenogenesis.

Acknowledgements

We thank Raffaela Wettl (Austrian Research Centre for Forests, Austria) for performing the soil analyses and Karl-Manfred Schweinzer, Georg Kindermann (Austrian Research Centre for Forests, Austria) and Dietmar Moser (University of Vienna, Austria) for helping to retrieve bioclimatic and topographic data, and Christof Endl, who took care of the plants in the common garden at the University of Vienna, Austria, during summer 2015. We are also grateful to Simon Stifter and Julian Ananda Haider (University of Vienna, Austria), Thomas Wilhalm (Museum of Nature South Tyrol, Italy), Juraj Paule (Senckenberg Museum Frankfurt, Germany) and Roswitha Schmickl (Heidelberg University, Germany) for their sampling effort collecting data in the field. Finally, we thank two anonymous reviewers for their constructive comments on the manuscript. The Austrian Science Fund (FWF) supported this research, grant number: P27688 to Christoph Dobeš.

Conflict of interest

The authors have no conflict of interest.

Author contributions

CD, KH, FDN and HAM designed the study. HAM, FDN, CD, SS, and AT carried out the field data collection. CD, HAM and FDN performed the flow cytometric measurements. KH, FDN and HAM performed the statistical analyses. HAM, FDN, KH and CD wrote the manuscript. All authors critically commented the results and the final version of the manuscript.

Data accessibility

All flow cytometric and ecological data acquired during this research and supporting this manuscript were deposited at the public repository Dryad. (http://dx.doi.org/10.5061/dryad.bj0436h).

REFERENCES

Adams, K.L., Wendel, J.F. 2005. Polyploidy and genome evolution in plants. *Curr. Opin. Pl. Biol.* 8, 135–141. https://doi.org/10.1016/J.PBI.2005.01.001

Allee, W.C., Emerson, A.E., Park, O., Park, T., Schmidt, K.P. 1949. Principles of animal ecology. W. B. Saunders Company, Philadelphia and London.

Asker, S.E. 1970. Apomixis and sexuality in the *Potentilla argentea* complex I. Crosses with other species. *Hereditas* 66, 127–143. https://doi.org/10.1111/J.1601-5223.1970.TB02339.X

Asker, S.E., Jerling, L. 1992. Apomixis in plants. CRC Press, Boca Raton.

Baduel, P., Bray, S., Vallejo-Marín, M., Kolář, F., Yant, L. 2018. The "polyploid hop": shifting challenges and opportunities over the evolutionary lifespan of genome duplications. *Front. Ecol. Evol.* 6, 117. https://doi.org/10.3389/fevo.2018.00117

Baker, H.G. 1955. Self-compatibility and establishment after "long-distance" dispersal. *Evolution (N. Y).* 9, 347–349. https://doi.org/10.1111/j.1558-5646.1955.tb01544.x

Barrett, S.C.H. 2002. Evolution of sex: The evolution of plant sexual diversity. *Nat. Rev. Genet.* 3, 274–284. https://doi.org/10.1038/nrg776

Bayer, R.J., 1997. *Antennaria rosea* (Asteraceae) - a model group for the study of the evolution of polyploid agamic complexes. *Opera Bot.* 132, 53–65.

Bayer, R.J., Purdy, B.G., Lebedyk, D.G. 1991. Niche differentiation among eight sexual species of *Antennaria* Gaertner (Asteraceae: Inuleae) and *A. rosea*, their allopolyploid derivative. *Evol. Trends Plants* 5, 109–123.

Bell, G. 1982. *The masterpiece of nature: the evolution and genetics of sexuality*. Croom Helm, London.

Bierzychudek, P. 1985. Patterns in plant parthenogenesis. *Experientia* 41, 1255–1264. https://doi.org/10.1007/BF01952068

Blanc, G., Wolfe, K.H. 2004. Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. *Plant Cell* 16, 1679–1691.

Blume, H.-P., Sukopp, H. 1976. Ökologische Bedeutung anthropogener

Bodenveränderungen. Schriftenr. für Veg. 10, 75–89.

Böcher, T.W. 1951. Cytological and embryological studies in the amphi-apomictic *Arabis holboellii* complex. *Biol. Skr*. 6, 1–59.

Bretagnolle, F., Thompson, J.D. 2001. Phenotypic plasticity in sympatric diploid and autotetraploid *Dactylis glomerata*. *Int. J. Plant Sci.* 162, 309–316. https://doi.org/10.1086/319572

Brown, J.H., Stevens, G.C., Kaufman, D.M. 1996. The geographic range: size, shape, boundaries, and internal structure. *Annual Rev. Ecol. Syst.* 27, 597–623. https://doi.org/10.1146/annurev.ecolsys.27.1.597

Campbell, C.S., Wright, W.A. 1996. Apomixis, hybridization, and taxonomic complexity in eastern north American *Amelanchier* (Rosaceae). *Folia Geobot. Phytotaxon.* 31, 345–354. https://doi.org/10.1007/BF02815379

Caperta, A.D., Castro, S., Loureiro, J., Róis, A.S., Conceição, S., Costa, J., Rhazi, L., Espírito Santo, D., Arsénio, P. 2017. Biogeographical, ecological and ploidy variation in related asexual and sexual *Limonium* taxa (*Plumbaginaceae*). *Bot. J. Linn. Soc.* 183, 75–93. https://doi.org/10.1111/boj.12498

Carman, J.G. 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol. J. Linn. Soc*. 61, 51–94. https://doi.org/10.1111/j.1095-8312.1997.tb01778.x

Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6, 836–846. https://doi.org/10.1038/nrg1711

Cosendai, A.-C., Wagner, J., Ladinig, U., Rosche, C., Hörandl, E. 2013. Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Heredity (Edinb).* 110, 560–569. https://doi.org/10.1038/hdy.2013.1

Coughlan, J.M., Han, S., Stefanović, S., Dickinson, T.A. 2017. Widespread generalist clones are associated with range and niche expansion in allopolyploids of Pacific Northwest Hawthorns (*Crataegus* L.). *Mol. Ecol.* 26, 5484–5499. https://doi.org/10.1111/mec.14331

Coughlan, J.M., Stefanović, S., Dickinson, T.A. 2014. Relative resource allocation to

dispersal and competition demonstrates the putative role of hybridity in geographical parthenogenesis. *J. Biogeogr.* 41, 1603–1613. https://doi.org/10.1111/jbi.12316

Crandall, S.G., Gilbert, G.S. 2017. Meteorological factors associated with abundance of airborne fungal spores over natural vegetation. *Atmos. Environ.* 162, 87–99. https://doi.org/10.1016/j.atmosenv.2017.05.018

Crowe, D.R., Parker, W.H. 1981. Hybridization and agamospermy of *Bidens* in Northwestern Ontario. *Taxon* 30, 749–760. https://doi.org/10.2307/1220076

de Kovel, C., de Jong, G. 1999. Responses of sexual and apomictic genotypes of *Taraxacum officinale* to variation in light. *Plant Biol.* 1, 541–546. https://doi.org/10.1055/s-2007-978550

de Kovel, C.G.F., de Jong, G. 2000. Selection on apomictic lineages of *Taraxacum* at establishment in a mixed sexual-apomictic population. *J. Evol. Biol.* 13, 561–568. https://doi.org/10.1046/j.1420-9101.2000.00211.x

Dickinson, T.A., Lo, E.Y.Y., Talent, N. 2007. Polyploidy, reproductive biology, and Rosaceae: Understanding evolution and making classifications. *Plant Syst. Evol.* 266, 59–78. https://doi.org/10.1007/s00606-007-0541-2

Didan, K. 2015. MOD13Q1 MODIS/Terra Vegetation Indices 16-Day L3 Global 250m SIN Grid V006. Distrib. by NASA EOSDIS LP DAAC. https://doi.org/10.5067/MODIS/MOD13Q1.006

Dobeš, C. 1999. Die Karyogeographie des *Potentilla verna* agg. (Rosaceae) in Österreich -mit ergänzenden Angaben aus Slowenien, Kroatien, der Slowakei und Tschechien. *Ann. Naturhist. Mus. Wien, B.* 101, 599–629.

Dobeš, C., Koch, M.A., Sharbel, T.F. 2006. Embryology, karyology, and modes of reproduction in the north american genus *Boechera* (Brassicaceae): a compilation of seven decades of research. *Ann. Missouri Bot. Gard.* 93, 517–534. https://doi.org/10.3417/0026-6493(2007)93[517:EKAMOR]2.0.CO;2

Dobeš, C., Milosevic, A., Prohaska, D., Scheffknecht, S., Sharbel, T.F., Hülber, K. 2013. Reproductive differentiation into sexual and apomictic polyploid cytotypes in *Potentilla puberula* (Potentilleae, Rosaceae). *Ann. Bot.* 112, 1159–1168.

https://doi.org/10.1093/aob/mct167

Dobeš, C., Scheffknecht, S., Fenko, Y., Prohaska, D., Sykora, C., Hülber, K. 2018. Asymmetric reproductive interference: The consequences of pollination on reproductive success in sexual – apomictic populations of *Potentilla puberula* (Rosaceae). *Ecol. Evol.* 8, 365–381. https://doi.org/10.1002/ece3.3684

Dobeš, C., Sharbel, T.F., Koch, M. 2007. Towards understanding the dynamics of hybridization and apomixis in the evolution of the genus *Boechera* (Brassicaceae). *Syst. Biodivers.* 5, 321–331. https://doi.org/10.1017/S1477200007002423

Doležel, J., Bartoš, J. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Ann. Bot.* 95, 99–110. https://doi.org/10.1093/aob/mci005

Doležel, J., Greilhuber, J., Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nat. Protoc*. 2, 2233–2244. https://doi.org/10.1038/nprot.2007.310

Eckert, C.G., Kalisz, S., Geber, M.A., Sargent, R., Elle, E., Cheptou, P.-O., Goodwillie, C., Johnston, M.O., Kelly, J.K., Moeller, D.A., Porcher, E., Ree, R.H., Vallejo-Marín, M., Winn, A.A. 2010. Plant mating systems in a changing world. *Trends Ecol. Evol*. 25, 35–43. https://doi.org/10.1016/J.TREE.2009.06.013

Fawcett, J.A., Maere, S., Van de Peer, Y. 2009. Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event. *Proc. Natl. Acad. Sci. U. S. A.* 106, 5737–5742. https://doi.org/10.1073/pnas.0900906106

Gaston, K.J. 2003. *The stucture and dynamics of geographic ranges*. Oxford University Press, Oxford, New York.

Grimanelli, D., Leblanc, O., Perotti, E., Grossniklaus, U. 2001. Developmental genetics of gametophytic apomixis. *Trends Genet.* 17, 597–604. https://doi.org/10.1016/S0168-9525(01)02454-4

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111, 1169–1194. https://doi.org/10.1086/283244

Grime, J.P. 1974. Vegetation classification by reference to strategies. *Nature* 250, 26–31. https://doi.org/10.1038/250026a0

Hao, J.H., Qiang, S., Chrobock, T., van Kleunen, M., Liu, Q.Q. 2011. A test of Baker's law: breeding systems of invasive species of Asteraceae in China. *Biol. Invas.* 13, 571–580. https://doi.org/10.1007/s10530-010-9850-4

Hörandl, E. 2010. The evolution of self-fertility in apomictic plants. *Sex. Plant Reprod.* 23, 73–86. https://doi.org/10.1007/s00497-009-0122-3

Hörandl, E. 2006. The complex causality of geographical parthenogenesis. *New Phytol.* 171, 525–538. https://doi.org/10.1111/j.1469-8137.2006.01769.x

Hörandl, E., Cosendai, A.-C., Temsch, E.M. 2008. Understanding the geographic distributions of apomictic plants: A case for a pluralistic approach. *Plant Ecol. Divers.* 1, 309–320. https://doi.org/10.1080/17550870802351175

Hörandl, E., Gutermann, W. 1999. Der *Ranunculus auricomus*-Komplex in Österreich. 2. Die *R. cassubicus*-, *R. monophyllus*- und *R. fallax*-Sammelgruppe. *Bot. Jahrb. Syst.* 120, 545–598.

Jalas, J. 1955. Hemerobe und hemerochore Pflanzenarten. Ein terminologischer Reformversuch. *Acta Soc. Fauna Fl. Fenn.* 72, 1–15.

Kao, R.H. 2008**.** Origins and widespread distribution of co-existing polyploids in *Arnica cordifolia* (Asteraceae). *Ann. Bot.* 101, 145–52. https://doi.org/10.1093/aob/mcm271

Kao, R.H., Parker, I.M. 2010. Coexisting cytotypes of *Arnica cordifolia*: morphological differentiation and local-scale distribution. *Int. J. Plant Sci.* 171, 81–89. https://doi.org/10.1086/647924

Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P., Kessler, M. 2017a. Climatologies at high resolution for the earth's land surface areas. *Sci. Data* 4, 170122. https://doi.org/10.1038/sdata.2017.122

Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P., Kessler, M. 2017b. Data from: Climatologies at high resolution for the earth's land surface areas. Dryad Digit. Repos. https://doi.org/10.5061/dryad.kd1d4

Karunarathne, P., Schedler, M., Martínez, E.J., Honfi, A.I., Novichkova, A.,

Hojsgaard, D. 2018. Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. *Ann. Bot.* 121, 1183–1196. https://doi.org/10.1093/aob/mcy004

Kirchheimer, B., Schinkel, C.C.F., Dellinger, A.S., Klatt, S., Moser, D., Winkler, M., Lenoir, J., Caccianiga, M., Guisan, A., Nieto-Lugilde, D., Svenning, J.-C., Thuiller, W., Vittoz, P., Willner, W., Zimmermann, N.E., Hörandl, E., Dullinger, S. 2016. A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. *J. Biogeogr.* 43, 716–726. https://doi.org/10.1111/jbi.12663

Kurtto, A., Lampinen, R., Junikka, L. 2004. Atlas Florae Europaeae 13. Distribution of vascular plants in Europe. Rosaceae (*Spiraea* to *Fragaria*, excl. *Rubus*). The Commitee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki.

Kyogoku, D. 2015. Reproductive interference: ecological and evolutionary consequences of interspecific promiscuity. *Popul. Ecol.* 57, 253–260. https://doi.org/10.1007/s10144-015-0486-1

Levin, D.A. 1983. Polyploidy and novelty in flowering plants. *Am. Nat.* 122, 1–25. https://doi.org/10.1086/284115

Levins, R., Culver, D. 1971. Regional coexistence of species and competition between rare species. *Proc. Natl. Acad. Sci. U. S. A.* https://doi.org/10.2307/60316

Lo, E.Y.Y., Stefanović, S., Dickinson, T.A. 2013. Geographical parthenogenesis in Pacific Northwest hawthorns (*Crataegus*; Rosaceae). *Botany* 91, 107–116. https://doi.org/10.1139/cjb-2012-0073

Lovell, J.T., Aliyu, O.M., Mau, M., Schranz, M.E., Koch, M.A., Kiefer, C., Song, B.-H., Mitchell-Olds, T. & Sharbel, T.F. 2013. On the origin and evolution of apomixis in *Boechera*. *Plant Reprod.* 26, 309–315. https://doi.org/10.1007/s00497-013-0218-7

Luijten, S.H., Oostermeijer, J.G.B., Ellis-Adam, A.C., den Nijs, J.C.M. 1999. Variable herkogamy and autofertility in marginal populations of *Gentianella germanica* in the Netherlands. *Folia Geobot.* 34, 483–496. https://doi.org/10.1007/BF02914924

Lynch, M. 1984. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. *Q. Rev. Biol.* 59, 257–290. https://doi.org/10.1086/413902

Mable, B.K. 2004. Polyploidy and self-compatibility: is there an association? *New Phytol.* 162, 803–811. https://doi.org/10.1111/j.1469-8137.2004.01055.x

Mártonfiová, L. 2015. Hybridization in natural mixed populations of sexual diploid and apomictic triploid dandelions (*Taraxacum* sect. *Taraxacum*): Why are the diploid sexuals not forced out? *Folia Geobot.* 50, 339–348. https://doi.org/10.1007/s12224-015-9231-y

Mau, M., Lovell, J.T., Corral, J.M., Kiefer, C., Koch, M.A., Aliyu, O.M., Sharbel, T.F. 2015. Hybrid apomicts trapped in the ecological niches of their sexual ancestors. *PNAS* 112, E2357–E2365. https://doi.org/10.1073/pnas.1423447112

Meirmans, P.G., Calame, F.G., Bretagnolle, F., Felber, F., den Nijs, J.C.M. 1999. Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. *Ruderalia*. *Folia Geobot*. 34, 451–469. https://doi.org/10.1007/BF02914922

Mogie, M. 1992. *The evolution of asexual reproduction in plants.* Chapman and Hall, London.

Mráz, P., Šingliarová, B., Urfus, T., Krahulec, F. 2008. Cytogeography of *Pilosella officinarum* (Compositae): altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. *Ann. Bot.* 101, 59–71. https://doi.org/10.1093/aob/mcm282

Münzbergová, Z. 2007. No effect of ploidy level in plant response to competition in a common garden experiment. *Biol. J. Linn. Soc.* 92, 211–219. https://doi.org/10.1111/j.1095-8312.2007.00820.x

Nardi, F.D., Dobeš, C., Müller, D., Grasegger, T., Myllynen, T., Alonso-Marcos, H., Tribsch, A. 2018. Sexual intraspecific recombination but not de novo origin governs the genesis of new apomictic genotypes in *Potentilla puberula* (Rosaceae). *Taxon* 67, 1108–1131. https://doi.org/ 10.12705/676.8

Noyes, R.D., Gerling, H., Vandervoort, C. 2006. Sexual and apomictic prairie fleabane (*Erigeron strigosus*) in Texas: geographic analysis and a new combination

(*Erigeron strigosus* var. *traversii*, Asteraceae). SIDA, Contrib. to Bot. 22, 265–276. https://doi.org/10.2307/41968578

Parisod, C., Holderegger, R., Brochmann, C. 2010**.** Evolutionary consequences of autopolyploidy. *New Phytol*. 186, 5–17. https://doi.org/10.1111/j.1469-8137.2009.03142.x

Parker, E.D., Selander, R.K., Hudson, R.O., Lester, L.J., Lester, L.J. 1977. Genetic diversity in colonizing parthenogenetic cockroaches. *Evolution (N. Y)*. 31, 836–842. https://doi.org/10.2307/2407445

Paule, J., Dunkel, F.G., Schmidt, M., Gregor, T. 2018. Climatic differentiation in polyploid apomictic *Ranunculus auricomus* complex in Europe. *BMC Ecol.* 18, 16. https://doi.org/10.1186/s12898-018-0172-1

Paule, J., Scherbantin, A., Dobeš, C. 2012. Implications of hybridisation and cytotypic differentiation in speciation assessed by AFLP and plastid haplotypes - a case study of *Potentilla alpicola* La Soie. *BMC Evol. Biol.* 12, 132. https://doi.org/10.1186/1471-2148-12-132

Paun, O., Greilhuber, J., Temsch, E.M., Hörandl, E. 2006. Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). *Mol. Ecol.* 15, 897–910. https://doi.org/10.1111/j.1365-294X.2006.02800.x

Petit, C., Thompson, J.D. 1997. Variation in phenotypic response to light availability between diploid and tetraploid populations of the perennial grass *Arrhenatherum elatius* from open and woodland sites. *J. Ecol.* 85, 657–667. https://doi.org/10.2307/2960536

Price, R.A. 1980. *Draba streptobrachia* (Brassicaceae), a new species from Colorado. *Brittonia* 32, 160–169. https://doi.org/10.2307/2806784

Puškadija, Z., Štefanić, E., Mijić, A., Zdunić, Z., Paradžiković, N., Florijančić, T., Opačak, A. 2007. Influence of weather conditions on honey bee visits (*Apis mellifera carnica*) during sunflower (*Helianthus annuus* L.) blooming period. *Poljopr*. 13, 230–233.

QGIS Development Team. 2018. QGIS Geographic Information System. Open Source

Geospatial Foundation Project.

R Development Core Team. 2018**.** R: A language and environment for statistical computing.

Rambuda, T.D., Johnson, S.D. 2004**.** Breeding systems of invasive alien plants in South Africa: does Baker's rule apply? *Divers. Distrib.* 10, 409–416. https://doi.org/10.1111/j.1366-9516.2004.00100.x

Rivas, L.R. 1964. A reinterpretation of the concepts "Sympatric" and "Allopatric" with proposal of the additional terms "Syntopic" and "Allotopic." *Syst. Biol*. 13, 42–43. https://doi.org/10.2307/sysbio/13.1-4.42

Robertson, K., Goldberg, E.E., Igić, B. 2011. Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. *Evolution (N. Y).* 65, 139–155. https://doi.org/10.1111/j.1558-5646.2010.01099.x

Schinkel, C.C.F., Kirchheimer, B., Dellinger, A.S., Klatt, S., Winkler, M., Dullinger, S., Hörandl, E. 2016. Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. *AoB Plants* 8, plw064. https://doi.org/10.1093/aobpla/plw064

Sexton, J.P., McIntyre, P.J., Angert, A.L., Rice, K.J. 2009. Evolution and ecology of species range limits. *Annual Rev. Ecol. Evol. Syst.* 40, 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317

Shulman, M.J., Ogden, J.C., Ebersole, J.P., McFarland, W.N., Miller, S.L., Wolf, N.G. 1983. Priority effects in the recruitment of juvenile coral reef fishes. *Ecology* 64, 1508–1513. https://doi.org/10.2307/1937505

Siena, L.A., Sartor, M.E., Espinoza, F., Quarín, C.L., Ortiz, J.P.A. 2008. Genetic and embryological evidences of apomixis at the diploid level in *Paspalum rufum* support recurrent auto-polyploidization in the species. *Sex. Plant Reprod*. 21, 205–215. https://doi.org/10.1007/s00497-008-0080-1

Soltis, D.E., Soltis, P.S., Pires, J.C., Kovarik, A., Tate, J.A., Mavrodiev, E. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biol. J. Linn.* Soc. 82, 485–501. https://doi.org/10.1111/j.1095-8312.2004.00335.x

Soltis, P.S., Soltis, D.E. 2009. The role of hybridization in plant speciation. *Annual Rev. Plant Biol.* 60, 561–588. https://doi.org/10.1146/annurev.arplant.043008.092039

Soltis, P.S., Soltis, D.E. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proc. Natl. Acad. Sci. U. S. A.* 97, 7051–7057. https://doi.org/10.1073/PNAS.97.13.7051

Song, Y., Drossel, B., Scheu, S. 2011. Tangled Bank dismissed too early. *Oikos* 120, 1601–1607. https://doi.org/10.1111/j.1600-0706.2011.19698.x

Stebbins, G.L. 1985. Polyploidy, hybridization, and the invasion of new habitats. *Ann. Missouri Bot. Gard*. 72, 824–832. https://doi.org/10.2307/2399224

Stebbins, G.L. 1957. Self fertilization and population variability in the higher plants. *Am. Nat.* 91, 337–354. https://doi.org/10.2307/2458946

Suda, J., Krahulcová, A., Trávníček, P., Krahulec, F. 2006. Ploidy level versus DNA ploidy level: an appeal for consistent terminology. *Taxon* 55, 447–450. https://doi.org/10.2307/25065591

Talley, S.M., Coley, P.D., Kursar, T.A. 2002. The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. *BMC Ecol.* 2, 7. https://doi.org/10.1186/1472-6785-2-7

te Beest, M., Le Roux, J.J., Richardson, D.M., Brysting, A.K., Suda, J., Kubešová, M., Pyšek, P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Ann. Bot.* 109, 19–45. https://doi.org/10.1093/aob/mcr277

Temsch, E.M., Greilhuber, J., Krisai, R. 2010. Genome size in liverworts. *Preslia* 82, 63–80.

Tribsch, A., Schönswetter, P. 2003. Patterns of endemism and comparative phylogeography confirm palaeo-environmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon* 52, 477–497.

van Dijk, P.J. 2003. Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. *Philos. Trans., Ser. B* 358, 1113–21. https://doi.org/10.1098/rstb.2003.1302

Van Valen, L. 1973. A new evolutionary law. *Evol. Theory* 1, 1–30.

Vandel, A. 1940. La parthénogenèse géographique. IV. Polyploidie et distribution géographique. *Bull. Biol. Fr. Belg*. 74, 94–100.

Vandel, A. 1928. La parthénogenèse géographique. Contribution à l'étude biologique et cytologique de la parthénogenèse naturelle. *Bull. Biol. Fr. Belg*. 62, 164–182.

Verduijn, M.H., Van Dijk, P.J., Van Damme, J.M.M. 2004**.** Distribution, phenology and demography of sympatric sexual and asexual dandelions (*Taraxacum officinale s.l.*): geographic parthenogenesis on a small scale. *Biol. J. Linn. Soc*. 82, 205–218. https://doi.org/10.1111/j.1095-8312.2004.00325.x

Visger, C.J., Germain-Aubrey, C.C., Patel, M., Sessa, E.B., Soltis, P.S., Soltis, D.E. 2016. Niche divergence between diploid and autotetraploid *Tolmiea*. *Am. J. Bot.* 103, 1396–1406. https://doi.org/10.3732/ajb.1600130

Vrijenhoek, R.C. 1984. Ecological differentiation among clones: the frozen niche variation model, in: Woermann, K., Loeschcke, V. (Eds.), Population Biology and Evolution. Springer, Berlin, pp. 217–231. https://doi.org/10.1007/978-3-642-69646-6_18

Vrijenhoek, R.C., Parker, E.D. 2009. Geographical parthenogenesis: general purpose genotypes and frozen niche variation, in: Schön, I., Martens, K., van Dijk, P. (Eds.), Lost Sex. Springer, Dordrecht, Heidelberg, London, New York, pp. 99–131. https://doi.org/10.1007/978-90-481-2770-2_6

Watanabe, K. 1986. The cytogeography of the genus *Eupatorium* (Compositae): a review. *Plant Species Biol.* 1, 99–116. https://doi.org/10.1111/j.1442-1984.1986.tb00019.x

Wendel, J.F. 2000. Genome evolution in polyploids. *Plant Mol. Biol.* 42, 225–249.

Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Yang, X., Lascoux, M., Glémin, S. 2018. Variation in competitive ability with mating system, ploidy and range expansion in four *Capsella* species. *bioRxiv* 214866. https://doi.org/10.1101/214866

CHAPTER 2

Sexual intraspecific recombination but not *de novo* origin governs the genesis of new apomictic genotypes in *Potentilla puberula* (Rosaceae)

Flavia Domizia Nardi, Christoph Dobeš, Dorothee Müller, Tobias Grasegger, Tuuli Myllynen, Henar Alonso-Marcos and Andreas Tribsch

Status: Published in *Taxon*, November 2018

Contribution: field material collection, flow cytometric analysis, data analysis

Sexual intraspecific recombination but not *de novo* origin governs the genesis of new apomictic genotypes in *Potentilla puberula* (Rosaceae)

FLAVIA DOMIZIA NARDI, [1, 2] CHRISTOPH DOBEŠ, [1] DOROTHEE MÜLLER, [2] TOBIAS GRASEGGER, [2] TUULI MYLLYNEN, [1], HENAR ALONSO-MARCOS[1] AND ANDREAS TRIBSCH[2]

[1]Austrian Research Centre for Forests, Department of Forest Genetics, Seckendorff-Gudent-Weg 8, 1131 Vienna, Austria

[2]University of Salzburg, Department of Biosciences, Hellbrunnerstraße 34, 5020 Salzburg, Austria

Running title: Origin of apomictic *Potentilla puberula*

Correspondent author: Flavia Domizia Nardi, flavia.d.nardi@gmail.com

Nardi, F., Dobeš, D., Müller, D., Grasegger, T., Myllynen, T., Alonso-Marcos, H. & Tribsch, A. 2018. Sexual intraspecific recombination but not *de novo* origin governs the genesis of new apomictic genotypes in *Potentilla puberula* (Rosaceae). *Taxon* 67: 1108–1131. https://doi.org/10.12705/676.8

ABSTRACT

Apomixis –asexual reproduction via seeds– might arise *de novo* following polyploidisation events, or via reproductive transfer of apomixis. Both processes can be obtained within species or via hybridisation. We aimed to determine the origin of apomictic genotypes in *Potentilla puberula*, a rosaceous species showing reproductive differentiation with ploidy: sexual tetraploids and apomictic penta- to octoploids, which regularly co-occur in sympatry. The study is based on 726 individuals, comprising all cytotypes, collected from 138 populations in the Eastern European Alps. We established relationships of cytotypes based on AFLP fingerprinting and cpDNA sequencing to test (1) whether the apomicts are of recurrent allopolyploid origin or originated from within the species via autopolyploidy, and (2) whether there are indications for reproductive transfer versus *de novo* origin of apomixis. Three principal pathways were identified which explain the origin of new apomictic genotypes, all involving at least one apomictic parent and thus compatible with the idea of reproductive transfer of the apomictic trait to the progeny: (1) self-fertilisation of unreduced egg cells in apomicts; (2) cross-fertilisation among apomicts; and (3) occasionally, heteroploid crosses among sexuals and apomicts. Autopolyploids derived from tetraploid sexuals were repeatedly observed, but did not express apomixis. Finally, our results suggest no role of other species in the origin of extant apomictic genotypes of *P. puberula*, although local hybrids with *P. crantzii* were identified. In conclusion, our results show that the formation of new apomictic genotypes required a genetic contribution from at least one apomictic parent. This finding is in accordance with the idea that apomixis is inheritable in *P. puberula*. On the contrary, lack of apomixis in penta- and hexaploids derived from sexual backgrounds did not support the hypothesis of a *de novo* origin of apomixis. Relatively high frequency of remnant sexuality in the apomicts involving different cytological pathways of seed formation can explain their high cytological and genotypic diversity. Finally, lack of global introgression from a third taxon is in support of *P. puberula* as a concise, although highly diverse, species.

Keywords: AFLP; apomixis, autopolyploidy, cpDNA, origin; *Potentilla*

INTRODUCTION

Gametophytic apomixis (henceforth, “apomixis”) refers to reproductive pathways of seed formation in flowering plants which arise through modification of the normal sexual pathway (Rutishauser, 1969; Koltunow & Grossniklaus, 2003). These pathways include the circumvention or interruption of meiosis (i.e., apomeiosis) and the development of egg cells into an embryo without fertilisation (i.e., parthenogenesis; Asker, 1980; Asker & Jerling, 1992). Although rare examples of diploid apomictic taxa are known (*Boechera* Á.Löve & D.Löve: Böcher, 1951; Dobeš & al., 2006; and *Paspalum* L.: Siena & al., 2008), the great majority is polyploid (Asker & Jerling, 1992; Carman, 1997; Comai, 2005; Dickinson & al., 2007). Most apomicts are of allopolyploid origin (Asker & Jerling, 1992) and tend to form amphi-apomictic hybrid complexes with sexual diploids (e.g., Böcher, 1951; Asker, 1970; Campbell & Wright, 1996; Bayer, 1997; Hörandl & Gutermann, 1999). Sexual diploidautopolyploid apomictic complexes, however, do also exist (e.g., in *Paspalum*: Hojsgaard & al., 2008; *Ranunculus* L.: Cosendai & al., 2011; *Townsendia* Hook.: Thompson & Whitton, 2006). In rare cases, sexual-apomictic differentiation further occurs at the polyploid level (Savidan & al., 2001; Rotreklová & al., 2002; Dobeš & al., 2013b).

Several hypotheses have been coined on the functional basis of apomixis. It was proposed to (1) have a genetic basis and originate from co-selection of genes coding for the expression of apomeiosis and parthenogenesis, respectively (Savidan, 1982; Nogler, 1984; Van Dijk & Vijverberg, 2005). Apomeiosis and parthenogenesis may be unlinked and segregate independently (Van Dijk & al., 1999; Noyes & Rieseberg, 2000; Matzk & al., 2005) or could be inherited as monogenic Mendelian trait (Grimanelli & al., 2001; Grossniklaus & al., 2001; Ozias-Akins & Van Dijk, 2007; Hand & Koltunow, 2014). Alternatively, apomixis might result from (2) ectopic expression of the normal sexual pathway, which might be directly linked to genome duplication (i.e., nucleotypic effect) or to the presence of divergent genomes (often allopolyploidy) with asynchronous reproductive gene expression (the “duplicate-gene asynchrony hypothesis”, Carman, 1997; Sharbel & al., 2010).

Apomixis may originate *de novo* independently multiple times in taxa or lineages preadapted for the evolution of this reproductive mode. This is shown clearly by the great diversity of apomictic pathways (Asker & Jerling, 1992) and their polyphyletic

distribution among the angiosperm orders (Carman, 1997; Hörandl & Hojsgaard, 2012). Rates of *de novo* origins, however, can be assumed to be constrained by the developmental and genetic complexity of the trait. Additionally, the establishment of a functional apomict may require several generations (Van Dijk & Vijverberg, 2005; Hojsgaard & al., 2014). Alternatively, apomixis may be transferred to sexuals via introgression by their apomictic relatives (Van Dijk & Vijverberg, 2005). While egg cells from apomictic individuals are usually unreduced, their pollen is often meiotically reduced and at least partially viable (Van Dijk, 2003; Mogie & al., 2007). Thus, pollen from apomicts is able to fertilise egg cells produced by sexual individuals (Mogie & al., 2007). Since apomixis has been shown to be inheritable (Asker, 1980; Savidan, 1982; Nogler, 1984; Grossniklaus & al., 2001; Ozias-Akins & Van Dijk, 2007), such cross-fertilisations may lead to the formation of new apomictic clonal lineages. Hybridisation between apomicts and sexual relatives was repeatedly found, for instance in *Boehmeria spicata* Thunb. (Yahara, 1990) and in *Taraxacum* sect. *Ruderalia* Kirschner & al. (Menken & al., 1995). Extensive introgression and hybridisation might also be responsible for the interspecific transfer of apomixis in *Bothriochloa* Kuntze and closely related genera (De Wet & Harlan, 1970). To give another example, Lo & al. (2010) revealed that apomictic tetraploids of *Crataegus suksdorfii* (Sarg.) Kruschke originated from backcrossing of allotriploids to sexual diploids.

Geographical separation, which often occurs in amphiapomictic complexes in the form of geographical parthenogenesis (Bierzychudek, 1985; Hörandl, 2006), represents a barrier of gene flow among sexuals and apomicts. In accordance with this argument, it has been shown that apomictic populations which at least partly occur with sexuals in sympatry are often genotypically more diverse than fully allopatric ones (Hörandl & al., 2001; Daurelio & al., 2004; Hörandl & Paun, 2007). This observation suggests repeated recent origins of apomicts. Geographical parthenogenesis might be also the result of following reproductive interactions among sexuals and apomicts (Cosendai & Hörandl, 2010; Hülber & al., 2013; Kirchheimer & al., 2018). Unidirectional fertilisation of sexuals by apomicts and introgression of the apomictic trait into sexual populations may in fact lead to their replacement (Mogie, 1992; Joshi & Moody, 1995, 1998). Thus, study systems in which sexuals and their apomictic descendants occur in sympatry are expected to hold the clue for the understanding of mechanisms governing the evolution and dynamics of amphi-apomictic complexes.

Here we investigate the origin of apomictic genotypes of *Potentilla puberula*, a rosaceous species showing a remarkabledifferentiation of sexual tetraploids on the one hand and apomictic penta- to octoploids on the other hand. Both reproductive modes occur sympatrically in the Eastern European Alps. *Potentilla puberula* itself has been proposed as an allopolyploid species (Wolf, 1908; Ehrendorfer, 1970; Soják, 2010), and it is already known to be a parental species of *P. alpicola* La Soie, via multiple hybridisation events with hexaploid *P. argentea* L. (Paule & al., 2012). So far, it has remained unclear whether the apomictic cytotypes of *P. puberula* themselves did originate from hybridisation events with other closely related species (allopolyploid origin) or whether they evolved intraspecifically via autopolyploidisation. The latter has been tentatively suggested because of little molecular differentiation among sexual and apomictic cytotypes (Paule & al., 2012). We based this study on established individuals from natural populations and used molecular methods (Amplified Fragment Length Polymorphisms [AFLPs] and plastid DNA [cpDNA] sequencing) for estimating genetic relatedness and structure.

In particular, we address the following questions: (1) did apomictic genotypes of *P. puberula* originate via allopolyploidy from (multiple) hybridisation events of sexual genotypes with sympatric related apomictic and/or sexual *Potentilla* L. species? (2) or are the apomicts of intraspecific origin? Particularly we (2a) address the role of sexual recombination involving apomicts in the formation of new apomictic genotypes, and (2b) alternatively, ask whether there is evidence for a (re)current autopolyploid *de novo* origin of apomicts from sexuals. Since we are not able to reconstruct either the individual generations leading to apomixis from established populations/individuals, or the duration of the process, we determine the origin of genotypes in terms of molecular genetic relatedness. If apomicts arose *de novo* from sexual parents, we would expect finding apomictic genotypes being genetically similar to sexuals. On the contrary, we would expect apomictic genotypes deriving by crosses involving other apomicts to be genetically admixed (in case of crosses involving sexuals) or close to other apomictic genotypes (in case of crosses among apomicts).

MATERIALS AND METHODS

Study system

Potentilla puberula (Rosaceae; *Potentilla pusilla* Host; Soják, 2010) is an herbaceous species inhabiting mainly foothills to montane (rarely subalpine) xeric grasslands on shallow soil ranging from the Eastern European Alps to the Western Carpathians (Kurtto & al., 2004). Five ploidy cytotypes are known: tetraploids (x = 7, 2n = 28), pentaploids (2n = 35), hexaploids (2n = 42), heptaploids (2n = 49), and octoploids (2n = 56), although sporadic higher ploidy levels have been found as well (Dobeš, 1999). Tetraploids reproduce almost always sexually and are self-incompatible, whereas the other cytotypes are usually pseudogamous apomictic (i.e., the endosperm needs a preceding fertilisation event initiating seed development) and are self-compatible (Dobeš & al., 2013b). No embryological observations are explicitly available for apomictic *P. puberula* (but see Håkansson, 1946 for the embryology of a sexual tetraploid cytotype). However, both diplospory Asch. (e.g., Smith, 1963a), a taxon of a formerly used broader circumscription including our species (Meusel & al., 1965; Kurtto & al., 2004).

Ex-situ crossing experiments revealed homoploid pollen preference (Alonso-Marcos & al., 2018) and reduction of the seed set resulting from heteroploid crosses in the tetraploids (Dobeš & al., 2018). However, viability of pollen from apomicts, together with the insignificancy of the mentor effect and the evidence that seeds from heteroploid crosses are still produced (Dobeš & al., 2018) – even in presence of competitive homoploid pollen (Alonso-Marcos & al., 2018) – suggest that natural fertilisation of tetraploids by apomicts is possible.

Plant material

A total of 2038 individuals of *P. puberula* were collected from 138 populations in the Eastern Alps (Appendix 1). Six additional species closely related to *P. puberula* (Wolf, 1908; Dobeš & Paule, 2010; Paule & al., 2012) and occurring in sympatry in the study area with the species (Kurtto & al., 2004) were included to test for their possible role in the origin of apomictic genotypes: *Potentilla argentea* L., *P. aurea* L., *P. brauneana* Hoppe, *P. crantzii* (Crantz) Beck ex Fritsch, *P. frigida* Vill., *P. grandiflora* L. and morphologically intermediate forms of *P. crantzii* × *P. puberula* (Table 1; Appendix 1). In total, 122 individuals from 36 populations were sampled. Three species are known to reproduce sexually (Dobeš & al., 2015) at the di- (*P. aurea*) and tetraploid level (*P.*

frigida, *P. grandiflora*). No evidence on reproductive mode is available for *P. brauneana* yet, but diploidy suggests that it reproduces sexually since apomixis is bound to polyploidy in the genus *Potentilla* (Dobeš & al., 2015). *Potentilla argentea* and *P. crantzii* show reproductive differentiation among their cytotypes. Diploid *P. argentea* reproduces sexually, whereas its hexaploid cytotype is preferentially apomictic (Paule & al., 2011; Dobeš & al., 2015). In *P. crantzii*, tetraploids are sexual (Czapik, 1961, 1962) and higher ploidy cytotypes preferentially apomictic (Smith, 1963a, b). *Potentilla argentea* is repeatedly found growing together with *P. puberula* in the study area; *P. aurea* and *P. crantzii* get occasionally in contact with *P. puberula* at higher elevations, while the remaining species hardly get into direct contact with *P. puberula* due to their preference for alpine to nival habitats (Wolf, 1908; Polatschek, 2000; Kurtto & al., 2004). Additionally, 15 individuals of *Potentilla incana* G.Gaertn. & al., which is a sexual tetraploid in Central Europe (Dobeš & al., 2015), from 3 populations in eastern Austria and 3 individuals from a single population at the southern limit of our study area were included in the analysis as a reference (Appendix 1). This species is closely related to *P. puberula*, both belonging to the *P. verna* L. agg. (Wolf, 1908; Ehrendorfer, 1973). Herbarium vouchers were submitted to the herbaria of the Natural History Museum of Vienna (W) and of the University of Göttingen (GOET; Appendix 2 and Electr. Suppl. 1: Table S1).

Table 1. Ploidies and associated reproductive modes in *Potentilla* L. species studied.

Species	Ploidy	Reproductive mode	References
P. argentea L.	2*x*	Sexual	Paule & al. (2011); Dobeš & al. (2015)
	6*x*	Apomictic	Paule & al. (2011); Dobeš & al. (2015)
P. aurea L.	2*x*	Sexual	Dobeš & al. (2015)
P. brauneana Hoppe	2*x*	Likely sexual	–
P. crantzii (Crantz) Beck ex Fritsch	4*x*	Sexual	Czapik (1961, 1962)
	5–9*x*	Apomictic	Smith (1963a, b)
P. frigida Vill.	4*x*	Sexual	Dobeš & al. (2015)
P. grandiflora L.	4*x*	Sexual	Dobeš & al. (2015)
P. incana G.Gaertn. & al.	4*x*	Sexual	Dobeš & al. (2015)
P. puberula Krašan	4*x*	Sexual	Dobeš & al. (2013b)
	5–8*x*	Apomictic	Dobeš & al. (2013b)

DNA ploidy level estimation

DNA ploidy was determined for all 2038 individuals of *P. puberula*, as well as 52 individuals of other species with multiple ploidy levels (i.e., *P. argentea* and *P. crantzii*). We used a Partec ML Ploidy Analyser (Partec, Münster, Germany) and followed the

protocol of Doležel & al. (2007): leaf material was chopped together with the internal standard *Solanum pseudocapsicum* L. (2C-value = 2.59 pg, Temsch & al., 2010) in 500 µl Otto I buffer (Otto, 1990), filtered through a 20 µm nylon mesh (Partec CellTrics, Partec, Münster, Germany) and stained with 1 ml Otto II buffer (Otto, 1990) containing 4 µg DAPI (4′-6-diamidino-2-phenylindole). The mean fluorescence intensities of sample and standard from each measurement were calculated using FloMax v.2.9 (Quantum Analysis, 2014). The ploidy of each individual was estimated calculating the sample/standard fluorescence ratio and using plants of known ploidy level as karyological reference (Ptl4048, 2*n* = 4*x* = 28; Ptl4184, 2*n* = 5*x* = 35; Ptl4133, 2*n* = 7*x* = 49; Paule & al., 2012).

Reproductive mode screening

The reproductive origin of 501 fruitlets (referred to as seeds in the following) from 464 individuals of *P. puberula* was determined via flow cytometric seed screen (FCSS, Matzk & al., 2000). Since apomictic-sexual reproductive differentiation in *P. puberula* is strong both at the level of cytotypes and individuals (Dobeš & al., 2013b, 2018), one seed per individual was generally measured. However, exceptionally, sexual penta- to octoploids were also observed. When a seed from a penta- to octoploid individual was estimated to be sexually derived, we, therefore, analysed more seeds at the individual level. Seeds were chopped in 300 µl Otto I buffer (Otto, 1990) together with *Pisum sativum* L. 'Kleine Rheinländerin' (Greilhuber & Ebert, 1994) and placed 30 min on ice. The material was then ultrasonicated for 1 min, filtered through a 20 µm mesh filter (Partec CellTrics) and finally stained in 1200 µl Otto II buffer (Otto, 1990) containing 0.2 µg/ ml DAPI. After 15 min, the samples were measured with a Partec ML Ploidy Analyser. The mean fluorescence intensities of embryo, endosperm and standard from each measurement were calculated using FloMax v.2.9 (Quantum Analysis, 2014).

The FCSS method is based on different ploidy levels of endosperm and embryo occurring in a seed: in most angiosperms, a triplophasic endosperm is formed by fertilisation of two haplophasic polar nuclei. The ploidy ratio among endosperm and embryo (i.e., the peak index) thus differs depending on the reproductive mode of seed formation: in regular sexuality a diplophasic embryo (1*n* + 1*n*) and a triplophasic endosperm (2*n* + 1*n*) are expected, whereas in pseudogamous apomixis a diplophasic embryo (2*n* + 0*n*) and a penta- or hexaphasic endosperm (4*n* + 1–2*n*, depending on the

male gamete) are usually formed, resulting in peak indices of respectively 3/2 and 5(or 6)/2 (Matzk & al., 2000). However, these ratios only apply under the assumption of equal ploidy of parents, regularity of meiosis, and functional male meiosis, which do not necessarily apply outside a controlled crossing study. We therefore allowed for continuous variation of peak indices and inferred the origin of the megagametophyte calculating the female genomic contribution: peak indices lower and higher than 2 indicate a zygotic and a parthenogenetic origin, respectively (Dobeš & al., 2013a). However, an endosperm with a ploidy twice that of the embryo is practically often indistinguishable from the embryo's G2 phase; therefore, we considered only peak indices < 1.9 and > 2.1. The maternal genomic contribution, calculated according to Dobeš & al. (2013a), was used to infer the megagametophyte origin: megagametophyte ploidies equal to the mother plant ploidy and equal to its half indicated an apomeiotic and a meiotic megagametophyte development, respectively. The combination of the two categories led to the identification of four main reproductive pathways: regular sexuality (meiosis and fertilisation), irregular sexuality (here defined as apomeiosis followed by fertilisation and giving rise to BIII hybrids, Rutishauser, 1948; or SIII progeny in case of selfing, Bicknell & al., 2003), haploid parthenogenesis (meiosis and parthenogenesis) and apomixis (apomeiosis and parthenogenesis; Table 2). The reproductive origin of additional 201 seeds from 38 individuals from populations 9, 13, 15 and 17 was taken from Dobeš & al. (2013b).

Table 2. Reproductive pathways of seed formation and their inference.

Embryo development	Megagametophyte formation	
	Meiosis	Apomeiosis
Fertilisation	Regular sexuality $P_{♀}$ = 1/2 M pi < 1.9	Irregular sexuality (B_{III} hybrids formation) $P_{♀}$ = M pi < 1.9
Parthenogenesis	Haploid parthenogenesis $P_{♀}$ = 1/2 P pi > 2.1	Apomixis $P_{♀}$ = P pi > 2.1

M: ploidy of the mother plant; $P_{♀}$: ploidy of the megagametophyte; pi: peak index.

DNA extraction, cpDNA amplification and sequencing

For all molecular analyses, four individuals per cytotype per population (726 of *P. puberula* and a total of 140 of the other taxa) were randomly selected. Genomic DNA was extracted from silica gel-dried leaves taken directly in the field using the CTAB

method, following the protocol by Doyle & Doyle (1987) with minor modifications: ground leaf material was washed twice in Sorbitol buffer (100 mM Tris-Cl pH 8.0, 0.35 M Sorbitol, 5 mM EDTA, 1% PVP-40) and incubated for 10 min at 65°C in 3× CTAB buffer (100 mM Tris-Cl pH 8.0, 3 M NaCl, 20 mM EDTA, 3% CTAB, 2% PVP-40) and 2 µl RNase A (10 mg/ml, Thermo Scientific, Massachusetts, U.S.A); at the end of the extraction, the DNA pellet was washed in 70% ethanol and resuspended in ddH2O.

For each individual of *P. puberula*, the plastid *trnH(gug)- psbA* intergenic spacer (IGS) and *rps16* intron were amplified using the primers trnH(gug) and psbA (Aldrich & al., 1988; Shaw & al., 2005) and rpS16F and rpS16R (Oxelman & al., 1997; Shaw & al., 2005) respectively. PCR reactions were performed in 30 µl master mix containing 5× Green GoTaq buffer (Promega, Madison, Wisconsin, U.S.A.), 0.2 µM of each primer, 0.2 mM dNTP mix, 0.83 U GoTaq DNA polymerase (Promega), and approximately 10 ng of template DNA using a My Cycler (Bio-Rad Laboratories, Vienna, Austria) thermal cycler. Thermal cycling started with a denaturation step at 95°C lasting 2 min, followed by 35 cycles each of 30 s denaturation at 95°C, 30 s annealing at 50°C, 1 min 20 s elongation at 72°C, and lasted with 5 min final elongation at 72°C and a final hold at 4°C.

The sequences of the two cpDNA markers were edited and combined on Geneious v.8.0 (Kearse & al., 2012; Biomatters, 2014). Haplotypes from the uncut sequences were collapsed using FaBox v.1.41 (Villesen, 2007) and deposited in GenBank (Electr. Suppl. 1: Table S2; Benson & al., 2005).

AFLP fingerprinting

Individuals selected for the molecular analyses (726 of *P. puberula* and a total of 140 of the other taxa) – including replicated samples following Bonin & al. (2004) – were genotyped using AFLP fingerprinting according to the protocol established by Vos & al. (1995) with the following modifications: approximately 300 ng of DNA was digested and ligated in a 11 µl reaction mix containing 10× T4-ligase buffer, 68.7 mM NaCl, 30.8 µg BSA, 0.9 U T4- ligase (Promega), 1 U MseI (New England Biolabs, Ipswich, Massachusetts, U.S.A.), 8 U EcoRI (Promega), and 6.25 µM MseI-adaptor and 0.63 µM EcoRI-adaptor. The reaction mix was incubated for 3 h at 37°C, and the restriction-ligation product was subsequently diluted 1: 20.

In the pre-selective PCR, 2 µl of the diluted restrictionligation product was used in a total reaction volume of 10 µl, containing: 5× Green GoTaq Buffer (Promega), 0.29

mM dNTP mix, 0.38 μM EcoRI-A primer (5′-GAC TGC GTA CCA ATTCA-A-3′), 0.38 μM MseI-C primer (5′-GAT GAG TCC TGA GTA AC-C-3′), and 0.13 U GoTaq G2 polymerase (Promega). The reactions were held at 72°C for 2 min followed by 30 cycles of: 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. For the selective PCR, 2 μl of 1 : 20-diluted pre-selective PCR product was used as a template in three different reactions including differently labelled primer combinations, for a reaction volume of 10 μl each containing: 5× Green GoTaq Buffer (Promega), 0.28 mM dNTP mix, 0.34 μM EcoRIfluorescence- labelled primer, 0.34 μM MseI primer (EcoRIAGG [VIC]/MseI-CTC, EcoRI-AAC [6-FAM]/MseI-CTT, EcoRI-AGC [NED]/MseI- CTG), and 0.2 U GoTaq G2 polymerase (Promega). The reactions were held at 94°C for 2 min followed by 10 cycles of: 94°C for 20 s, 66°C → 57°C (−1°C per cycle) for 30 s and 72°C for 2 min, followed by 20 cycles of: 94°C for 20 s, 56°C for 30 s and 72°C for 2 min, with a final 30 min extension at 60°C. The product of each step (restrictionligation, pre-selective and selective amplifications) was run on 1% agarose gel together with a negative control.

For each sample, the three different PCR products were combined, and the fragments were separated on a MegaBACE 1000 DNA capillary-sequencer together with an ET-ROX 400 size standard (GE Healthcare Biosciences, Pittsburgh, Pennsylvania, U.S.A.). In each run, 48 samples including negative and carryover controls (Bonin & al., 2004) were analysed. Raw data were visualised and quality checked with Fragment Profiler v.1.2 (Amersham Biosciences, Amersham, U.K.) and the fragments manually scored as binary presence-absence data using DAx v.9.0 (Van Mierlo Software Consultancy, Eindhoven, The Netherlands) and following the procedure described by Bendiksby & al. (2011). Two final presence-absence matrices were created by two different scoring procedures: to have a higher resolution at the specific level, all the 726 individuals of *P. puberula* only were included (intraspecific dataset), and to better identify species-specific markers, a subset of 163 individuals of *P. puberula* covering the whole sampling area and all ploidy levels (Appendix 1) was scored together with all 140 samples of the other taxa (interspecific dataset).

Data analyses

Since genotyping errors might overestimate the genotypic differences among genotypes (Bonin & al., 2004), a threshold was estimated from both the error rate (Pompanon & al., 2005) and the distributions of pairwise genotypic distances within

populations of individuals of the same ploidy, calculated with the R script AFLPdat v.2010 (Ehrich, 2006; R Development Core Team, 2018). AFLP phenotypes showing pairwise distances not exceeding the threshold were merged into unique genotypes ("genotype" in the following) and only those were used in the analyses. Principal coordinate analyses (PCoAs) calculated with Dice dissimilarity index were run using the R packages ape (Paradis & al., 2004) and vegan v.2.5-2 (Oksanen & al., 2018) to evaluate the genetic similarity among all individuals of *P. puberula* alone (intraspecific dataset) and among all studied species (interspecific dataset).

The Bayesian clustering-based software STRUCTURE v.2.3.4 (Pritchard & al., 2000) was used to detect genetic admixture among groups. To test for introgression from sympatrically occurring species in apomictic individuals of *P. puberula*, we used the admixture model with independent allele frequencies, and 10 replicates were run for each pre-defined number of clusters (K) ranging from 1–10. A burn-in period of 2 × 105 and Markov chain Monte Carlo (MCMC) of 5 × 105 iterations was chosen. Null alleles were defined to account for genotypic ambiguity (Falush & al., 2007). To assess the level of admixture among sexual and apomictic *P. puberula*, we ran a STRUCTURE analysis with ploidy as prior information (USEPOPINFO = 1) for all tetraploids. This function is available only for diploids, thus we renounced to set the individuals as polyploids. Although studies on intraspecific ploidy variation often attempt a differential ploidy definition (Stöck & al., 2010), no simulation has ever been run to test the best way to deal with different ploidy levels (Meirmans & al., 2018) and the superiority of this approach over the standard diploid definition has not been proven yet. On the contrary, the prior information approach – with the limitation of a diploid definition of the data – allows assisting genetic clustering in genetically low-structured groups.

This approach is meaningful in our context, because the tetraploid group, reproducing sexually, can be seen as a single genetic population. We thus ran a STRUCTURE analysis with 10 replicates for each K from 1 to 5 with burn-in of 2 × 105 and MCMC of 5 × 105 iterations. Both the admixture and the correlated allele frequencies model were used, and we allowed a migration prior of 0.1. Since a phylogeographical signal was detected in the results, we ran a similar additional analysis including all and only the 105 genotypes from Eastern Tyrol (see Appendix 1), with the following differences: we ran 2 × 105 iterations after a 105 burn-in, and the migration prior was set to 0.05. All markers monomorphic in these selected genotypes were removed. The most

likely values of K were estimated from the likelihood distribution of single runs per K value. For each chosen K, the runs with the highest likelihood were chosen and graphically displayed with the R package pophelper v.2.2.7 (Francis, 2017). All graphs were created in R (R Development Core Team, 2018), using the R package ggplot2 v.3.0.0 (Wickham, 2016).

RESULTS

DNA ploidy of adults and reproductive mode screening

Five distinct classes of sample: standard fluorescence ratios were identified for *P. puberula*. The classes corresponded to the tetra- (966 individuals; 47.40 %), penta- (613; 30.08%), hexa- (116; 5.69%), hepta- (280; 13.74%) and octoploid (63; 3.09%) DNA ploidy levels. Of the 138 populations, 77 were of mixed ploidy, the others were uniformly tetra- (40 populations), penta- (17), hexa- (2), or heptaploid (2; Appendix 1). Fourteen individuals (58.33%) of *P. argentea* were determined as diploid, whereas ten were hexaploid (41.67%). One population out of four was cytologically mixed (Appendix 1). Individuals of *P. crantzii* were determined as tetra- (5 individuals; 21.74%), penta- (2; 8.70%), hexa- (8; 34.78%) and heptaploid (8; 34.78%). Two populations out of six were cytologically mixed (Appendix 1). The individual measurements are provided in Table S3 (Electr. Suppl. 2).

The intra-specific reproductive differentiation by ploidy in *P. puberula* observed by Dobeš & al. (2013b) was basically confirmed, with tetraploids forming 97.46% (192) of the seeds by regular sexuality and penta- to octoploids 84.01% (247) of the seeds apomictically (Table 3). Irregular sexuality (BIII hybrids) was found in equal percentages in tetraploids (4 seeds, 2.03% of seeds) and penta- to octoploids (6 seeds, 2.04%), whereas haploid parthenogenesis was present exclusively in penta- to octoploids (10 seeds, 3.40% of seeds). Among the higher ploidy levels, 13 hexaploid individuals which were assigned to the tetraploid cluster by the STRUCTURE analysis (see below) produced all seeds (15) by regular sexuality, and no parthenogenetically derived seed was found in the same genetic group (Table 3). On the contrary, 92.02% (369) of seeds produced by individuals assigned to the high ploidy level genetic cluster was apomictically derived, whereas 2.74% (11), 3.74% (15) and 1.50% (6) were derived respectively by haploid parthenogenesis, regular and irregular sexuality (Table 3). For individuals admixed among the two clusters no clear tendency was observed, with 41.67% (5) of their seeds

produced by apomixis, 33.33% (4) by regular sexuality and 25.00% (3) by irregular sexuality (Table 3). Sample histograms for each reproductive mode of seed formation are provided in Fig. S1 (Electr. Suppl. 1). Individual measurements are provided in Table S4 (Electr. Suppl. 2).

Table 3. Association among genetic clusters as defined by trained STRUCTURE analysis (see text and Fig. 5), ploidy and reproductive mode of seed formation (N = 491[a]) inferred by FCSS in *Potentilla puberula* Krašan.

Genetic cluster[b]	N individuals	N genotypes	N seeds	A	HP	S_{reg}	S_{irreg}
Cluster 1 ("Tetraploids")							
4*x*	253	253	95 (236)	0 (0)	0 (0)	191 (231)	4 (5)
5*x*	1	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6*x*	13	13	15 (15)	0 (0)	0 (0)	15 (15)	0 (0)
Admixed							
5*x*	9	9	10 (10)	4 (4)	0 (0)	3 (3)	3 (3)
6*x*	3	3	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)
7*x*	1	1	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)
Cluster 2 ("High ploidy")							
4*x*	3	2	2 (2)	1 (1)	0 (0)	1 (1)	0 (0)
5*x*	225	132	124 (192)	119 (185)	2 (2)	2 (2)	1 (3)
6*x*	42	25	20 (67)	19 (63)	0 (1)	1 (2)	0 (1)
7*x*	131	84	89 (94)	79 (83)	1 (1)	7 (8)	2 (2)
8*x*	45	31	34 (46)[a]	25 (37)[a]	7 (7)[a]	2 (2)	0 (0)

Number of seeds for each reproductive pathway are reported: A: apomixis (apomeiosis and parthenogenesis); HP: haploid parthenogenesis; S_{reg}: regular sexuality (female meiosis and fertilisation); S_{irreg}: irregular sexuality (B_{III} hybrids formation). Numbers out of parentheses refer to measurements within this study only. Numbers in parentheses include both seeds measured within this study and seeds measured by Dobeš & al. (2018)

a Ten additional seeds produced by octoploid individuals were found to be parthenogenetically derived, but the exact reproductive pathway (i.e., apomixis vs. haploid parthenogenesis) could not be determined.

b Genetic cluster as resulted by the intraspecific STRUCTURE analysis for K = 2. Genotypes were assigned to the two clusters (Cluster 1 "Tetraploids" and Cluster 2 "High ploidy") when their estimated membership to the pre-defined tetraploid genetic population was respectively over 90% and below 10%. Genotypes were considered admixed among the two clusters when their assignation to Cluster 1 ("Tetraploids") was over 10% and below 90%.

Genotypic and haplotypic variation within *P. puberula*

The AFLP scoring of 726 successfully genotyped individuals of *P. puberula* resulted in the identification of 370 polymorphic markers ranging between 62 and 401 bp after removal of non- reproducible and non- informative fragments, with an error rate of 2.35% (the presence-absence matrix is provided as Table S5 in Electr. Suppl. S2). We identified 554 genotypes applying a pragmatic threshold of 12 mismatches, derived from the observed error rate of 2.35% and the distribution of pairwise genotypic distances (Electr. Suppl. 1: Fig. S2). All tetraploid individuals were represented by unique genotypes (DGT = 1), while 300 unique genotypes representing 471 individuals were found for the penta- to octoploids. The proportion of polymorphic fragments and the number of private fragments was comparable among tetraploids (14 fragments) and the

group of high ploidy levels (12 fragments), but the private fragments were largely shared among the high-ploidy cytotypes, since only one private fragment each in the penta- and heptaploids, respectively, remained when cytotypes were considered separately.

Interestingly, 2.71% of the genotypes was shared among cytoypes (Table 4) and involving almost exclusively high-ploidy cytotypes. In particular, penta- and hepta-/octoploids, and hexaand octoploids shared the same genotype. In total, 719 individuals of *P. puberula* were successfully amplified for both *trnH-psbA* and *rps16* markers (Electr.Suppl. S2: Table S5). The length of the combined sequences ranged from 1108 bp to 1177 bp.

Geno-type	4*x*	5*x*	6*x*	7*x*	8*x*	N clones	N cytotypes sharing genotype
g027	–	1	–	1	–	2	2
g065	–	1	–	3	–	4	2
g100	–	3	–	1	–	4	2
g109	–	2	–	1	–	3	2
g182	–	1	–	1	–	2	2
g198	–	1	–	–	1	2	2
g256	–	–	–	2	1	3	2
g262	1	3	–	1	–	5	3
g302	–	–	2	–	1	3	2
g321	–	1	–	2	–	3	2
g365	–	1	–	–	2	3	2
g389	–	–	1	–	3	4	2
g488	–	3	–	2	–	5	2
g512	–	–	1	–	4	5	2
g527	–	1	1	–	–	2	2

Table 4. AFLP genotypes (defined by a threshold of 12 mismatches) shared among cytotypes of Potentilla puberula Krašan. Number of clones are reported by cytotype. The shared genotypes present 2.71% of the genotypes observed.

Overall, the haplotype sharing among cytotypes was high (Table 5). Out of 49 haplotypes in total, 29 were found to be shared between at least two cytotypes and represented the striking majority of the studied individuals (94.58%). Of the remaining 20 unshared haplotypes, 9 were found only in the tetraploids (representing 2.23% of all individuals). On the contrary, 15 haplotypes (4 shared among the other cytotypes and 11 found in single cytotypes, representing 7.92% of all individuals) were not present in the tetraploids.

Table 5. Number of individuals of Potentilla puberula Krašan sharind the same cpDNA haplotype, reported by ploidy level.

Haplotype	4*x*	5*x*	6*x*	7*x*	8*x*	N	N_c
H18	82	91	18	58	24	273	5
H01	38	38	–	10	7	93	4
H19	24	22	2	1	6	55	5
H17	18	11	1	2	–	32	4
H02	7	2	19	1	–	29	4
H03	12	3	–	7	–	22	3
H31	8	–	–	11	–	19	2
H28	10	–	2	2	–	14	3
H30	3	5	1	–	4	13	4
H29	–	9	–	2	–	11	2
H42	1	7	–	3	–	11	3
H23	3	4	1	2	–	10	4
H26	–	5	–	5	–	10	2
H04	2	2	4	–	–	8	3
H14	–	7	–	1	–	8	2
H08	2	–	3	–	2	7	3
H21	2	5	–	–	–	7	2
H33	1	–	4	2	–	7	3
H41	1	4	–	2	–	7	3
H06	1	1	–	4	–	6	3
H15	5	–	1	–	–	6	2
H37	5	–	1	–	–	6	2
H22	4	1	–	–	–	5	2
H25	–	2	–	1	2	5	3
H32	2	1	1	–	1	5	4
H40	5	–	–	–	–	5	1
H07	–	4	–	–	–	4	1
H24	3	1	–	–	–	4	2
H34	–	4	–	–	–	4	1
H39	–	–	–	4	–	4	1
H27	1	–	–	2	–	3	2
H48	–	–	–	3	–	3	1
H10	2	–	–	–	–	2	1
H16	2	–	–	–	–	2	1
H20	1	–	–	1	–	2	2
H35	–	2	–	–	–	2	1
H38	2	–	–	–	–	2	1
H47	1	–	–	1	–	2	2
H05	–	–	–	–	1	1	1
H09	–	–	1	–	–	1	1
H11	1	–	–	–	–	1	1
H12	–	1	–	–	–	1	1
H13	1	–	–	–	–	1	1
H36	–	–	1	–	–	1	1
H43	1	–	–	–	–	1	1
H44	–	–	1	–	–	1	1
H45	–	1	–	–	–	1	1
H46	1	–	–	–	–	1	1
H49	1	–	–	–	–	1	1

N: total number of individuals per haplotype; N_c: number of cytotypes sharing the same haplotype.

Testing for recurrent allopolyploidisation events involving third species

The AFLP scoring of the 303 individuals of *P. puberula* and its closely related species resulted in 335 polymorphic markers after removal of non-reproducible and non-informative fragments, with an error rate of 1.09% (data accessible as Table S6 in Electr. Suppl. S2). Duplicated genotypes defined by a threshold of seven pairwise distances were excluded from further analyses (Table S6 in Electr. Suppl. S2). The interspecific PCoA supported the distinctness of species (Fig. 1), with the first two coordinates explaining 26.66% and 7.90% of the genetic variation, respectively. Only *P. incana* overlapped *P. puberula*, and no distinction was found among tetraploid and penta- to octoploid *P. puberula.*

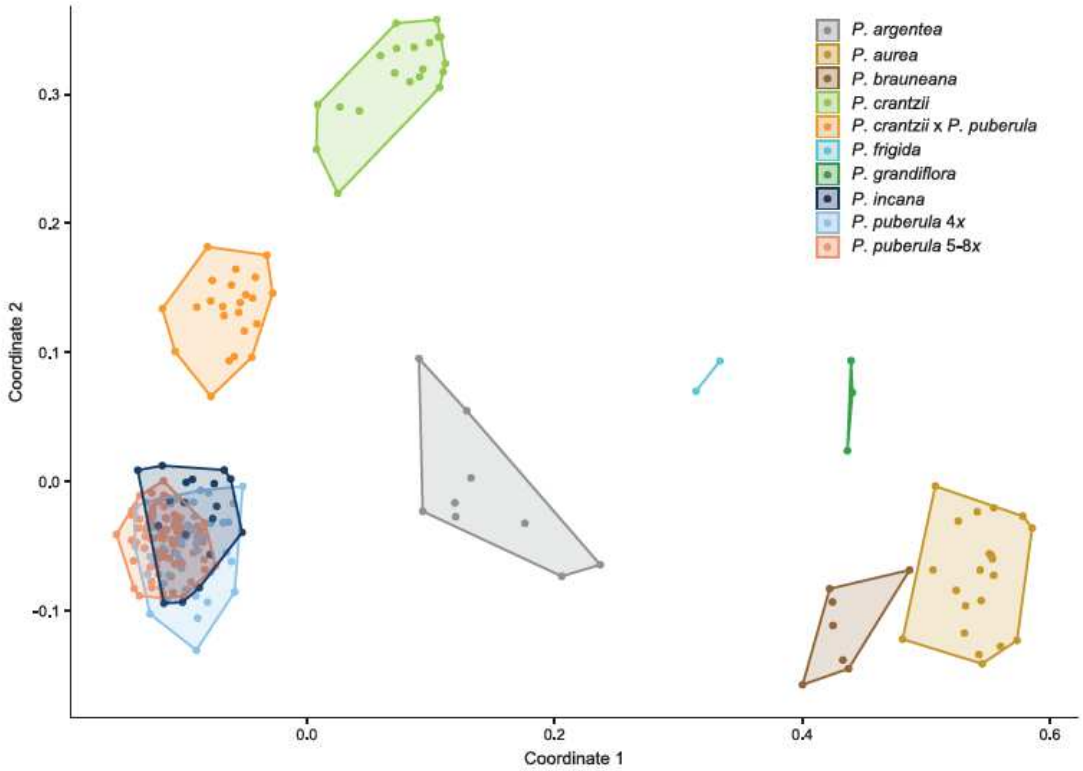


Fig. 1. Principal Coordinate Analysis of 251 unique genotypes of nine *Potentilla* L. taxa based on 335 AFLP markers. The coordinates 1 and 2 explain 26.66% and 7.90% of the total genetic variation, respectively.

Twenty-three additional unique genotypes originally classified as *P. puberula* formed a separate group intermediate to *P. puberula* and *P. crantzii* consistent with a hybrid origin. The result was confirmed by the interspecific STRUCTURE analysis (Fig. 2). At K = 4, all cytotypes of *P. puberula* as well as *P. incana* were assigned to one cluster, while *P. argentea*, *P. aurea*, and *P. crantzii* were assigned to the remnant three clusters. *Potentilla brauneana*, *P. frigida* and *P. grandiflora* were assigned mainly to the *P. argentea* cluster; although they were assigned to the *P. aurea* cluster in most of the other (less likely) runs (Electr. Suppl. 1: Fig. S3), probably because of the low number of individuals. Two tetraploid individuals of *P. puberula* showed some introgression from a species belonging to the *P. argentea* cluster, but no penta- to octoploid *P. puberula* showed any sign of introgression from other species. The putative hybrids *P. crantzii* × *P. puberula* were clearly admixed among the two assumed parental species. K > 4 yielded only admixed clusters with individual assignments below 5%.

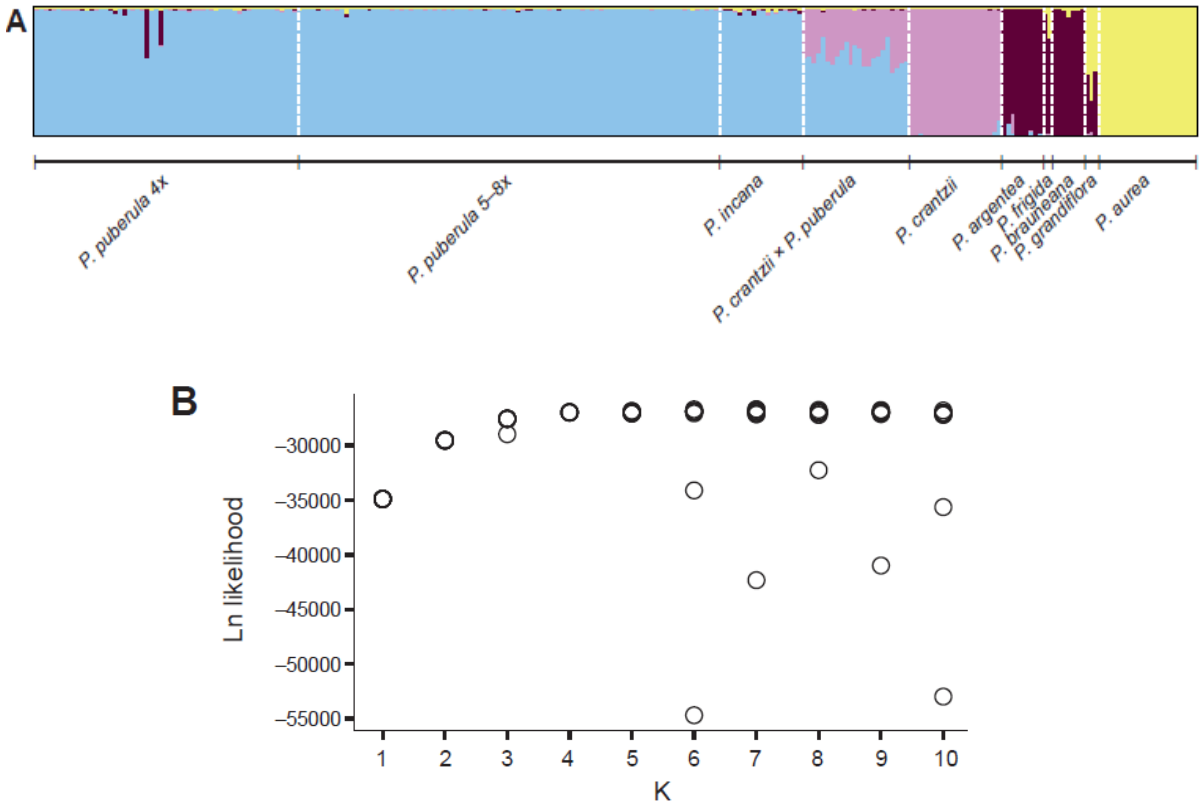


Fig. 2. Individual cluster assignment of 251 single genotypes of nine *Potentilla* L. taxa resulting from a STRUCTURE analysis based on 335 AFLP markers. **A,** K = 4, run with the highest likelihood; **B,** Logarithmic likelihood of each run per K value.

Molecular relationships among intraspecific cytotypes and reproductive modes

In *P. puberula*, three fragments (170 VIC, 219 FAM, 286 FAM) were present at constantly high frequencies in the high-ploidy cytotypes (respectively, 93.31%, 91.64% and 90.30% of genotypes) but low frequencies in the tetraploids (respectively, 3.14%, 1.18% and 8.24%). The fragments tended to co-occur within individuals, as 86.29% of penta- to octoploid genotypes had all three markers, while 89.02% of the tetraploids did not have any of those. Moreover, among 435 genotypes for which reproductive information was available, all genotypes which formed seeds parthenogenetically (independently on the megagametophyte origin) had at least two of the three fragments and 93.27% had all three, whereas 86.89% of genotypes which formed seeds by regular sexuality had none (Fig. 3). Only five high-ploidy genotypes for which only zygotic embryo development was found (corresponding to 1.94% of sexual, 16.67% of BIII and 1.67% of high- ploidy genotypes) had all three fragments.

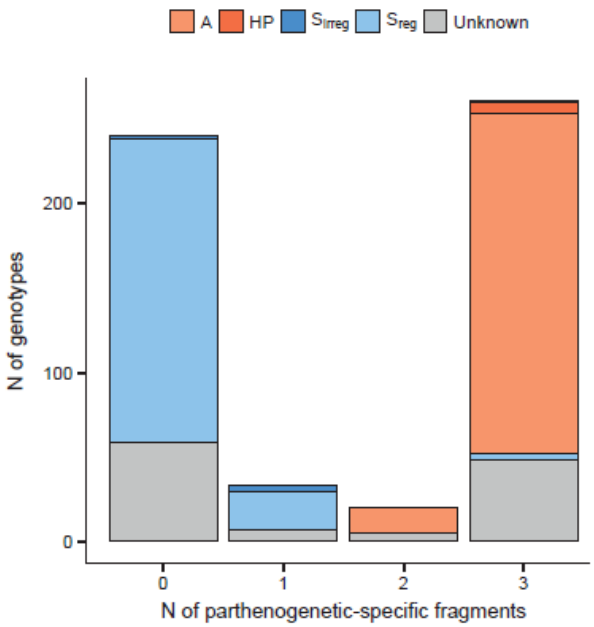
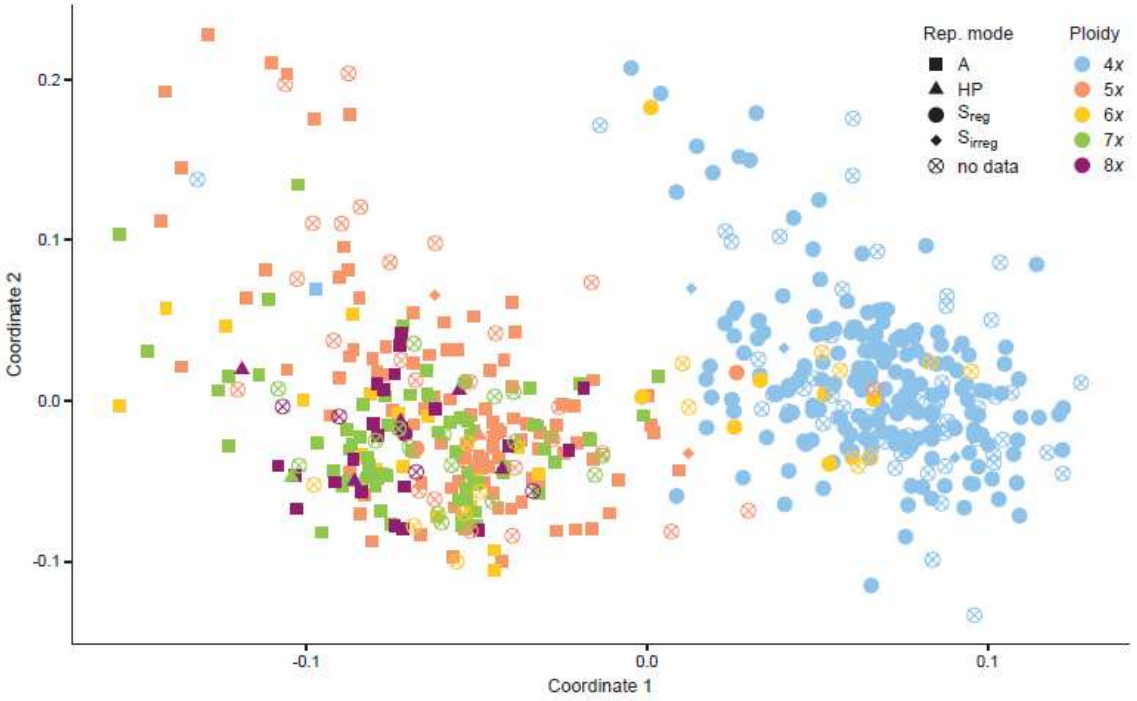


Fig. 3. Occurrence of three (presumably) parthenogenetic-specific AFLP fragments (170 VIC, 219 FAM, 286 FAM) in genotypes of *Potentilla puberula* Krašan classified by reproductive mode.
A: apomixis; HP: haploid parthenogenesis; Sirreg: irregular sexuality (BIII hybrids formation); Sreg: regular sexuality.

The first two coordinates of the PCoA accounted for only 4.04% and 2.51% of the total variation, respectively (Fig. 4). The first coordinate basically separated the sexual tetraploids from the high-ploidy genotypes. Exceptions are all the hexaploids and two of the pentaploids sampled together with tetraploids in natural populations, which clustered with the first. On the contrary, only two tetraploid genotypes were assigned to the group of the high-ploidy levels. The differentiation was probably mainly due to the three presumably parthenogenetic-specific fragments and largely disappeared when these were removed from the analysis (Electr. Suppl. 1: Fig. S4). None of the highploidy cytotypes formed a distinct subgroup. Rather, cytotypes were fairly mixed in the PCoA.

At K = 2, the intraspecific STRUCTURE analysis well differentiated the tetraploids and most high-ploidy cytotypes (Fig. 5). Exceptions involved two tetraploid genotypes (one apomictic and one for which no seeds were available) assigned to the high-ploidy cluster and 14 sexual penta- and hexaploids which were assigned to the tetraploid cluster with a membership higher than 90% (Table 3). Among the high-ploidy genotypes, 13 were variously admixed by the tetraploid cluster (Table 3). Most of the other high-ploidy genotypes formed an alternative defined genetic cluster (i.e., with assignation to the tetraploid cluster lower than 10%).

Fig. 4. Principal coordinate analysis of 554 unique genotypes of *Potentilla puberula* Krašan based on 370 AFLP markers. The coordinates 1 and 2 explain 4.04% and 2.51% of the total genetic variation, respectively. A: apomixis; HP: haploid parthenogenesis; Sirreg: irregular sexuality (BIII hybrids formation); Sreg: regular sexuality.



At higher values of K, a second tetraploid cluster was found, mostly associated to phylogeographical signal in East Tyrol, and was shared with apomictic individuals from the same geographical area (Fig. 5). At K = 4 and K = 5, additional high-ploidy clusters were obtained, and several genotypes showed admixture among two or more of these clusters (Fig. 5).

Since the second tetraploid cluster found at K > 2 might reflect a different phylogeographical history, rather than representing a real admixture among the tetraploids and the high ploidy genotypes assigned to this group, we ran a STRUCTURE analysis including only the 105 unique genotypes from Eastern Tyrol (Electr. Suppl. 1: Fig. S5). This second analysis resulted in the same pattern evidenced by the general analysis, confirming an effect of geographical structure. At K = 2, the tetraploids were clearly distinct from the penta- to octoploids, with three tetraploids assigned to the high ploidy cluster, while at higher values of K only additional apomictic clusters were found (Electr. Suppl. 1: Fig. S5A).

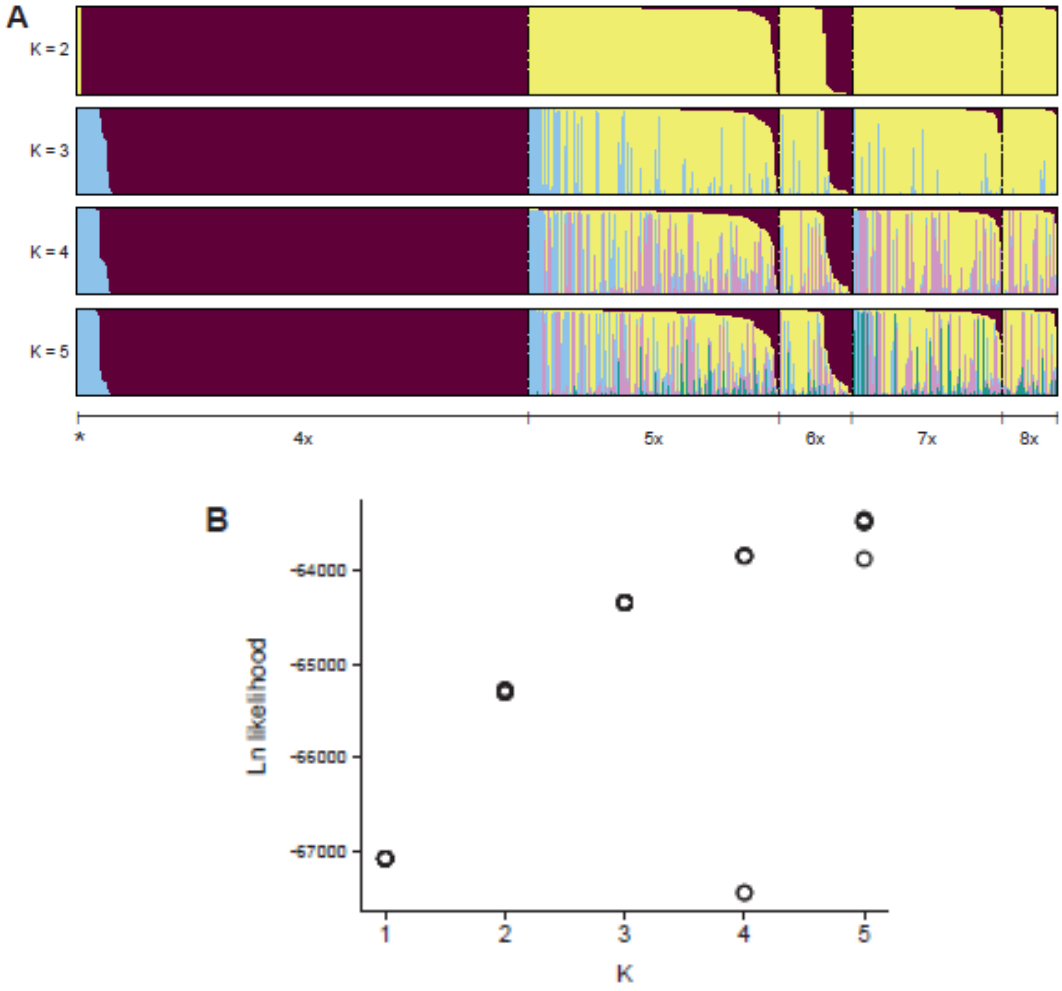


Fig. 5. Results of the intraspecific STRUCTURE analysis of 554 single genotypes of *Potentilla puberula* Krašan. **A,** Individual cluster membership based on the run with the highest likelihood per K. The asterisk indicates apomictic tetraploid genotypes. **B,** Logarithmic likelihood of single runs per K value.

DISCUSSION

In this study, we aimed to identify the origin of extant apomictic genotypes of *Potentilla puberula* collected in the field, discriminating in particular among an allopolyploid and an intraspecific origin, and searching for indications for reproductive transfer of apomixis versus its *de novo* origin. Both the PCoA and the intraspecific STRUCTURE analyses evidenced a slight but clear genetic differentiation of sexuals – mostly tetraploids – from apomictic penta- to octoploids ("high ploidy levels"). Main cause of this differentiation is the association of three AFLP alleles with parthenogenetic genotypes (Figs. 3, 4; Electr. Suppl. 1: Fig. S4). In general, our data on neutral genetic variation clearly shows that polyploidisation in *P. puberula* does not involve

contributions of other sympatric species, but they allow for drawing conclusions on the ongoing reproductive interactions in this sexual-apomictic system.

Autopolypoidisation of sexuals did not involve a change in reproductive mode

Although our study did not investigate the intra-individual variation in reproductive modes, we are nevertheless able to draw conclusions at the level of the identified genetic clusters. In particular, no progeny was found to be parthenogenetically derived from the individuals clustering with the sexual group, but sexually derived seeds were found from apomictic penta- to octoploid individuals clustering with the apomictic group. This suggests that the highploidy individuals, mostly hexaploids that clustered with the sexual tetraploids and formed seeds by regular sexuality were derived by spontaneous autopolyploidisation of tetraploids. Auto-hexaploids would originate by sexual fusion of a reduced and an unreduced gamete of tetraploid individuals (i.e., BIII, sensu Bicknell & al., 2003). The production of unreduced gametes has been observed in *P. puberula*: in an ex-situ crossing experiment, Dobeš & al. (2018) found the occasional production of seeds from unreduced egg cells (1.09%, Table 6) in sexual tetraploids. Even 2.03% of tetraploids seeds measured within our study fall into this category (Table 3). Simple backcrossing of auto-hexaploids with tetraploids might be responsible for the origin of the single exceptional auto-pentaploid we found.

The result is in line with co-occurrence patterns of cytotypes, since the sexual auto-hexaploids mostly occurred in tetraploid populations only. However, frequencies of hexaploids were remarkable (5.00%–9.52%, Appendix 1), an observation which indicates that functional sexuality might allow them to be relatively stable in a population, in spite of minority cytotype disadvantages (Levin, 1975). Vice-versa, irregularities in meiosis rare and ephemeral. Spontaneous polyploidisation of diploids via fertilisation of unreduced egg cells by reduced pollen was observed in natural populations of *Ranunculus kuepferi* (Schinkel & al., 2017). However, triploids reproduce in this species mostly by apomixis and occur only in the contact zone of sexual diploids and apomictic tetraploids (Schinkel & al., 2016), suggesting an inter-cytotype origin and that they might represent the first step towards the establishment of new apomictic tetraploids (Schinkel & al., 2017). In contrast, no apomictically derived seed was found in auto-hexaploids of *P. puberula* and their absence from apomictic populations suggests that they are not “hexaploid bridges” leading to the formation of apomictic lineages. In conclusion, the

evidence of sexuality in autopolyploids does not support the hypothesis that apomixis arises directly via polyploidisation events as nucleotypic effect.

Cytological pathways to new apomictic lineages involve apomicts

The PCoA, the intraspecific STRUCTURE analysis and the haplotype sharing revealed that none of the apomictic cytotypes represents an independent lineage. On the contrary, the high genetic similarity of apomictic cytotypes strongly supports the hypothesis of frequent changes in ploidy. Overall, we found indication for three principal pathways explaining the origin of newly formed apomicts within *P. puberula*, importantly all involving high ploidy apomicts at least as one parent: self-fertilisation of unreduced egg cells, sexual recombination within apomicts and crosses among apomicts and sexual tetraploids.

Self-fertilisation of unreduced egg cells (i.e., formation of SIII, sensu Bicknell & al., 2003), is suggested by the finding of sporadic inter-ploidal clonality (Table 4). This particular type of clonality can be explained by fusion of an apomeiotically derived (i.e., unreduced and non-recombined) and a meiotically recombined copy of the same genome. Since dosage of AFLP markers was not diagnosed, this pathway would not result in visible genotyping changes. The ploidy levels of individuals sharing the same genotype in this study mirrored very well this hypothesis: the most frequent cases of genotype sharing were among penta- and hepta-/octoploids (Table 4), in which unreduced pentaploid egg cells might have been fertilised by (irregularly) reduced di- to triploid self-pollen. Fertilisation of unreduced egg cells is relatively likely in apomictic *P. puberula*, since it was the most frequent mode of seed formation among the aberrant pathways observed by Dobeš & al. (2018) involving 3.56% of the progeny of the apomicts.

Sexual recombination among apomicts involving both selfing and outcrossing emerged from our data as a major source of genotypic variation within the apomictic group. In the first instance, the intra-population distribution of pairwise genotypic distances among apomicts was not bi-modal as expected in presence of clones and fully recombinant (i.e., derived from outcrossing) genotypes but showed intermediate values (Electr. Suppl. 1: Fig. S2). Although the skewness of the left peak (the clonal distribution) might be also explained with the presence of somatic mutations (as in the case of *Ranunculus carpaticola* Soó, Paun & al., 2006), this cannot account for inter-individual distances intermediate between the two peaks. We hypothesise, thus, that some

individuals originated by self-fertilisation involving reduced egg cells (i.e., SII, sensu Bicknell & al., 2003), a process which would not induce a change in ploidy, but recombines the maternal apomictic genotype. The result is in line with Dobeš & al. (2018), who found that the ploidy of apomictic mothers is often retrieved in sexually derived progeny of high-ploidy individuals (2.70% of the progeny). Penta- to octoploid *P. puberula* is self-compatible and selfing was frequent despite application of cross-pollen on the stigmata of non-emasculated flowers (Dobeš & al., 2013b), which suggests that selfing might play a relevant role in the origin of new genotypes.

A second evidence for sexual genetic recombination within the apomictic ploidy levels comes from the admixture analysis, which partially assigned several individuals to different apomictic clusters. The absence of cytotype-specific genetic clusters makes impossible to reveal the concrete cytological pathways giving rise to new genotypes in terms of ploidy of involved parents and gametes. However, the overall high genetic similarity of the different apomictic cytotypes strongly supports the hypothesis of frequent heteroploid crosses resulting in the formation of new cytotypes. Although meiosis in odd-ploids is expected to be irregular, female reduced gametes are nevertheless produced side by side with unreduced ones and both types of gametes can be fertilised by heteroploid pollen, also as a result of the tolerance of unbalanced genomic ratios in the endosperm (Dobeš & al., 2018). The large cpDNA haplotype sharing among apomictic cytotypes (regardless of the sharing with tetraploids) gives additional support to this scenario. Data obtained by Dobeš & al. (2018) indicate that, although most of apomicts reproduce sexually only at a low rate (3.03 % of the measured seeds was sexually derived), sexuality is maintained at significant rates (20.38% of the seeds) in some facultative apomicts (Table 6), which might play a key role for maintenance of the genetic variability of the population and the formation of new genotypes within the apomictic group.

Heteroploid crosses involving sexual tetraploids and apomictic penta- to octoploids were revealed by the admixture analyses: some penta- to heptaploid individuals were at least partially admixed with the sexual tetraploid cluster. Changes in ploidy in the progeny of tetraploids observed within heteroploid. Crossing experiments (Dobeš & al., 2018), showed that penta- and hexaploid embryos are usually produced when high- ploidy individuals fertilise tetraploids, although up to octoploid embryos may be formed in case of unreduced egg cells. No unreduced pollen has been found to successfully fertilise

tetraploids, probably because of unsustainable genomic imbalance (Dobeš & al., 2018). However, successful cross-fertilisation of tetraploids and high ploidy individuals appears uncommon in wild populations, because of the low frequency of genotypes genetically admixed between the tetra- and the high ploidy genetic cluster (Fig. 5). Ex-situ heteroploid crossing experiments with tetraploids as mothers revealed that viable out-crossing-derived seeds could be produced, but the resulting seed set was significantly lower compared to homoploid tetraploid crosses (Dobeš & al., 2018). In addition, fertilisation of sexuals by apomicts was nearly non-existent in reproductively mixed populations in their natural habitats, an observation explained by activity of prezygotic crossing barriers (Dobeš & al., 2013b). Our results, thus, are in line with the existence of a reproductive, although incomplete, barrier among tetraploids and penta- to octoploids in natural populations. Interestingly, all except one individual which showed 10%–90% of assignation to the sexual cluster in the STRUCTURE analysis were sampled from populations in which tetra- and penta- to octoploids co-occurred. This confirms the importance of sympatry in a strict sense in shaping the relationships of sexuals with apomicts and supports the idea of possible progressive replacement of sexuals by apomicts via heteroploid crosses. Such process would be delayed by the evolution of reproductive barriers between the two groups (Mogie, 1992; Joshi & Moody, 1995, 1998). In addition, selection against non-functional apomicts arising from the meiotic and sexual recombination of their genomes resulting from hybridisation, may explain the low level of admixture by sexuals, and in turn maintenance of the slight but significant differentiation among reproductive modes.

Although the ongoing heteroploid origin of apomicts involving sexual tetraploids has not been highly relevant in the last generations, the large cpDNA haplotype sharing of tetraploids with penta- to octoploids suggests that recombination events have occurred in historic times extensively and possibly constantly. Spatial and reproductive contact among sexuals and apomicts might have been even more frequent in different ecological conditions, compared to the recent situation (Hülber & al., 2013). The effect of sympatry might have been amplified in glacial refugia, during the postglacial expansion of dry grasslands, fragmentations after postglacial forestation and grassland expansion due to human land-use. Certain situations might have favoured the high-ploidy apomicts as they are expected to have an advantage in environmental conditions at the margin of the ecological range of a certain taxon and in a colonisation phase, due to higher and faster

seed production, and to independence from pollinators and mating partners (Hörandl, 2006). It is therefore likely that the apomictic trait has been positively selected in such environmental conditions (Mogie, 1992) and that the relative frequencies of sexuals and apomicts might have been different from nowadays, influencing the rates of heteroploid reproductive success. The cpDNA variation would thus be the result of past admixture, which is no more visible at the nuclear level, because of mutations and the confounding effect of sexuality within the apomictic group.

Apomictic genotypes did not originate by recurrent interspecific hybridisation

Among the studied species, we found substantial rates of hybridisation of *P. puberula* only with *P. crantzii*. With all other related and sympatric taxa, no sign of hybridisation was found. Only a tetraploid population of *P. puberula* (population 95) showed introgression from a taxon among or related to *P. argentea*, *P. braunenana*, *P. frigida* and *P. grandiflora.* The genetic proximity of the largely allopatric *P. incana* to *P. puberula* reflects their very close relatedness. The two species both belong to the *P. verna* agg., a complex of cryptic species (Wolf, 1908; Ehrendorfer, 1973; Kurtto & al., 2004), therefore our AFLP markers probably did not have the resolution to discriminate them.

Potentilla crantzii hybridised frequently (in 14 populations) with *P. puberula*. However, the *P. crantzii* × *P. puberula* hybrids were genetically clearly distinct (probably mainly F1 hybrids) and we found no relevant introgression from *P. crantzii* in *P. puberula*. Moreover, neither *P. crantzii* nor the other sympatric species presented the three loci possibly associated with apomixis (170 VIC, 219 FAM, 286 FAM), with the exception of two genotypes of *P. crantzii* (from population 224) sampled together with *P. puberula* (population 69).

Based on this study, we can exclude recent recurrent allopolyploid origins of apomictic *P. puberula*, which we confirm as a genetically variable but coherent species (Wolf, 1908; Ehrendorfer, 1973; Paule & al., 2012). Nevertheless, we cannot fully exclude that apomixis was originally historically introduced into *P. puberula* after introgression and gene capture – or alternatively as direct effect of allopolyploidisation (Carman, 1997) – from another now extinct or not sampled species from outside the study area, as the case in other systems (e.g. De Wet & Harlan, 1970; Roche & al., 1999; Sharbel & Mitchell- Olds, 2001).

Final remarks

The sexual-apomictic species *Potentilla puberula* offers opportunities to study the relationships and interactions of reproductively differentiated cytotypes. Sympatry and reproductively compatible cytotypes may have drastic effects on the maintenance of obligate sexual populations and culminate in their displacement. However, other factors, such as ecological differentiation or the evolution of stronger reproductive barriers might allow for coexistence of the two groups. Phylogeographical investigations on the study area, together with estimation of the age of the apomicts might help to investigate whether sexual displacement partially already occurred in some populations.

Additionally, the discovery of three parthenogenetic-specific AFLP fragments might lay the foundations for genetic mapping studies on apomixis, which are still underrepresented in the Rosaceae family (Ozias-Akins & Van Dijk, 2007). Such studies, together with careful investigation of inheritance of apomixis and its components, are necessary to shed light on the genetics of apomixis in this species and, consequently, to understand the roles of recombination and outcrossing in the genesis of apomicts.

Author contributions

FDN, CD and AT developed the scientific framework of the study and wrote the article. FDN, HAM and TM conducted the flow cytometric analyses. FDN, DM and TG performed the molecular study. FDN took care of the data analyses. — ORCID: FDN, https://orcid.org/0000-0001-7548- 3420; CD, https://orcid.org/0000-0002-8631-219X; HAM, https://orcid.org/0000-0002-9726-9019; AT: https://orcid.org/0000-0002-5081-1675

Acknowledgements

We would like to thank Franz Hadacek, Elvira Hörandl, Stefan Hörandl, University of Göttingen, Julian Ananda Haider, Karl Hülber and Simon Stifter, University of Vienna, for providing plant material. We are grateful to Ernst Vitek and the volunteers of the Herbarium of the Natural History Museum Vienna (W) for taking care of the herbarium vouchers and to Matthias Affenzeller, University of Salzburg, for his kind help with the laboratory work and analyses. The STRUCTURE analyses were performed by the computer cluster Doppler-Cluster, IT Services, University of Salzburg. Carlos Trujillo-Moya, Federal Research Centre for Forest, Juraj Paule, Senckenberg Research Institute, Elvira Hörandl and two anonymous reviewers provided useful comments to improve the

manuscript. This study was supported by the Austrian Science Fund (project P27688 to Christoph Dobeš).

REFERENCES

Aldrich, J., Cherney, B.W. & Merlin, E. 1988. The role of insertions/deletions in the evolution of the intergenic region between *psbA* and *trnH* in the chloroplast genome. *Curr. Genet.* 14: 137–146. https://doi.org/10.1007/BF00569337

Alonso-Marcos, H., Hülber, K., Myllynen, T., Pérez Rodríguez, P., & Dobeš, C. 2018. Pollen precedence in sexual *Potentilla puberula* and its role as a protective reproductive barrier against apomictic cytotypes. *Taxon* 67: x-xx. https://doi.org/10.12705/676

Asker, S. 1970. Apomixis and sexuality in the *Potentilla argentea* complex II. Crosses within the complex. *Hereditas* 66: 189–204. https://doi.org/10.1111/j.1601-5223.1970.tb02345.x

Asker, S.E. 1980. Gametophytic apomixis: Elements and genetic regulation. *Hereditas* 93: 277–293. https://doi.org/10.1111/j.1601-5223.1980.tb01367.x

Asker, S.E. & Jerling, L. 1992. *Apomixis in plants*. Boca Raton: CRC Press.

Bayer, R.J. 1997. *Antennaria rosea* (Asteraceae): A model group for the study of the evolution of polyploid agamic complexes. *Opera Bot.* 132: 53–65.

Bendiksby, M., Tribsch, A., Borgen, L., Trávníček, P. & Brysting, A.K. 2011. Allopolyploid origins of the *Galeopsis* tetraploids: Revisiting Müntzing's classical textbook example using molecular tools. *New Phytol.* 191: 1150–1167. https://doi.org/10.1111/j.1469-8137.2011.03753.x

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Wheeler, D.L. 2005. GenBank. *Nucl. Acids Res.* 33: D34–D38. https://doi.org/10.1093/nar/gki063

Bicknell, R.A., Lambie, S.C. & Butler, R.C. 2003. Quantification of progeny classes in two facultatively apomictic accessions of *Hieracium*. *Hereditas* 138: 11–20. https://doi.org/10.1034/j.1601-5223.2003.01624.x

Bierzychudek, P. 1985. Patterns in plant parthenogenesis. *Experientia* 41: 1255–1264. https://doi.org/10.1007/BF01952068

Biomatters 2014. Geneious, version 8. Auckland: Biomatters. https:// www.geneious.com/

Böcher, T.W. 1951. Cytological and embryological studies in the amphiapomictic *Arabis holboellii* complex. *Biol. Skr.* 6: 1–59.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C. & Taberlet, P. 2004. How to track and assess genotyping errors in population genetics studies. *Molec. Ecol.* 13: 3261–3273. https://doi.org/10.1111/j.1365-294X.2004.02346.x

Campbell, C.S. & Wright, W.A. 1996. Apomixis, hybridization, and taxonomic complexity in eastern North American *Amelanchier* (Rosaceae). *Folia Geobot. Phytotax.* 31: 345–354. https://doi.org/10.1007/BF02815379

Carman, J.G. 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol. J. Linn. Soc.* 61: 51–94. https://doi.org/10.1111/j.1095-8312.1997.tb01778.x

Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6: 836–846. https://doi.org/10.1038/nrg1711

Cosendai, A.-C. & Hörandl, E. 2010. Cytotype stability, facultative apomixis and geographical parthenogenesis in *Ranunculus kuepferi* (Ranunculaceae). *Ann. Bot. (Oxford)* 105: 457–470. https://doi.org/10.1093/aob/mcp304

Cosendai, A.-C., Rodewald, J. & Hörandl, E. 2011. Origin and distribution of autopolyploids via apomixis in the Alpine species *Ranunculus kuepferi* (Ranunculaceae). *Taxon* 60: 355–364.

Czapik, R. 1961. Badania embriologiczne nad rodzajem *Potentilla* L. I. *P. crantzii* = Embryological studies in the genus *Potentilla* L. I. *P. crantzii*. *Acta Biol. Cracov., Ser. Bot.* 4: 97–119.

Czapik, R. 1962. Badania embriologiczne nad rodzajem *Potentilla* L. III. Mieszance miedzy *P. crantzii* i *P. arenaria* = Embryological studies in the genus *Potentilla* L. III. Hybrids between *P. crantzii* and *Potentilla arenaria*. *Acta Biol. Cracov., Ser. Bot.* 5: 43–61.

Daurelio, L.D., Espinoza, F., Quarin, C.L. & Pessino, S.C. 2004. Genetic diversity in sexual diploid and apomictic tetraploid populations of *Paspalum notatum* situated in sympatry or allopatry. *Pl. Syst. Evol.* 244: 189–199. https://doi.org/10.1007/s00606-003-0070-6

De Wet, J.M.J. & Harlan, J.R. 1970. Apomixis, polyploidy, and speciation in *Dichanthium*. *Evolution (Lancaster)* 24: 270–277. https://doi.org/10.1111/j.1558-5646.1970.tb01760.x

Dickinson, T.A., Lo, E. & Talent, N. 2007. Polyploidy, reproductive biology, and Rosaceae: Understanding evolution and making classifications. *Pl. Syst. Evol.* 266: 59–78. https://doi.org/10.1007/s00606-007-0541-2

Dobeš, C. 1999. Die Karyogeographie des *Potentilla verna* agg. (Rosaceae) in Österreich – Mit ergänzenden Angaben aus Slowenien, Kroatien, der Slowakei und Tschechien. *Ann. Naturhist. Mus. Wien B* 101: 599–629.

Dobeš, C. & Paule, J. 2010. A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): Implications for its geographic origin, phylogeography and generic circumscription. *Molec. Phylogen. Evol.* 56: 156–175. https://doi.org/10.1016/j.ympev.2010.03.005

Dobeš, C., Koch, M. & Sharbel, T.F. 2006. Embryology, karyology, and modes of reproduction in the North American genus *Boechera* (Brassicaceae): A compilation of seven decades of research. *Ann. Missouri Bot. Gard.* 93: 517–534. https://doi.org/10.3417/0026-6493(2007)93[517:EKAMOR]2.0.CO;2

Dobeš, C., Lückl, A., Hülber, K. & Paule, J. 2013a. Prospects and limits of the flow cytometric seed screen – Insights from *Potentilla* sensu lato (Potentilleae, Rosaceae). *New Phytol.* 198: 605–616. https://doi.org/10.1111/nph.12149

Dobeš, C., Milosevic, A., Prohaska, D., Scheffknecht, S., Sharbel, T.F. & Hülber, K. 2013b. Reproductive differentiation into sexual and apomictic polyploid cytotypes in *Potentilla puberula* (Potentilleae, Rosaceae). *Ann. Bot. (Oxford)* 112: 1159–1168. https://doi.org/10.1093/aob/mct167

Dobeš, C., Lückl, A., Kausche, L., Scheffknecht, S., Prohaska, D., Sykora, C. & Paule, J. 2015. Parallel origins of apomixis in two diverged evolutionary lineages in tribe Potentilleae (Rosaceae). *Bot. J. Linn. Soc.* 177: 214–229. https://doi.org/10.1111/boj.12239

Dobeš, C., Scheffknecht, S., Fenko, Y., Prohaska, D., Sykora, C. & Hülber, K. 2018. Asymmetric reproductive interference: The consequences of pollination on reproductive success in sexualapomictic populations of *Potentilla puberula* (Rosaceae). *Ecol. Evol.* 8: 365–381. https://doi.org/10.1002/ece3.3684

Doležel, J., Greilhuber, J. & Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature, Protoc.* 2: 2233–2244. https://doi.org/10.1038/nprot.2007.310

Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. Bot. Soc. Amer.* 19: 11–5.

Ehrendorfer, F. 1970. Mediterran-mitteleuropäische Florenbeziehungen im Lichte cytotaxonomischer Befunde. *Feddes Repert.* 81: 3–32. https://doi.org/10.1002/fedr.19700810103

Ehrendorfer, F. (ed.) 1973. *Liste der Gefäßpflanzen Mitteleuropas*, ed. 2. Stuttgart: Fischer.

Ehrich, D. 2006. AFLPdat: A collection of R functions for convenient handling of AFLP data. *Molec. Ecol. Notes* 6: 603–604. https://doi.org/10.1111/j.1471-8286.2006.01380.x

Falush, D., Stephens, M. & Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molec. Ecol. Notes* 7: 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Francis, R.M. 2017. pophelper: An R package and web app to analyse and visualize population structure. *Molec. Ecol. Resources* 17: 27–32. https://doi.org/10.1111/1755-0998.12509

Greilhuber, J. & Ebert, I. 1994. Genome size variation in *Pisum sativum*. *Genome* 37: 646–655. https://doi.org/10.1139/g94-092

Grimanelli, D., Leblanc, O., Perotti, E. & Grossniklaus, U. 2001. Developmental genetics of gametophytic apomixis. *Trends Genet.* 17: 597–604. https://doi.org/10.1016/S0168-9525(01)02454-4

Grossniklaus, U., Nogler, G.A. & Van Dijk, P.J. 2001. How to avoid sex: The genetic control of gametophytic apomixis. *Pl. Cell* 13: 1491–1498. https://doi.org/10.1105/tpc.13.7.1491

Håkansson, A. 1946. Untersuchungen über die Embryologie einiger *Potentilla*-Formen. *Lunds Univ. Årsskrift., 2*, 42: 1–70.

Hand, M.L. & Koltunow, A.M.G. 2014. The genetic control of apomixis: Asexual seed formation. *Genetics* 197: 441–450. https://doi.org/10.1534/genetics.114.163105

Hojsgaard, D., Schegg, E., Valls, J.F.M., Martínez, E.J. & Quarin, C.L. 2008. Sexuality, apomixis, ploidy levels, and genomic relationships among four *Paspalum* species of the subgenus *Anachyris* (Poaceae). *Flora* 203: 535–547. https://doi.org/10.1016/j.flora.2007.09.005

Hojsgaard, D., Greilhuber, J., Pellino, M., Paun, O., Sharbel, T.F. & Hörandl, E. 2014. Emergence of apospory and bypass of meiosis via apomixis after sexual hybridisation and polyploidisation. *New Phytol.* 204: 1000–1012. https://doi.org/10.1111/nph.12954

Hörandl, E. 2006. The complex causality of geographical parthenogenesis. *New Phytol.* 171: 525–538. https://doi.org/10.1111 /j.1469-8137.2006.01769.x

Hörandl, E. & Gutermann, W. 1999. Der *Ranunculus auricomus*- Komplex in Österreich. 2. Die *R. cassubicus*-, *R. monophyllus*- und *R. fallax*-Sammelgruppe. *Bot. Jahrb. Syst.* 120: 545–598.

Hörandl, E. & Hojsgaard, D.H. 2012. The evolution of apomixis in angiosperms: A reappraisal. *Pl. Biosyst.* 146: 681–693. https://doi.org/10.1080/11263504.2012.756073

Hörandl, E. & Paun, O. 2007. Patterns and sources of genetic diversity in apomictic plants: Implications for evolutionary potentials. Pp. 169–194 in: Hörandl, E., Van Dijk, P.J., Grossniklaus, U. & Sharbel, T.F. (eds.), *Apomixis: Evolution, mechanisms and perspectives*. Ruggell: Gantner.

Hörandl, E., Jakubowsky, G. & Dobeš, C. 2001. Isozyme and morphological diversity within apomictic and sexual taxa of the *Ranunculus auricomus* complex. *Pl. Syst. Evol.* 226: 165–185. https://doi.org/10.1007/s006060170064

Hülber, K., Scheffknecht, S. & Dobeš, C. 2013. Partitioning the factors explaining the eco-geography in the amphi-apomictic species *Potentilla puberula* (Rosaceae). P. 98 in: Kroh, A., Berning, B., Haring, E., Harzhauser, M., Sattmann, H., Walochnik, J., Zimmermann, D. & Zuschin, M. (eds.), *BioSyst.EU 2013 Global systematics!*, 18–22 February 2013, Abstract volume. Vienna: NOBIS Austria.

Joshi, A. & Moody, M.E. 1995. Male gamete output of asexuals and the dynamics of populations polymorphic for reproductive mode. *J. Theor. Biol.* 174: 189–197. https://doi.org/10.1006/JTBI.1995.0091

Joshi, A. & Moody, M.E. 1998. The cost of sex revisited: Effects of male gamete output of hermaphrodites that are asexual in their female capacity. *J. Theor. Biol.* 195: 533–542. https://doi.org/10.1006/JTBI.1998.0811

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. https://doi.org/10.1093/ bioinformatics/bts199

Kirchheimer, B., Wessely, J., Gattringer, A., Hülber, K., Moser, D., Schinkel, C.C.F., Appelhans, M., Klatt, S., Caccianiga, M., Dellinger, A., Guisan, A., Kuttner, M., Lenoir, J., Maiorano, L., Nieto-Lugilde, D., Plutzar, C., Svenning,

J.-C., Willner, W., Hörandl, E. & Dullinger, S. 2018. Reconstructing geographical parthenogenesis: Effects of niche differentiation and reproductive mode on Holocene range expansion of an alpine plant. *Ecol. Letters* 21: 392–401. https://doi.org/10.1111/ele.12908

Koltunow, A.M. & Grossniklaus, U. 2003. Apomixis: A developmental perspective. *Annual Rev. Pl. Biol.* 54: 547–574. https://doi.org/10.1146/annurev.arplant.54.110901.160842

Kurtto, A., Lampinen, R. & Junikka, L. 2004. *Atlas Florae Europaeae: Distribution of vascular plants in Europe*, vol. 13, *Rosaceae (Spiraea to Fragaria, excl. Rubus).* Helsinki: The Commitee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo.

Levin, D.A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43. https://doi.org/10.2307/1218997

Lo, E.Y.Y., Stefanović, S. & Dickinson, T.A. 2010. Reconstructing reticulation history in a phylogenetic framework and the potential of allopatric speciation driven by polyploidy in an agamic complex in *Crataegus* (Rosaceae). *Evolution (Lancaster)* 64: 3593–3608. https://doi.org/10.1111/j.1558-5646.2010.01063.x

Matzk, F., Meister, A. & Schubert, I. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant J.* 21: 97–108. https://doi.org/10.1046/j.1365-313X.2000.00647.x

Matzk, F., Prodanovic, S., Bäumlein, H. & Schubert, I. 2005. The inheritance of apomixis in *Poa pratensis* confirms a five locus model with differences in gene expressivity and penetrance. *Pl. Cell* 17: 13–24. https://doi.org/10.1105/tpc.5.10.1425

Meirmans, P.G., Liu, S. & Van Tienderen, P.H. 2018. The analysis of polyploid genetic data. *J. Heredity* 109: 283–296. https://doi.org/10.1093/jhered/esy006

Menken, S.B.J., Smit, E. & Den Nijs, H.(J.)C.M. 1995. Genetical population structure in plants: Gene flow between diploid sexual and triploid asexual dandelions

(*Taraxacum* section *Ruderalia*). *Evolution (Lancaster)* 49: 1108–1118. https://doi.org/10.2307/2410435

Meusel, H., Jäger, E. & Weinert, E. 1965. *Vergleichende Chorologie der zentraleuropäischen Flora*, Text. Jena: Fischer.

Mogie, M. 1992. *The evolution of asexual reproduction in plants.* London: Chapman and Hall.

Mogie, M., Britton, N.F. & Stewart-Cox, J.A. 2007. Asexuality, polyploidy and the male function. Pp. 195–214 in: Hörandl, E., Grossniklaus, U., Van Dijk, P.J. & Sharbel, T.F. (eds.), *Apomixis: Evolution, mechanisms and perspectives*. Ruggell: Gantner.

Nogler, G.A. 1984. Genetics of apospory in apomictic *Ranunculus auricomus*. V, Conclusion. *Bot. Helv.* 94: 411–422. https://doi.org/10.5169/seals-65890

Noyes, R.D. & Rieseberg, L.H. 2000. Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics* 155: 379–390.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. 2018. vegan: Community Ecology Package. https://cran.r-project.org/ package=vegan

Otto, F. 1990. DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. *Meth. Cell Biol.* 33: 105–110. https://doi.org/10.1016/S0091-679X(08)60516-6

Oxelman, B., Lidén, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410. https://doi.org/10.1007/BF00987959

Ozias-Akins, P. & Van Dijk, P.J. 2007. Mendelian genetics of apomixis in plants. *Annual Rev. Genet.* 41: 509–537. https://doi.org/10.1146/annurev.genet.40.110405.090511

Paradis, E., Claude, J. & Strimmer, K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20: 289–290. https://doi.org/10.1093/bioinformatics/btg412

Paule, J., Sharbel, T.F. & Dobeš, C. 2011. Apomictic and sexual lineages of the *Potentilla argentea* L. group (Rosaceae): Cytotype and molecular genetic differentiation. *Taxon* 60: 721–732.

Paule, J., Scherbantin, A. & Dobeš, C. 2012. Implications of hybridisation and cytotypic differentiation in speciation assessed by AFLP and plastid haplotypes: A case study of *Potentilla alpicola* La Soie. *B. M. C. Evol. Biol.* 12: 132. https://doi.org/10.1186/1471-2148-12-132

Paun, O., Greilhuber, J., Temsch, E.M. & Hörandl, E. 2006. Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). *Molec. Ecol.* 15: 897–910. https://doi.org/10.1111/j.1365-294X.2006.02800.x

Polatschek, A. 2000. *Flora von Nordtirol, Osttirol und Vorarlberg*, Band 3, *Samenpflanzen: Fabaceae bis Rosaceae.* Innsbruck: Tiroler Landesmuseum Ferdinandeum.

Pompanon, F., Bonin, A., Bellemain, E. & Taberlet, P. 2005. Genotyping errors: Causes, consequences and solutions. *Nat. Rev. Genet.* 6: 847–846. https://doi.org/10.1038/nrg1707

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Quantum Analysis 2014. FloMax: Software for multiparameter data analysis, instrument control, and data acquisition for flow cytometry for Windows. Münster: Qunatum Analysis. http://quantumanalysis.com/

R Development Core Team 2018. R: A language and environment for statistical computing. Vienna. http://www.r-project.org

Roche, D., Cong, P., Chen, Z., Hanna, W.W., Gustine, D.L., Sherwood, R.T. & Ozias-Akins, P. 1999. An apospory-specific genomic region is conserved between Buffelgrass (*Cenchrus ciliaris* L.) and *Pennisetum squamulatum* Fresen. *Plant J.* 19: 203–208. https://doi.org/10.1046/j.1365-313X.1999.00514.x

Rotreklová, O., Krahulcová, A., Vaňková, D., Peckert, T. & Mráz, P. 2002. Chromosome numbers and breeding systems in some species of *Hieracium* subgen. *Pilosella* from Central Europe. *Preslia* 74: 27–44.

Rutishauser, A. 1948. Pseudogamie und Polymorphie in der Gattung *Potentilla*. *Arch. Julius Klaus-Stiftung. Vererbungsf.* 23: 267–424. https://doi.org/10.1007/978-3-7091-8222-2

Rutishauser, A. 1969. *Embryologie und Fortpflanzungsbiologie der Angiospermen: Eine Einführung*. Wien & New York: Springer.

Savidan, Y. 1982. Nature et hérédité de l'apomixie chez *Panicum maximum* Jacq. *Trav. Doc. O. R. S. T. O. M.* 153: 1–159.

Savidan, Y., Carman, J.G. & Dresselhaus, T. (eds.) 2001. *The flowering of apomixis: From mechanisms to genetic engineering.* Mexico, D.F.: CIMMYT, IRD, European Commission DG VI (FAIR). https://repository.cimmyt.org/handle/10883/588

Schinkel, C.C.F., Kirchheimer, B., Dellinger, A.S., Klatt, S., Winkler, M., Dullinger, S. & Hörandl, E. 2016. Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. *AoB Plants* 8: plw064. https://doi.org/10.1093/aobpla/plw064

Schinkel, C.C.F., Kirchheimer, B., Dullinger, S., Geelen, D., De Storme, N. & Hörandl, E. 2017. Pathways to polyploidy: Indications of a female triploid bridge in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Pl. Syst. Evol.* 303: 1093–1108. https://doi.org/10.1007/s00606-017-1435-6

Sharbel, T.F. & Mitchell-Olds, T. 2001. Recurrent polyploid origins and chloroplast phylogeography in the *Arabis holboellii* complex (Brassicaceae). *Heredity* 87: 59–68. https://doi.org/10.1046/j.1365-2540.2001.00908.x

Sharbel, T.F., Voigt, M.-L., Corral, J.M., Galla, G., Kumlehn, J., Klukas, C., Schreiber, F., Vogel, H. & Rotter, B. 2010. Apomictic and sexual ovules of *Boechera* display heterochronic global gene expression patterns. *Pl. Cell* 22: 655–671. https://doi.org/10.1105/tpc.109.072223

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142–166. https://doi.org/10.3732/ajb.92.1.142

Siena, L.A., Sartor, M.E., Espinoza, F., Quarin, C.L. & Ortiz, J.P.A. 2008. Genetic and embryological evidences of apomixis at the diploid level in *Paspalum rufum* support recurrent autopolyploidization in the species. *Sexual Pl. Reprod.* 21: 205–215.https://doi.org/10.1007/s00497-008-0080-1

Smith, G.L. 1963a. Studies in *Potentilla* L. I. Embryological investigations into the mechanism of agamospermy in British *P. tabernaemontani* Aschers. *New Phytol.* 62: 264–282. https://doi.org/10.1111/j.1469-8137.1963.tb06334.x

Smith, G.L. 1963b. Studies in *Potentilla* L. II. Cytological aspects of apomixis in *P. crantzii* (Cr.) Beck ex Fritsch. *New Phytol.* 62: 283–300. https://doi.org/10.1111/j.1469-8137.1963.tb06335.x

Soják, J. 2010. Origin of *Potentilla crantzii*, *P. verna* and *P. puberula* (Rosaceae) with a note on the nomenclature of *P. pusilla*. *Feddes Repert.* 121: 112–116. https://doi.org/10.1002/fedr.201000010

Stöck, M., Ustinova, J., Lamatsch, D.K., Schartl, M., Perrin, N. & Moritz, C. 2010. A vertebrate reproductive system involving three ploidy levels: Hybrid origin of triploids in a contact zone of diploid and tetraploid paleartic green toads (*Bufo viridis* subgroup). *Evolution (Lancaster).* 64: 944–959. https://doi.org/10.1111/j.1558-5646.2009.00876.x

Temsch, E.M., Greilhuber, J. & Krisai, R. 2010. Genome size in liverworts. *Preslia* 82: 63–80.

Thompson, S.L. & Whitton, J. 2006. Patterns of recurrent evolution and geographic parthenogenesis within apomictic polyploid Easter daises (*Townsendia hookeri*). *Molec. Ecol.* 15: 3389–3400. https://doi.org/10.1111/j.1365-294X.2006.03020.x

Van Dijk, P.J. 2003. Ecological and evolutionary opportunities of apomixis: Insights from *Taraxacum* and *Chondrilla*. *Philos. Trans., Ser. B.* 358: 1113–1121. https://doi.org/10.1098/rstb.2003.1302

Van Dijk, P.J. & Vijverberg, K. 2005. The significance of apomixis in the evolution of the angiosperms: A reappraisal. Pp. 101–116 in: Bakker, F., Chatrou, L., Gravendeel, B. & Pelzer, P. (eds.), *Plant species-level systematics: New perspectives on pattern & process.* Regnum Vegetabile 143. Ruggell: Gantner.

Van Dijk, P.J., Tas, I.C.Q., Falque, M. & Bakx-Schotman, T. 1999. Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83: 715–721. https://doi.org/10.1046/j.1365-2540.1999.00620.x

Villesen, P. 2007. FaBox: An online toolbox for fasta sequences. *Molec. Ecol. Notes* 7: 965–968. https://doi.org/10.1111/j.1471-8286.2007.01821.x

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. van de, Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M. & Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucl. Acids Res.* 23: 4407–4414. https://doi.org/10.1093/nar/23.21.4407

Wickham, H. 2016. *ggplot2: Elegant graphics for data analysis.* New York: Springer.

Wolf, T. 1908. *Monographie der Gattung Potentilla*. Stuttgart: E. Schweizerbartsche Verlagsbuchhandlung.

Yahara, T. 1990. Evolution of agamospermous races in *Boehmeria* and *Eupatorium*. *Pl. Spec. Biol.* 5: 183–196. https://doi.org/10.1111/j.1442-1984.1990.tb00203.x

CHAPTER 3

Pollen precedence in sexual *Potentilla puberula* and its role as a protective reproductive barrier against apomictic cytotypes

Henar Alonso-Marcos, Karl Hülber, Tuuli Myllynen,
Patricia Pérez Rodríguez and Christoph Dobeš

Status: Published in *Taxon*, November 2018

Contribution: crossing experiments, flow cytometric analysis, data analysis and visualization, writing – original draft manuscript, writing – review and editing

Pollen precedence in sexual *Potentilla puberula* and its role as a protective reproductive barrier against apomictic cytotypes

HENAR ALONSO-MARCOS[1], KARL HÜLBER[2], TUULI MYLLYNEN[2],
PATRICIA PÉREZ RODRÍGUEZ[2] AND CHRISTOPH DOBEŠ[1]

[1]Department of Forest Genetics, Austrian Research Centre for Forests, Seckendorff-Gudent-Weg 8, A-1131, Vienna, Austria

[2]Department of Conservation Biology, Vegetation Ecology and Landscape Ecology, University of Vienna, Rennweg 14, 1030 Vienna, Austria

Running title: Ecological parthenogenesis in *Potentilla puberula*

Corresponding author: Henar Alonso-Marcos henaralonsomarcos@gmail.com

Alonso-Marcos, H., Hülber, K., Myllynen, T., Pérez Rodríguez, P., & Dobeš, C. 2019. Pollen precedence in sexual *Potentilla puberula* and its role as a protective reproductive barrier against apomictic cytotypes. *Taxon* 67: 1132–1142. https://doi.org/10.12705/676.9

ABSTRACT

Cross-pollination is a major factor determining the demographic dynamics of mixed-ploidy populations. Typically, rare cytotypes are suppressed due to reduced female fertility by losing gametes in heteroploid crosses (i.e., through minority cytotype exclusion). In species with reproductive differentiation into sexual and apomictic cytotypes, sexuals might be reproductively suppressed by apomicts (or transformed due to introgression of apomixis genes). Pollen precedence potentially acts as a post-pollination pre-fertilization barrier protecting sexuals against their apomictic counterparts. We estimated the role of pollen precedence as a barrier against cross-fertilization of tetraploid sexuals by penta- and heptaploid gametophytic apomicts in *Potentilla puberula* (Rosaceae) by means of controlled crosses, and inference of the paternity through DNA ploidy estimation of embryos. Individuals from five regions spanning an elevational and biogeographic gradient were used to account for the variation in the relative frequencies of reproductive modes across the study area. We tested (1) whether the application of heteroploid pollen (sexual × apomictic) causes a reduction of seed yield compared to homoploid crosses (sexual × sexual), and (2) if so, whether pollen precedence recovers the seed yield in simultaneous applications of pollen from sexuals and apomicts (mixed-ploidy). Seed yield was significantly lower in hetero- than in homoploid crosses. We found clear evidence for precedence of homoploid pollen, despite a 13% to 15% of embryos experienced a change in ploidy due to heteroploid fertilizations. Thus, our study indicates that pollen precedence operates as a barrier against intercytotype fertilization in *P. puberula*, promoting the integrity of the sexual cytotype and their co-existence with apomictic individuals.

Keywords: apomixis; Eastern European Alps; flow cytometric seed screen; pollen precedence; reproductive isolation; Rosaceae.

INTRODUCTION

Reproductive isolation is fundamental to maintain the integrity of diverged evolutionary lineages of plants. In sympatry, sequential mechanisms operate to restrict gene flow and, hence, limit hybrid formation. Post-zygotic barriers thereby decrease the reproductive potential of populations via abortion or reduced viability of the progeny due

to genic or genomic incompatibilities (Dobzhansky, 1936; Muller, 1932). In contrast, pre-zygotic barriers act against the formation of a hybrid zygote. They include spatial and microhabitat differentiation (Hülber & al., 2009; Sonnleitner & al., 2010), temporal isolation via asynchronous flowering time (Petit & al., 1997; Lamont & al., 2003; Botes & al., 2008), differential flower architecture (Grant, 1994; Kay, 2006) leading to assortative pollinator behaviour (Husband & Schemske, 2000; Lowry & al., 2008), and pollen precedence (Howard, 1999). These reproductive barriers act in concert to establish and strengthen genetic boundaries.

Pollen precedence is defined as differential fertilization success of pollen from different species upon their simultaneous deposition on a recipient's stigma(s) (Howard, 1999). Reduced germination of pollen on the stigma and/or slower pollen tube growth of foreign compared to conspecific pollen limit or avoid hybridization despite cross-pollination (Campbell & al., 2003; Chapman & al., 2005). Conspecific pollen precedence thus has been identified as an important component of reproductive isolation in closely related species (Arnold & al., 1993; Rieseberg & al., 1995; Brock, 2009; Abadie & al., 2012; Lepais & al., 2013; Brys & al., 2014). However, competitive interactions among pollen might also occur at the intraspecific level like in systems exhibiting cytological differentiation by ploidy. Whole genome duplication events, or polyploidization, occur either due to genome duplication within a species (autopolyploid) or hybridization of two species (allopolyploid). Intraspecific pollen precedence was indeed shown for species showing differentiation by ploidy as di- and tetraploid individuals of *Chamerion angustifolium* (L.) Holub (Husband & al., 2002) and *Ranunculus adoneus* A.Gray (Baack, 2005). Polyploidization is considered as a common driver of lineage divergence, deducible as ancient events preceding angiosperm radiation, and in more recent time scales, from increased diversification rates in periods of environmental instability (Stebbins, 1947; Grant, 1981; Otto & Whitton, 2000; Comai, 2005; Soltis & al., 2007; Baker & al., 2016; Soltis & Soltis, 2016; Landis & al., 2018). However, maintenance and divergence of novel ploidy differentiated lineages depends on their reproductive isolation (Kolár & al., 2017).

In the absence of complete prezygotic reproductive isolation, difference in ploidy among mating partners typically leads to a disturbed development or even the abortion of the progeny (Lin, 1984; Koltunow & Grossniklaus, 2003). This is because heteroploid fertilizations cause loss of zygotes or later ontological stages (Coyne & Orr, 1998; Brown

& Mitchell, 2001; Hersh & al., 2016) and can constrain the maintenance or co-occurrence of cytotypes through the minority cytotype exclusion principle (Levin, 1975). A specific situation arises in sexual-apomictic systems in which ploidy differentiation is usually accompanied by a differentiated reproductive mode. Typically, diploid (or low-ploidy) cytotypes are sexual while higher-ploidy individuals are apomictic (Bayer, 1997; Hojsgaard & al., 2008; Cosendai & al., 2011). In contrast to the sexuals, the embryos of apomicts develop autonomously without fertilization (Nogler, 1984; Asker & Jerling, 1992; Hörandl & Hojsgaard, 2012), effectively counteracting loss of zygotes. This asexual embryo development in apomictic individuals may lead to asymmetric reproductive interference in mixed-cytotype populations (e.g., Hersh & al., 2016; Dobeš & al., 2018) because by expectation, only sexual individuals suffer from reduced fitness of progeny through heteroploid fertilizations. Furthermore, apomixis might introgress as a heritable trait (Ozias-Akins & Van Dijk, 2007), and reproductively transform sexuals (Joshi & Moody, 1995, 1998).

In this study, we investigated the role of pollen precedence as a barrier against fertilization of sexuals by apomictic ploidy cytotypes in *Potentilla puberula* (Rosaceae). We applied homo- and heteroploid pollen as well as a pollen mixture containing both types of pollen (mixed-ploidy pollination) onto the stigmas of emasculated flowers of sexuals in a controlled ex situ crossing experiment. Furthermore, we estimated and quantified, based on the ploidy of embryos, the paternity of seeds derived from the mixed-ploidy pollinations using flow cytometric seed screen (FCSS). By this approach, we verified the following expectations: (1) Heteroploid pollinations cause a reduction in seed yield compared to homoploid pollinations; and, if so, (2) homoploid pollen precedence is a means to avoid or reduce such losses of progeny in mixed-ploidy pollinations.

MATERIALS AND METHODS

The study species

The rosaceous species *Potentilla puberula* Krašan (= *Potentilla pusilla* Host; Soják, 2010) is a perennial herb distributed in the European Alps and the Carpathians (Kurtto & al., 2004). The yellow pentamerous insect-pollinated flowers are polyandrous and present more than one ovule – on average 28 ± 9.7 ovules (N = 251 individuals) – with each of the ovaries giving rise to a one-seeded fruitlet (for convenience, we consistently use the term seed when referring to both, fruitlets and isolated seeds as used in the FCSS). The

species exhibits a series of ploidy levels comprising tetra- (x = 7; 2n = 28), penta- (2n = 35), hexa- (2n = 42), hepta- (2n = 49), octo- (2n = 56), and occasionally nonaploids (2n = 63) (Dobeš, 1999). Tetraploid individuals are almost exclusively sexual and self-incompatible, whereas higher polyploids are self-compatible and predominantly apomictic. Apomixis is gametophytic and pseudogamous; i.e., fertilization is still required for endosperm development and successful seed formation (Dobeš & al., 2013, 2018). Reproductive modes are ecologically and biogeographically differentiated (Nardi & al., 2018): tetraploids preferentially inhabit pristine sites in areas that likely served as a glacial refugium (Tribsch & Schönswetter, 2003), while higher ploids prefer secondary habitats and higher elevations in formerly glaciated areas. However, intermixture at the population level occurs regularly (in 60 out of 269 sites tetraploids co-occurred with higher ploids in a screen within an area approximately congruent to the study area. as described in the following (Hülber & al., 2013; Nardi & al., 2018).

Study area and plant material

The study is based on plants collected during spring 2016 from five regions spanning an elevational and biogeographic gradient, which largely covers the ecological range realized by the species. Within each region, sexual and apomictic individuals grow at similar frequencies. Three of the five studied regions were inhabited by tetra- and pentaploid individuals: Zabernig (47°00′16.812″ N/ 12°31′09.119″E, at 1340 m a.s.l.) and Obersteiner (47°01′04.33″ N/ 12°24′31.39″ E, at 1460 m a.s.l.), both located in East Tyrol, Austria, and Bodenalm (46°55′23.124″ N/11°41′53.195″ E, at 1700 m a.s.l.) in South Tyrol, Italy. Each of the two remaining regions of the study comprise in turn two similar and spatially close sites: with tetraploids in the first site, and both penta- and heptaploids in the second site. This way, Raas with tetraploids (46°44′49.49″ N/11°39′12.60″ E, at 750 m a.s.l.) was combined with penta- and heptaploids from Fortezza (46°46′35.58″ N/ 11°37′47.46″ E, at 730 m a.s.l.), in South Tyrol, Italy, whereas Ossenigo with tetraploids (45°40′27.95″ N/10°54′34.16″ E, at 250 m a.s.l.) was combined with Scaiola penta- and heptaploid individuals (45°32′03.26″ N/10°21′50.43″ E, at 200 m a.s.l.), Trentino/Lombardia, Italy.

Homoploid, heteroploid and mixed-ploidy crossing experiment

A controlled ex situ crossing experiment was carried out from April to June 2017 in the experimental garden of the Austrian Research Centre for Forests

(https://bfw.ac.at/), located in the grounds of the Schönbrunn Palace Park in Vienna (48°10′36″ N/16°18′34″ E at 259 m a.s.l.). Plants were grown from cuttings collected in the field in 2016, in 14 cm pots. Out of the plants, we randomly selected from each of the five regions 12 individuals as pollen recipients and 190 plants as pollen donors. More specifically, sexual and apomictic pollen donors were used per each region, respectively, as follows: Zabernig 8/11; Obersteiner 16/13; Ossenigo/Scaiola 14/43; Raas/Fortezza 16/39 and Bodenalm 18/12, in the homoploid and the heteroploid treatment, respectively. Plants were planted in plots spaced in minimum 10 meters from each other. Prior to pollination, flowers were carefully emasculated to prevent self-fertilization and bagged using bridal veil, as this material has the least effect on the microclimate of the bagged flowers (Wyatt & al., 1992). At stigma maturity, flowers were pollinated by gently rubbing the anthers of pollen donors over the pollen recipients' stigmas.

Four treatments were applied: (i) Homoploid pollinations among sexuals: each of three flowers per individual was pollinated with pollen from one randomly selected donor of the same (tetraploid) ploidy; (ii) Heteroploid pollinations of sexuals by apomicts: three flowers (but six at sampling sites harboring not only pentaploid but also heptaploids) were treated with pollen of a heteroploid donor; and (iii) Mixed-ploidy pollinations: six flowers per recipient (but twelve at sampling sites including penta- and heptaploids) were pollinated with both, pollen from one sexual and one apomictic donor. In the latter case, we applied the pollen by rubbing the anthers of both donors over the recipients' stigmas. Application of about equal amounts of pollen from both pollen donors was secured by visual inspection of stigmas using a binocular. To avoid bias due to the order of pollination, we alternated sexual versus apomictic pollen donors when starting a pollination (Electr. Suppl.: Table S1). In addition, (iv) two flowers per pollen recipient served as open control. At maturity, reproductive success was determined as the number of obtained viable seeds (i.e., filled with a fleshy embryo). We used seed yield as a measure of reproductive success, since no relationship between number of ovules and number of mature seeds was found in previous crossing experiment in P. puberula (Dobeš & al., 2018). The estimation of the number of ovules (required for defining the seed set) is destructive in our model system (Dobeš & al., 2018). Thus, by using seed yield, we maximized the number of flowers available for pollination. Moreover, effects of the variation in the number of ovules among individuals were removed by performing all treatments on every pollen recipient. Furthermore, seed yield has been used in other crossing experiments (e.g., Behrend & al., 2015; Sutherland & Galloway, 2017),

including *Potentilla* (Trunschke & Stöcklin, 2017). Data derived from crosses with penta- and heptaploid pollen donors were pooled for each treatment, since penta- and heptaploid individuals did not show differences in their behaviour among treatments and considered in the following together as apomicts (Electr. Suppl.: Table S2).

To compare the number of seeds (i.e., seed yield) among treatments, Poisson Generalized Linear Mixed Models (GLMMs), as implemented in the package glmmTMB v.0.2.0 (Magnusson & al., 2017) of the R statistical environment (R Development Core Team 2017), were applied. These models comprise a conditional and a zero-inflated model component (Brooks & al., 2017). Treatment was used as the fixed-effects predictor in the conditional model component, and random intercepts for each maternal and paternal individual, both nested in population, were included to account for potential differences in pollination success among individual pollen recipients and pollen donors. Because many flowers produced no viable seed (in all treatments, including the open control), we allowed for random intercepts in the zero-inflation component. We compared (i) the homo- against the heteroploid treatment to test for confounding effects of interploidy fertilization on seed yield, as well as (ii) the pooled data of these two against the mixed ploidy treatment by using Helmert contrasts. This comparison was done in order to test for the existence of pollen precedence, i.e., if homo- and heteroploid pollen have an unequal probability to fertilize an ovule, resulting in a seed yield not intermediate to the mixed-ploidy treatment. (iii) We compared seed yield in the mixed-ploidy treatment (as the baseline) to that in the homoploid one, as well as to the heteroploid treatment in order to test whether, at least, partial recovery due to the pollen precedence from crosses with heteroploid pollen occurs. These comparisons among treatments were also applied separately to the data of each region to examine whether the detected pattern is idiosyncratic or generic.

To test whether pollen quality affected seed formation, the number of vital pollen grains (i.e., in the sense of stainable in a physiological vitality test) per flower was estimated for 157 pollen donors. Two anthers per individual were stained with a solution of Malachite green, acid fuchsin and Orange G for at least 12 hours (Peterson & al., 2010, vitality stain) and diluted in 50 µl of distilled water. A homogenized 2 µl aliquot was analysed using the light microscope Axioskop 1 (Zeiss, Jena, Germany) and bright-field illumination at 400-fold magnification. Stained and regular-shaped (i.e., physiologically and morphologically intact) grains were considered as viable and counted (Electr. Suppl.:

Fig. S1). The number of obtained pollen grains were compared among treatments using zero-inflated GLMMs as described above, but assuming pollen quality to be a Gaussian-distributed response variable.

Flow cytometry seed screen

The ploidy of functional (successful in heteroploid crosses) male gametes of penta- and heptaploids was 1.6-times (ca. corresponding to triploidy) and 1.9-times (ca. tetraploid), respectively, higher than those of sexual tetraploids (Dobeš & al., 2018). Thus, embryos derived from heteroploid crosses (fertilization with pollen from penta- and heptaploid donor plants) are expected to have a higher ploidy than those obtained in homoploid crosses (tetraploid donors). To estimate the proportions of progeny sired by sexual (i.e., homoploid) and apomictic (i.e., heteroploid) pollen donors, we compared the embryo-to-standard fluorescence ratio of each seed derived from the mixed-ploidy crosses to the distribution of such ratios obtained from the following reference samples: Thirty-five seeds (sampled from 13 individuals) drawn from crosses with tetraploid pollen donors were used as a homoploid reference sample. As heteroploid reference samples, 37 seeds (from 17 individuals) and 30 seeds (from 7 individuals) obtained in crosses with pentaploid and heptaploid pollen donors were used, respectively. Seeds of the mixed-ploidy crosses with embryo-to-standard fluorescence ratios below the lower limit of the 95% confidence interval (i.e., the 2.5% confidence limit computed as arithmetic mean − 1.96 × standard deviation) of the distribution of embryo-to-standard fluorescence ratios obtained from (each of) the heteroploid reference sample, were regarded as emerged from a homoploid fertilization. Accordingly, embryo-to-standard fluorescence ratios above the upper limit of the 95% confidence interval (i.e., the 97.5% limit) of the homoploid reference sample identifies seeds originated by heteroploid fertilisation. Calculations were done separately for pentaploid and heptaploid pollen donors. Normality distribution of the values observed for the homo- and heteroploid references was tested with the Shapiro-Wilk test.

Then, from the mixed-ploidy treatment, 1001 randomly selected seeds were analysed using FCSS (Matzk & al., 2000) from all the five studied regions. The two-steps protocol followed Doležel & al. (2007) and modifications by Dobeš & al. (2013). In brief, seeds were analysed using Otto I + II buffers (Otto, 1990), DAPI (4′-6-diamidino-2-phenylindole) as DNA-specific fluorescence stain and *Pisum sativum* '*Kleine Rheinländerin*' as internal biological standard (Greilhuber & Ebert, 1994). First, fleshy

seeds freed from the fruit wall/testa were chopped together with the internal standard in 200 µl Otto I extraction buffer using a razor blade in a petri dish, and 200 µl more were added before placing the sample on ice for 30 minutes. Subsequently, the petri dishes were ultrasonicated for 1 minute and then filtered through a 20 µm nylon mesh filter (Partec CellTrics, Partec Münster, Germany). Prior to the measurements, 1.2 ml Otto II containing 0.2 µg DAPI/ml was added to the samples. After five minutes, samples were measured with a Partec ML Ploidy Analyser. The embryo to standard fluorescence ratio was calculated from the means of the fluorescence histograms using FloMax (v.2.9, Quantum Analysis, 2014).

RESULTS

Crossing experiment

A total of 638 pollinations were carried out for the three treatments. At maturity, 1410 seeds (146 pollinations, mean ± SD 9.65 ± 8.77) were obtained for the homoploid treatment, 930 seeds (193 pollinations, 4.89 ± 5.94) for the heteroploid treatment and 2124 seeds (299 pollinations, 7.1 ± 7.26) for the mixed-ploidy treatment (Table 1).

Table 1. Reproductive success of sexual tetraploid Potentilla puberula Krašan individuals from five regions by homoploid and heteroploid pollen as well as a pollen mixture containing both, and open controls.

Region	Treatment	Flowers pollinated	Flowers failed	Seed yield	Mean ± SD	Median
Zabernig	Homoploid	14	5	37	2.64 ± 3.10	1.5
	Control	12	6	86	4.77 ± 4.62	5.0
	Heteroploid	14	6	16	1.14 ± 1.35	1.0
	Mixed-ploidy	17	13	23	1.35 ± 3.02	0.0
Obersteiner	Homoploid	37	3	401	10.83 ± 8.18	9.0
	Control	21	5	212	8.15 ± 7.52	7.0
	Heteroploid	24	4	188	7.83 ± 8.09	10.0
	Mixed-ploidy	46	2	411	8.90 ± 6.20	8.5
Ossenigo/Scaiola	Homoploid	29	10	159	5.48 ± 6.84	5.0
	Control	14	11	80	3.20 ± 4.69	1.0
	Heteroploid	58	18	225	3.80 ± 5.42	2.0
	Mixed-ploidy	68	30	299	4.40 ± 7.04	1.5
Raas/Fortezza	Homoploid	31	0	343	11.06 ± 5.65	10.0
	Control	13	9	128	5.82 ± 10.10	1.0
	Heteroploid	61	12	289	4.74 ± 4.77	4.0
	Mixed-ploidy	102	15	715	7.01 ± 6.40	5.0
Bodenalm	Homoploid	35	1	470	13.43 ± 10.76	8.5
	Control	17	8	172	6.88 ± 10.78	2.0
	Heteroploid	36	10	212	5.88 ± 10.30	5.0
	Mixed-ploidy	66	8	676	10.24 ± 8.33	9.0

"Flowers failed" represent the number of flowers not producing a single seed. "Mean" and "Median" refer to the number of seed per flower.

Pooled over the five regions, seed yield obtained in the homoploid treatment was significantly higher than in the heteroploid treatment (Fig. 1; zero-inflated GLMM: coefficient ± SE: −0.33 ± 0.04, z = −7.83, p < 0.001).

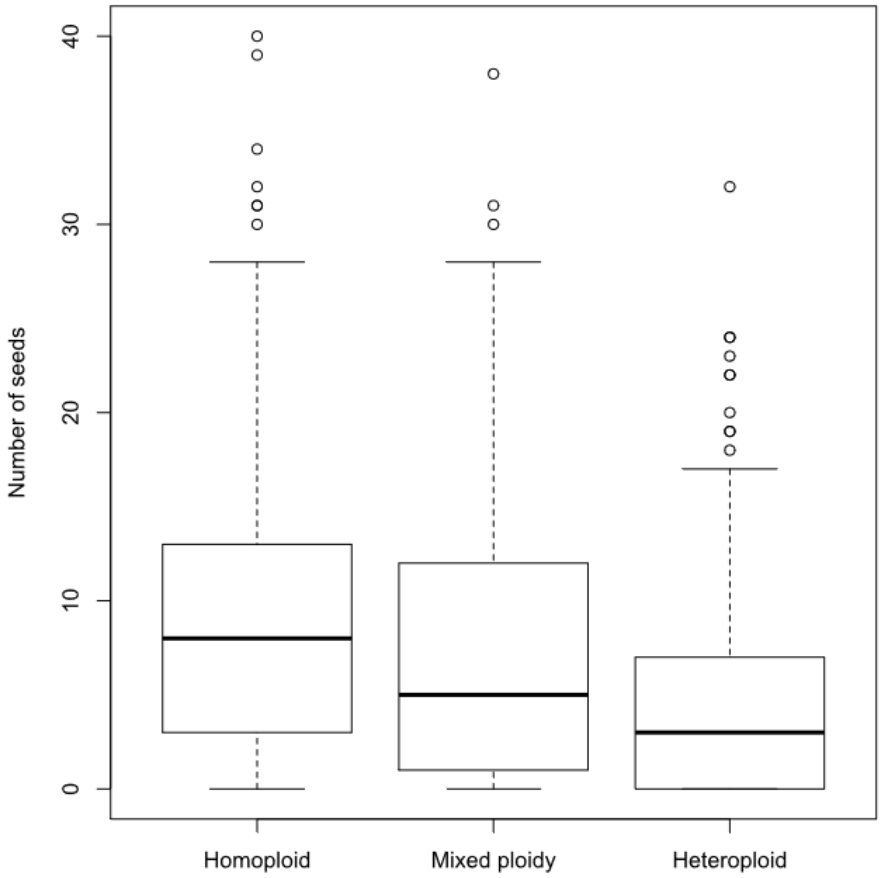


Fig. 1. Seed yield of 60 sexual tetraploid *Potentilla puberula* Krašan individuals crossed with pollen derived from sexual (i.e., homoploid) and apomictic (i.e., heteroploid) donor plants as well as with a mixture of both pollen types. Significant differences (p< 0.001) were found for the comparison among all treatments.

The seed yield derived from the mixed-ploidy treatment was significantly lower (0.31 ± 0.08, z = 3.73, p < 0.001) and higher (−0.35 ± 0.08, z = −4.34, p < 0.001) than the homoploid and heteroploid treatment, respectively, but did not significantly differ from the pooled data of these two treatments (0.01 ± 0.02, z = 0.33, p = 0.781). These patterns of seed yield (homoploid > mixed-ploidy > heteroploid but mixed-ploidy = pooled homoploid + heteroploid) were confirmed for most of the five studied regions when tested separately (Table 2).

Table 2. Fixed effects of zero-inflated Poisson Generalized Linear Mixed Models relating the seed yield of sexual tetraploid *Potentilla puberula* Krašan individuals to three pollination treatments applied in an ex situ crossing experiment.

Region	Sample size	Pollen donors	Comparison of treatments	Estimate ± SE	*z* value	*p* value
Zabernig	45/7	8/11	homo ↔ hetero	−0.38 ± 0.16	−2.340	**0.019**
			mixed ↔ (homo, hetero)	−0.26 ± 0.10	2.458	**0.014**
			mixed ↔ homo	0.89 ± 0.52	1.720	0.085
			mixed ↔ hetero	0.07 ± 0.54	0.140	0.886
Obersteiner	107/12	16/13	homo ↔ hetero	−0.12 ± 0.13	−0.884	0.376
			mixed ↔ (homo, hetero)	−0.02 ± 0.06	−0.401	0.689
			mixed ↔ homo	0.20 ± 0.22	0.906	0.365
			mixed ↔ hetero	−0.04 ± 0.23	−0.180	0.857
Ossenigo/Scaiola	155/12	14/43	homo ↔ hetero	−0.41 ± 0.09	−4.350	**<0.001**
			mixed ↔ (homo, hetero)	−0.02 ± 0.06	−0.273	0.784
			mixed ↔ homo	0.45 ± 0.21	2.137	**0.032**
			mixed ↔ hetero	−0.36 ± 0.18	−1.951	0.051
Raas/Fortezza[a]	194/12	16/39	homo ↔ hetero	−0.39 ± 0.06	−6.417	**<0.001**
			mixed ↔ (homo, hetero)	−0.05 ± 0.03	−1.380	0.168
			mixed ↔ homo	0.55 ± 0.13	4.334	**<0.001**
			mixed ↔ hetero	−0.24 ± 0.13	−1.913	0.055
Bodenalm	137/12	18/12	homo ↔ hetero	−0.26 ± 0.11	−2.444	**0.014**
			mixed ↔ (homo, hetero)	0.05 ± 0.05	1.204	0.228
			mixed ↔ homo	0.09 ± 0.15	0.611	0.541
			mixed ↔ hetero	−0.43 ± 0.19	−2.21	**0.027**

"Sample size" refers to the number of pollinated flowers/number of pollen recipient plants. "Pollen donors" indicate the number of sexual/number of apomictic individuals used for the pollinations. The first treatment given in each comparison was used as baseline. Significant differences ($\alpha = 0.05$) between the treatments are highlighted by bold *p*-values.
a For the Raas/Fortezza region the zero-inflation term was removed from the model due its non-significance and a numerical problem.

The mean number of vital pollen grains per sample (Electr. Suppl.: Table S3) was 522.30 ± 330.64 SD for the tetraploids (range 69 to 1415), 347.72 ± 209.37 for the pentaploids (64 to 892) and 234.25 ± 219.68 for the heptaploids (1 to 921). Pollen quality decreased in tendency with increasing ploidy: tetraploids 94.59 ± 7.15% (71.45%–100%), pentaploids 90.76 ± 9.56% (56.49%–100%) and heptaploids 86.24% ± 14.08% (35.37%–100%). Pollen quality differed among reproductive modes, but did not significantly affect the number of obtained seeds neither in the homoploid (zero-inflated GLMM: coefficient ± SE: 0.02 ± 0.13, z = 0.15, p = 0.878) nor in the heteroploid (0.04 ± 0.04, z = 1.12, p = 0.263) or the mixed-ploidy treatment (−0.04 ± 0.06, z = −0.67, p = 0.506).

Flow cytometric seed screen

Good-quality fluorescence signals were obtained for 871 seeds out of the 1001 analysed from the mixed-ploidy treatment. More precisely, the number of screened seeds per region were 15, 183, 336, 134 and 203 from Zabernig, Obersteiner, Raas/Fortezza, Ossenigo/Scaiola and Bodenalm, respectively (Electr. Suppl.: Table S4). The number of

embryo nuclei counted per sample and the coefficient of variation (CV) of the peaks ranged between 155 and 6367 (mean ± SD 1406 ± 821) and 3.32–8.22 (mean ± SD 5.22 ± 0.76), respectively. Examples of flow cytometric histograms are shown in Fig. 2 for homoploid, heteroploid and mixed-ploidy analysed embryos.

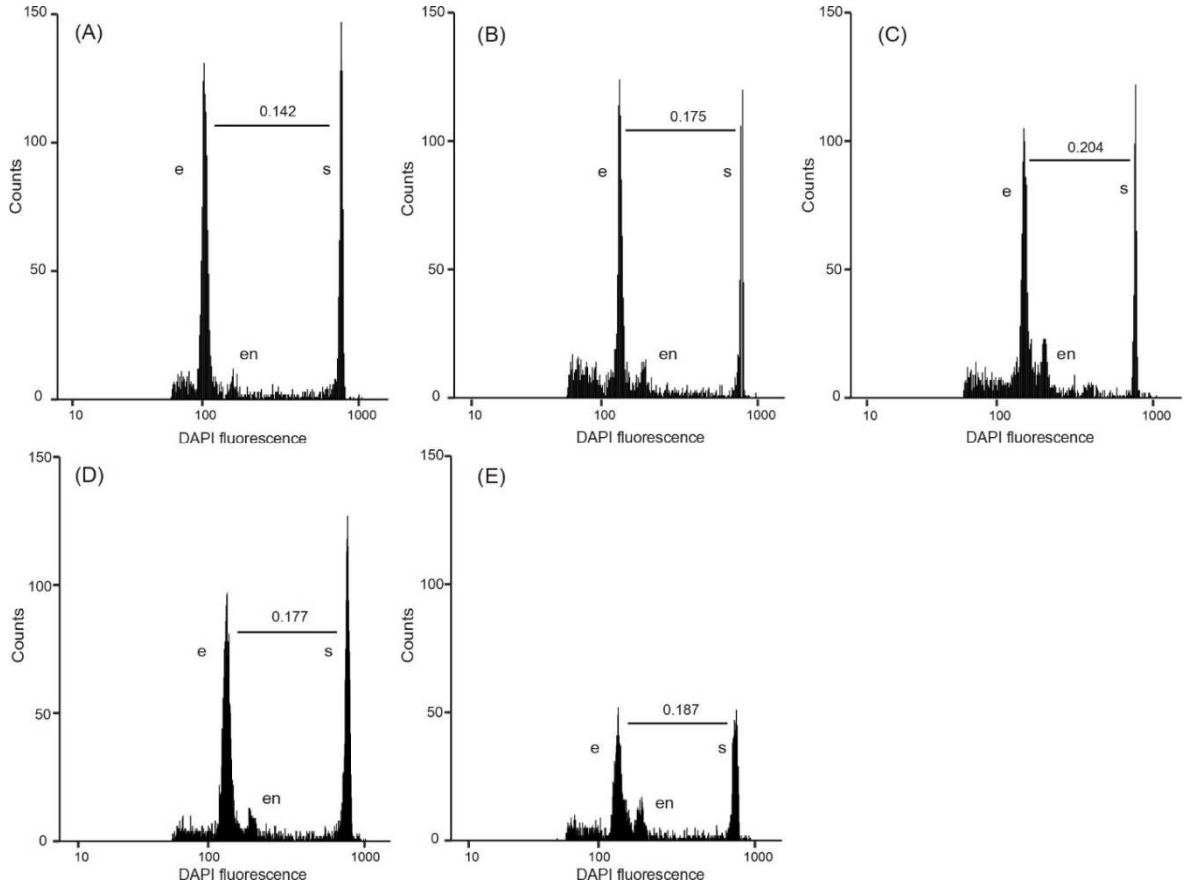


Fig. 2. Flow cytometric sample histograms for *Potentilla puberula* Krašan seeds obtained from sexual tetraploid mother plants crossed with (A) a tetraploid sexual father, (B) a pentaploid apomictic father, (C) a heptaploid apomictic father, (D) a mixture of pollen of tetra-and pentaploid fathers, and (E) a mixture of pollen of tetra-and heptaploid fathers. Letters indicate the signals for the embryo (e), the endosperm (en) and the internal standard Pisum sativum (s). The embryo-to-standard fluorescence ratio was calculated for each seed. Indicated ratios of 0.142, 0.175 and 0.204 are representative for a pollination with (A) DNA-tetraploid, (B) DNA-pentaploid and (C) DNA-hexaploid donor plants. Accordingly, embryos in (d, e) are of heteroploid origin.

In the homoploid reference sample, we observed a single peak of embryo-to-standard fluorescence ratios (Electr. Suppl.: Table S4; mean 0.142 ± 0.0022 SD; normality of the distribution was confirmed by a Shapiro-Wilk-test: W = 0.957, p = 0.191). The 97.5% confidence limit used as a threshold against embryos of higher ploidy expected from heteroploid fertilizations was 0.146. However, in the heteroploid reference sample of pentaploids, embryo-to-standard fluorescence ratios were non-normally distributed (Shapiro-Wilk, W = 0.925, p = 0.032) and showed a tentatively bimodal distribution with 35.1% of the values forming the lower peak (Electr. Suppl.: Table S4; mean ± SD 0.145 ± 0.0033) and the remaining 64.9% constituting the upper peak (0.172 ± 0.0104). Only ploidies of the lower peak overlapped with the embryo ploidies determined for the homoploid reference. We therefore inferred the threshold against progeny of homoploid origin only from seeds belonging to the lower peak, which itself was normally distributed (Shapiro-Wilk, W = 0.976, p-value = 0.955). This limit was calculated as 0.138 (Fig. 3).

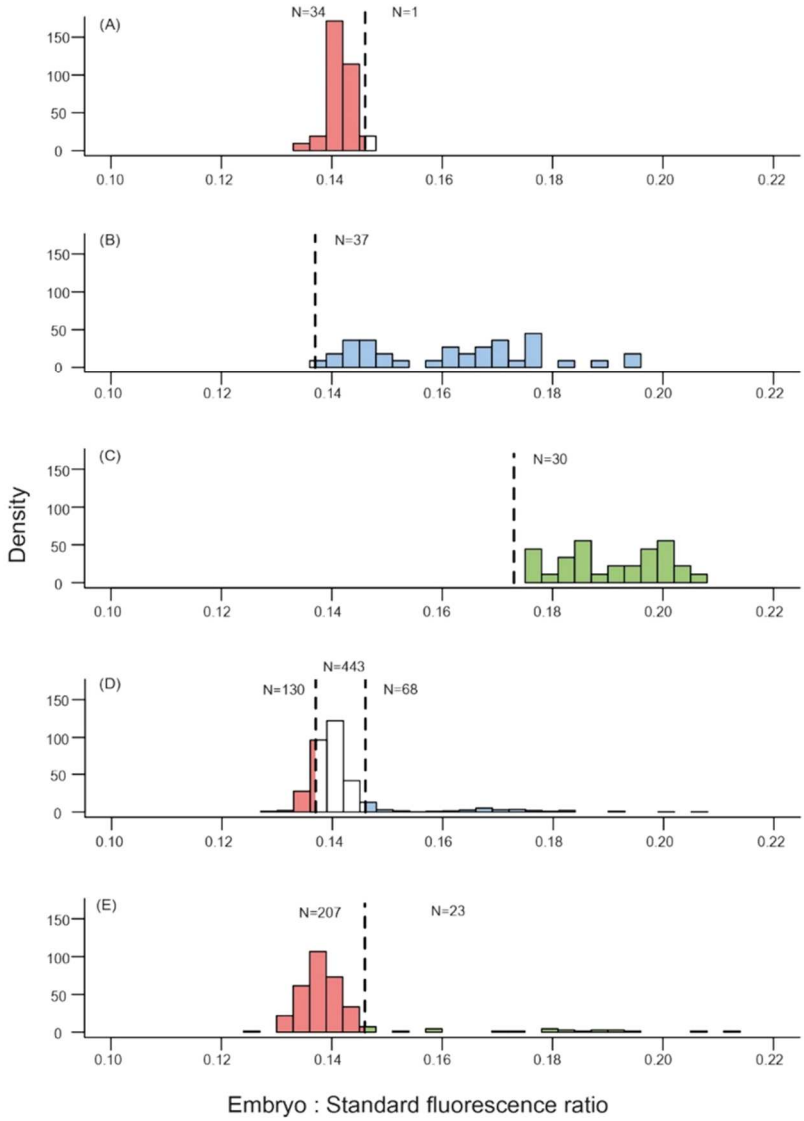


Fig. 3. Frequency distributions of embryo-to-standard fluorescence ratios observed for *Potentilla puberula* seeds obtained in fertilizations of tetraploid sexual mothers with (A) homoploid sexual tetraploid pollen donors (B) with pentaploid apomictic pollen donors, (C) with heptaploid apomictic pollen donors, (D) with a mixture of pollen from tetra- and pentaploid donors, and (E) with a mixture of pollen from tetra- and heptaploid donors. The three reference samples (A–C) were used to infer the paternity of seeds obtained from the mixed-ploidy pollinations. To differentiate between fertilizations by sexuals and apomicts, the upper (i.e., 97.5%) and lower (i.e., 2.5%) limit of the 95% confidence interval (given as dashed lines) of the homoploid and each of the two heteroploid reference samples, respectively, served as thresholds. Seed with a ploidy below (red bars) and above (blue and green bars) these limits were inferred as of homo- and heteroploid origin, respectively. In contrast, seeds having a ploidy in between the limits (white bars) could not be unambiguously.

Nevertheless, 75.7 % of the embryo-to-standard fluorescence ratios in the heteroploid pentaploid reference were higher than the homoploid threshold of 0.146. Sixty-eight out of 641 embryos (10.6%) measured for the mixed-ploidy pollinations, including pentaploids, were inferred to have experienced a change in ploidy in accordance with an heteroploid origin (i.e., had a ploidy higher than the homoploid reference), 130 (20.28%) were derived from homoploid fertilization (ploidies lower than the heteroploid reference), and 443 embryos (69.11%) fell within the range of overlap.

However, under the reasonable assumption that ploidy distribution of successful male gametes formed by the pentaploids was similar in both heteroploid and mixed-ploidy pollinations, the 68 before-mentioned seeds should represent 75.7 % of the total number of seeds of heteroploid origin, which we accordingly calculated to be 90 (N = 90), or 14.4% of the total seeds screened from the mixed-ploidy pollinations involving pentaploids. We used this percentage as an approximation for the fertilization success of apomicts in the mixed-ploidy treatment.

For the heteroploid heptaploid reference, the 2.5% confidence limit was 0.173 (0.191 ± 0.009; Shapiro-Wilk, W = 0.938, p = 0.083), a value allowing for unambiguous inference of paternity of seeds. Twenty-three out of 230 embryos (10.0%) measured for mixed-ploidy pollinations including heptaploids showed ploidies from within the distribution of values observed for the heteroploid reference sample, i.e., were of heteroploid origin, and 207 embryos had values indicative of a homoploid origin (90.0%) (Fig. 3).

DISCUSSION

Our data indicate for the first time the existence of homoploid pollen precedence in *Potentilla puberula*. In line with Dobeš & al. (2018), we found the seed yield in heteroploid crosses to be lower than in homoploid crosses, suggesting that heteroploid pollen negatively affects the reproductive success of sexual individuals. However, our current novel investigations with mixed-ploidy pollinations revealed that these losses of sexual progeny due to heteroploid fertilizations were partially restored when homo- and heteroploid pollen was simultaneously applied onto the recipient flower. Seed yield derived from mixed-ploidy fertilizations was intermediate to those obtained in the homo- and heteroploid crosses, but did not significantly differ from the average of these two treatments, suggesting a similar fertilization success of homo- and heteroploid pollen.

However, this equal performance contrasted with the high proportion of homoploid fertilizations (an estimated 85%–87 %) derived from seeds obtained in the mixed-ploidy crosses. These proportions are much higher than expected under a scenario of equal fertilization success of pollen from sexuals and apomicts after correcting for the differential seed yield observed in the homo- versus heteroploid crosses. Thus, under the hypothetical scenario that homo- and heteroploid pollen tubes fertilized egg cells in equal numbers, and the assumption that observed difference in seed yield among these single-pollen treatments is due to unsuccessful fertilizations (see discussion below), homo- and heteroploid progeny would be expected to be 60% (1410 seeds obtained) and 40% (930 seeds). The seed yield was actually lower than these hypothetical values, which might be explained by mutual developmental inhibition of the two applied pollen types. Negative pollen-pollen interactions can arise at different developmental stages of the male gametophyte after pollination. For instance, pollen germination on *Clarkia unguiculata* Lindl stigmas was higher with both pure self and outcrossed pollen than in mixed-pollen pollinations (Németh & Smith-Huerta, 2002). Likewise, interference among growing pollen tubes could occur if a tube grows into a stylar region already occupied by a previous tube, or if multiple tubes attempt to reach the same space/resources at once (Harder & al., 2016), indicating existence of a quantitative effect by number of pollen limiting tube growth.

Similar to our observation of homoploid pollen precedence, Ramsey & al. (2003), observed that interspecific pollinations between two sister species of monkeyflowers (*Mimulus lewisii* Pursh, *M. cardinalis* Douglas ex Benth.) produced nearly 50% fewer

seeds than intraspecific crosses, but in mixed-pollen pollinations of *M. cardinalis*, less than 25% of the progeny were hybrids. Koutecký & al. (2011) obtained the same pattern in diploid *Centaurea pseudophrygia* C.A.Mey and tetraploid *C. jacea* L., with lower success in hetero- compared to homoploid treatments, and mixed-ploidy pollination success closer to homoploid values, suggesting that pollen precedence may enhance reproductive isolation among ploidies. An example for a sexual-apomictic system was provided by Mártonfiová (2006, 2015), who studied fertilization of sexual diploids individuals by pollen of triploid apomicts in *Taraxacum* Wigg sect. Taraxacum (Syn: *T.* sect. *Ruderalia* Kirschner & al.), and observed that, when sexual diploid individuals received different mixtures of pollen of diploids and apomictic triploids, only diploid progeny was produced, suggesting homoploid pollen precedence.

Various non-exclusive mechanisms can explain pollen precedence. The gametophytically controlled SI system operative in the Rosaceae allows the female reproductive organ to discriminate self-pollen and, consequently, to reduce inbreeding (Brewbaker, 1957; Kao & McCubbin, 1996; Franklin-Tong & Franklin, 2003; Hiscock & McInnis, 2003). Interspecific reproductive barriers recognizing and rejecting interspecific pollen can be mechanistically linked to the SI system, suggesting a possible overlap and a partially shared genetic basis between both mechanisms of post-mating prezygotic female choice (Baek & al., 2015; Tovar-Méndez & al., 2016; Hamlin & al., 2017). In addition to selectively refusing pollen, ovules can be selective in attracting pollen (Takeuchi & Higashiyama, 2012). Such attraction was found to be related to SI: ovules of SI *Solanum* L. species attracted more conspecific pollen compared to SC species (see Lafon-Placette & al., 2016). In *P. puberula* the SI system is functional in the sexual individuals (likely due to diploidization of its assumed allopolyploid genome: Dobeš & al., 2013) and they may well recognize heteroploid pollen, favouring homoploid fertilization.

The presence of heteroploid apomictic pollen reduced the reproductive success of sexual *Potentilla puberula* by means of two mechanisms: Firstly, the significantly lower seed set in the mixed-ploidy compared to the homoploid crosses suggests loss of progeny due to incompatible heteroploid fertilization. Thus, post-zygotic barriers prevent the development of egg cells fertilized by heteroploid pollen into a mature seed. Secondly, a considerable proportion of the progeny of sexuals (more than one-tenth of the developed embryos were derived from fertilizations by heteroploid pollen) was cytologically

transformed despite the high degree of homoploid pollen precedence. In sexually derived seeds, such losses by transformation are considered to cause more serious effects than those resulting from endosperm imbalance in apomictically derived seeds, since apomicts are able to avoid cross-fertilization through selfing.

The impact of foreign pollen on the reproductive success may also depend on the phylogeographic history and the biogeographic context, because plants may evolve local adaptive mechanisms. Arceo-Gómez & al. (2016) found that conspecific pollen performed better in terms of pollen tube growth in populations with historical contact of two *Clarkia* species compared to those without. In our case, sexual individuals of *P. puberula* from lowland regions in the south of the study area (region Raas/Fortezza and region Ossenigo/Scaiola), where mainly pure tetraploid populations occur, suffered from higher losses of seeds upon cross-fertilization. In these lower-elevation regions, seed yield from the mixed-ploidy crosses thus was significantly lower compared to the pooled crosses with homo- and heteroploid pollen, a difference that was not significant for the regions from higher elevations. This pattern may indicate that they are more sensitive to interactions with heteroploid pollen than sexual individuals at higher elevations in the north, where apomicts are more frequent.

Mentor effects, defined as the induction of self-fertilization of otherwise self-incompatible sexual plants upon co-application of self- and foreign pollen, are a protective mechanism to avoid cross-fertilization by both, other species and intra-specific cytotypes, including apomicts (Mráz, 2003; Hörandl & Temsch, 2009). In our study, the SI sexual pollen recipients were emasculated and, therefore, unintended selfing – which we would not have been able to discriminate from homoploid cross fertilizations without genotyping the progeny – was prevented. Consequently, we are not able to quantify the relative role of pollen precedence vs. mentor effects based on our study design. However, mentor effects have already been totally disproven for the mixed-ploidy pollinations including heptaploids and were at least partly excluded for the pentaploids (Dobeš & al., 2018). Hence, yet there is strong evidence that pollen precedence acts as an alternative protection against cross-fertilization, and plays the dominant role as prezygotic barrier in *P. puberula.*

We analysed seed yield as a measure of reproductive success instead of the more commonly used seed set (i.e., ratio mature seedsto ovules). However, we could remove the inter-individual variation in the number of ovules since all treatments were performed

in flowers of each pollen recipient. Still, it has to be assumed that there are no differences in the number of ovules among flowers of the same individual allocated to the different treatments. The absence of such a bias seems likely attributable to a random allocation of flowers for a sufficient number of individuals, underlined for *P. puberula* by Dobeš & al. (2018), suggesting non-structured differences in base line seed set among individuals. However, even in case of a violation of the assumption of equal ovule numbers, it is parsimonious to presume that the rate of seed abortion, and hence the discrepancy between seed yield and seed set, would be higher in heteroploid than in homoploid crosses due to reproductive incompatibilities among ploidy levels. Actually, this would rather accentuate than weaken the higher reproductive success found in homoploid crosses, identifying our results as conservative concerning the uncertainties in the determination of reproductive success. Consequently, estimation of seed set would not necessarily increase the reliability of our results since it also rests on an assumption: namely, that the number of ovules and reproductive success does not differ between flowers used to count both ovules and mature seeds. In conclusion, we found evidence for precedence of homoover heteroploid pollen in *P. puberula.*

However, a considerable proportion of the progeny of sexual plants was lost or transformed upon cross-pollination by their apomictic conspecifics. This likely affects their co-occurrence, because only a complete reproductive isolation avoids – in the long run – the competitive displacement of sexuals by apomicts, unless the former is favoured by other factors related to their reproductive system. In natural populations, the actual success of sexuals versus apomicts depends on a variety of decisive factors like the activity of additional reproductive barriers such as spatial isolation (Dobeš & al., 2013), shifts in flowering time, or assortative pollination, as well as the relative fertility and vigor of sexuals and apomicts. These factors need to be studied in concert with pollen precedence to allow for a quantitative assessment of the degree of pollen precedence necessary to allow a stable co-existence or even a competitive replacement of apomicts. Nevertheless, the observed precedence of homoploid pollen reduces the pressure upon the sexuals in mixed-ploidy populations.

Author contributions

CD designed the study. HAM, PPR and TM carried out the experiments and calculations. KH and CD designed the statistical analyses. HAM, KH and CD wrote the manuscript. All authors discussed the results and helped shape the research, analyses and

manuscript. ORCID: HA-M, https://orcid.org/0000-0002-9726-9019; CD, https://orcid.org/0000-0002-8631-219X; KH, https://orcid.org/0000-0001-9274-1647.

Acknowledgements

We are grateful to Susanne Scheffknecht, Institute of Botany, University of Natural Resources and Life Sciences, Vienna, for help and guidance with the hand-pollination technique, and Flavia Domizia Nardi, Department of Forest Genetics, Austrian Research Centre for Forests, Vienna, for her assistance with some FCSS samples. We are also thankful to Elvira Hörandl and two anonymous reviewers who helped us to improve the manuscript by their comments. The Austrian Science Foundation (FWF) supported this research, grant number: P27688 to Christoph Dobeš.

REFERENCES

Abadie, P., Roussel, G., Dencausse, B., Bonnet, C., Bertocchi, E., Louvet, J. M., Kremer, A. & Garnier-Géré, P. 2012. Strength, diversity and plasticity of postmating reproductive barriers between two hybridizing oak species (*Quercus robur* L. and *Quercus petraea* (Matt) Liebl.). *J. Evol. Biol.* 25: 157--173. http://doi.org/10.1111/j.1420-9101.2011.02414.x

Arceo-Gómez, G., Raguso, R.A. & Geber, M.A. 2016. Can plants evolve tolerance mechanisms to heterospecific pollen effects? An experimental test of the adaptive potential in *Clarkia* species. *Oikos* 125: 718--725. http://doi.org/10.1111/oik.02594

Arnold, M.L., Hamrick, J. L. & Bennet, B.D. 1993. Interspecific pollen competition and reproductive insolation in *Iris*. *J. Heredity*. 84: 13--16. http://doi.org/10.1093/oxfordjournals.jhered.a111269

Asker S.E. & Jerling, L. 1992. *Apomixis in plants*. Boca Raton: CRC Press.

Baack, E.J. 2005. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): minority cytotype exclusion and barriers to triploid formation. *Amer. J. Bot.* 92: 1827--1832. http://doi.org/10.3732/ajb.92.11.1827

Baek, Y.S., Covey, P.A., Petersen, J.J., Chetelat, R.T., McClure, B. & Bedinger, P.A. 2015. Testing the SI x SC rule: Pollen-pistil interactions in interspecific crosses

between members of the tomato clade (*Solanum* section *Lycopersicon*, Solanaceae). *Amer. J. Bot.* 102: 302--311. http://doi.org/10.3732/ajb.1400484

Baker, M.S., Arrigo, N., Baniaga, A.E., Li, Z. & Levin, D.A. 2016. On the relative abundance of autopolyploids and allopolyploids. *New Phytol*. 210: 391--398. http://doi.org/10.1111/nph.13698

Bayer, R.J. 1997. *Antennaria rosea* (Asteraceae) - a model group for the study of the evolution of polyploid agamic complexes. *Opera Bot*. 132: 53--65.

Behrend, A., Gluschak, A., Przybyla, A. & Hohe, A. 2015. Interploid crosses in heather (*Calluna vulgaris*). *Sci. Hortic.* 181: 162–167. https://doi.org/10.1016/j.scienta.2014.11.005

Botes, C., Johnson, S.D. & Cowling, R.M. 2008. Coexistence of succulent tree aloes: partitioning of bird pollinators by floral traits and flowering phenology. *Oikos* 117: 875--882. http://doi.org/10.1111/j.0030-1299.2008.16391.x

Brewbaker, J.I. 1957. Pollen cytology and self-incompatibility systems in plants. *J. Heredity* 48:271--277. https://doi.org/10.1093/oxfordjournals.jhered.a106742

Brock, M.T. 2009. Prezygotic barriers to gene flow between *Taraxacum ceratophorum* and the invasive *Taraxacum officinale* (Asteraceae). *Oecologia* 161: 241--251. http://doi.org/10.1007/s00442-009-1383-0

Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J. & Maechler, M. 2017. Modeling zero-inflated count data with glmmTMB. R J. 9: 1--14. *bioRxiv*, http://doi.org/10.1101/132753

Brown, B.J. & Mitchell, R.J. 2001. Competition for pollination: Effects of pollen of an invasive plant on seed set of a native congener. *Oecologia* 129: 43--49. http://doi.org/10.1007/s004420100700

Brys, R., Vanden Broeck, A., Mergeay, J., & Jacquemyn, H. 2014. The contribution of mating system variation to reproductive isolation in two closely related *Centaurium* species (Gentianaceae) with a generalized flower morphology. *Evolution (Lancaster)*. 68: 1281--1293. http://doi.org/10.1111/evo.12345

Campbell, D.R., Alarcón, R., & Wu, C.A. 2003. Reproductive isolation and hybrid pollen disadvantage in *Ipomopsis*. *J. Evol. Biol.* 16: 536--540.

https://doi.org/10.1046/j.1420-9101.2003.00538.x

Chapman, M.A., Forbes, D.G. & Abbot, R.J. 2005. Pollen competition among two species of *Senecio* (Asteraceae) that form a hybrid zone on Mt. Etna, Sicily. *Amer. J. Bot.* 92: 730--735. http://doi.org/10.3732/ajb.92.4.730

Comai, L. 2005. The advantages and disadvantages of being polyploid. *Amer. J. Bot.* 6: 836--846. http://doi.org/10.1038/nrg1711

Cosendai, A.-C, Rodewald, J. & Hörandl, E. 2011. Origin and distribution of autopolyploids via apomixis in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Taxon* 60: 355--364.

Coyne, J.A. & Orr, H.A. 1998. The evolutionary genetics of speciation. *Philos. Trans., Ser. B.* 353: 287--305. http://doi.org/10.1098/rstb.1998.0210

Dobeš, C. 1999. Die Karyogeographie des *Potentilla verna* agg. (Rosaceae) in Österreich – mit ergänzenden Angaben aus Slowenien, Kroatien, der Slowakei und Tschechien. *Ann. Naturhist. Mus. Wien, B* 101: 599--629.

Dobeš, C., Milosevic, A., Prohaska, D., Scheffknecht, S., Sharbel, T. F. & Hülber, K. 2013. Reproductive differentiation into sexual and apomictic polyploid cytotypes in *Potentilla puberula* (Potentilleae, Rosaceae). *Ann. Bot. (Oxford)* 112: 1159--1168. http://doi.org/10.1093/aob/mct167

Dobeš, C., Scheffknecht, S., Fenko, Y., Prohaska, D., Sykora, C. & Hülber, K. 2018. Asymmetric reproductive interference: The consequences of pollination on reproductive success in sexual – apomictic populations of *Potentilla puberula* (Rosaceae). *Ecol. Evol.* 00: 1--17. http://doi.org/10.1002/ece3.3684

Dobzhansky, T. 1936. Position effects on genes. *Biol. Rev. Cambridge Philos. Soc.* 11: 364--384. http://doi.org/10.1111/j.1469-185X.1936.tb00911.x

Doležel, J., Greilhuber, J. & Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nat. Protoc.* 2: 2233--2244. http://doi.org/10.1038/nprot.2007.310

Franklin-Tong, V.E. & Franklin, F.C.H. 2003. The different mechanisms of gametophytic self-incompatibility. *Philos. Trans., Ser. B.* 358: 1025--1032. http://doi.org/10.1098/rstb.2003.1287

Grant, V. 1981. *Plant speciation*. New York: Columbia University Press.

Grant, V. 1994. Modes and origins of mechanical and ethological isolation in Angiosperms. *Proc. Natl. Acad. Sci. U.S.A.* 91: 3--10.

Greilhuber, J. & Ebert, I. 1994. Genome size variation in *Pisum sativum*. *Genome* 37: 646--655. http://dx.doi.org/10.1007/BF00224067

Hamlin, J.A.P., Sherman, N.A. & Moyle, L.C. 2017. Two loci contribute epistastically to heterospecific pollen rejection, a postmating isolating barrier between species. *G3: Genes Genomes Genet.* 7: 2151--2159. http://doi.org/10.1534/g3.117.041673

Harder, L.D., Aizen, M.A., Richards, S.A., Joseph, M.A. & Busch, J.W. 2016. Diverse ecological relations of male gametophyte populations in stylar environments. *Amer. J. Bot.* 103: 484--497. https://doi.org/10.3732/ajb.1500269

Hersh, E., Grimm, J. & Whitton, J. 2016. Attack of the clones: reproductive interference between sexuals and asexuals in the *Crepis* agamic complex. *Ecol. Evol.* 6: 6473--6483. http://doi.org/10.1002/ece3.2353

Hiscock, S. & McInnis, S. 2003. The diversity of self-incompatibility system in flowering plants. *Pl. Biol. (Stuttgart)* 5: 23--32. http://doi.org/10.1055/s-2003-37981

Hojsgaard, D., Schegg, E., Valls, J.F.M., Martínez, E.J. & Quarin, C.L. 2008. Sexuality, apomixis, ploidy levels, and genomic relationships among four *Paspalum* species of the subgenus *Anachyris* (Poaceae). *Flora* 203: 535--547. https://doi.org/10.1016/j.flora.2007.09.005

Hörandl, E. & Hojsgaard, D. 2012. The evolution of apomixis in angiosperms: A reappraisal. *Pl. Biosyst.* 146:37--41.

Hörandl, E & Temsch, E.M. 2009. Introgression of apomixis into sexual species is inhibited by mentor effects and ploidy barriers in the *Ranunculus auricomus* complex. *Ann. Bot. (Oxford)* 104: 81--89. http://dx.doi.org/10.1093/aob/mcp093

Howard, D.J. 1999. Conspecific sperm and pollen precedence and speciation. *Annual Rev. Ecol. Evol. Syst*. 30: 109--132.

Hülber, K., Sonnleitner, M., Flatscher, R., Berger, A., Dobrovsky, R., Niessner, S., Nigl, T., Schneeweiss, G.M., Kubešová M., Rauchová J., Suda J. &

Schönswetter, P. 2009. Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. *Preslia* 81: 309--319.

Hülber , K., Scheffknecht, S. & Dobeš, C. 2013. Partitioning the factors explaining the eco-geography in the amphi-pomictic species *Potentilla puberula* (Rosaceae). P. 98 in: Kroh, A., Berning, B., Haring, E., Harzhauser, M., Sattmann, H., Walochnik, J., Zimmermann, D. & Zuschin, M (eds.). *BioSyst.EU 2013. Global systematics!* 18-22 February 2013. Abstract volume. Vienna: NOBIS Austria.

Husband, B.C. & Schemske, D.W. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *J. Ecol.* 88: 689--701. http://dx.doi.org/10.1046/j.1365-2745.2000.00481.x

Husband, B.C., Schemske, D.W., Burton, T.L. & Goodwillie, C. 2002. Pollen competition as a unilateral reproductive barrier between sympatric diploid and tetraploid *Chamerion angustifolium*. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 269: 2565--2571. http://dx.doi.org/10.1098/rspb.2002.2196

Joshi A. & Moody M.E. 1995. Male gamete output of asexuals and the dynamics of populations polymorphic for reproductive mode. *J. Theor. Biol.* 174: 189--197.

Joshi A. & Moody M.E. 1998. The cost of sex revisited: effects of male gamete output of hermaphrodites that are asexual in their female capacity. *J. Theor. Biol.* 195: 533--542.

Kao, T.H. & McCubbin, A.G. 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. *Proc. Natl. Acad. Sci. U.S.A.* 93: 12059--12065. https://doi.org/10.1073/pnas.93.22.12059

Kay, K.M. 2006. Reproductive isolation between two closely related hummingbird-pollinated neotropical gingers. *Evolution (Lancaster)* 60: 538--552.

Kolár, F., Čertner, M., Suda, J., Schönswetter, P. & Husband, B.C. 2017. Mixed-ploidy species: progress and opportunities in polyploid research. *Trends Pl. Sci*. 22: 1041--1055. http://doi.org/10.1016/j.tplants.2017.09.011

Koltunow, A.M. & Grossniklaus, U. 2003. Apomixis: a developmental perspective. *Annual Rev. Pl. Biol.* 54: 547--574. http://doi.org/10.1146/annurev.arplant.54.110901.160842

Koutecký, P., Badurová, T., Štech, M., Košnar, J. & Karásek, J. 2017. Hybridization between diploid *Centaurea pseudophrygia* and tetraploid *C . jacea* (Asteraceae): the role of mixed pollination, unreduced gametes, and mentor effects. *Biol. J. Linn. Soc.* 104: 93--106. http://doi.org/10.1111/j.1095-8312.2011.01707.x

Kurtto, A., Lampinen, R. & Junikka, L. 2004. *Atlas Florae Europaeae: Distribution of vascular plants in Europe*, vol 13, *Rosaceae (Spiraea to Fragaria, excl. Rubus)*. Helsinki: The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo

Lafon-Placette, C., Vallejo-Marín, M., Parisod, C., Abbott, R.J. & Köhler, C. 2016. Current plant speciation research: unravelling the processes and mechanisms behind the evolution of reproductive isolation barriers. *New Phytol.* 209: 29--33. http://doi.org/10.1111/nph.13756

Lamont, B.B., He, T., Enright, N.J., Krauss, S.L. & Miller, B.P. 2003. Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. *J. Evol. Biol.* 16: 551--557. http://doi.org/10.1046/j.1420-9101.2003.00548.x

Landis, J.B., Soltis, D.E., Li, Z., Marx, H.E., Barker, M.S., Tank, D.C. & Soltis, P.S. 2018. Impact of whole-genome duplication events on diversification rates in angiosperms. *Amer. J. Bot.* 105: 1--16. http://doi.org/10.1002/ajb2.1060

Lepais, O., Roussel, G., Hubert, F., Kremer, A. & Gerber, S. 2013. Strength and variability of postmating reproductive isolating barriers between four European white oak species. *Tree Genet. Genomes*. 9: 841--853. http://doi.org/10.1007/s11295-013-0602-3

Levin, D.A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35--43. http://doi.org/10.2307/1218997

Lin, B.Y. 1984. Ploidy barrier to endosperm development in maize. *Genetics* 107: 103--115.

Lowry, D.B., Modliszewski, J.L., Wright, K.M., Wu, C.A. & Willis, J.H. 2008. The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philos. Trans., Ser. B* 363: 3009--3021. http://doi.org/10.1098/rstb.2008.0064

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., Van

Bentham, K., Bolker, B. & Brooks, M. 2017. glmmTMB: Generalized Linear Mixed Models using Template Model Builder R package, version 0.1.1. https://CRAN.R-project.org/package=glmmTMB

Mártonfiová, L. 2006. Possible pathways of the gene flow in *Taraxacum* sect. *Ruderalia*. *Folia Geobot*. 41: 183--201. http://doi.org/10.1007/BF02806478

Mártonfiová, L. 2015. Hybridization in natural mixed populations of sexual diploid and apomictic triploid dandelions (*Taraxacum* set. *Taraxacum*): "Why are the diploid sexuals not forced out? *Folia Geobot*. 50: 339--348. http://doi.org/10.1007/s12224-015-9231-y

Matzk, F., Meister, A. & Schubert, I. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant J.* 21: 97--108. http://doi.org/10.1046/j.1365-313X.2000.00647.x

Mráz, P. 2003. Mentor effects in the genus *Hieracium* s.str. (*Compositae, Lactuceae*). *Folia Geobot*. 38: 345--350. https://doi.org/10.1007/BF02803204

Muller, H.J. 1939. Further studies on the nature and causes of gene mutations. Pp. 213–255 in: Jones, D.F. (ed.), *Proceedings of the Sixth International Congress of Genetics, Ithaca, New York, 1932*, vol. 1. Menasha, Wisconsin: Brooklyn Botanical Garden.

Nardi, F.D., Dobeš, C., Müller, D., Grasegger, T., Myllynen, T., Alonso-Marcos, H. & Tribsch, A. 2018. Sexual intraspecific recombination but not *de novo* origin governs the genesis of new apomictic genotypes in *Potentilla puberula* (Rosaceae). *Taxon* 67: 1108–1131. https://doi.org/10.12705/676.8

Németh, M.B. & Smith-Huerta, N.L. 2002. Effects on pollen load composition and deposition pattern on pollen performance in *Clarkia unguiculata* (Onagraceae). *Int. J. Pl. Sci.* 163: 795--802. http://doi.org/10.1086/342034

Nogler, G.A. 1984. Gametophytic apomixis. Pp. 475--518 in: Johri B.M. (ed.), *Embriology of Angiosperms*. Berlin: Springer. http://doi.org/10.1007/978-3-642-69302-1_10

Otto, F.J. 1990. DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. *Meth. Cell Biol.* 33: 105--110.

Otto, S.P. & Whitton, J. 2000. Polyploid incidence and evolution. *Annual. Rev. Genet*. 34: 401--437. http://dx.doi.org/10.1146/annurev.genet.34.1.401

Ozias-Akins, P. & Van Dijk, P.J. 2007. Mendelian genetics of apomixis in plants. *Annual Rev. Genet*. 41: 509--537. http://doi.org/10.1146/annurev.genet.40.110405.090511

Peterson, R., Slovin, J.P. & Chen, C. 2010. A simplified method for differential stainingof aborted and non-aborted pollen grains. *Int. J. Plant Biol*. 1:e13. https://doi.org/10.4081/pb.2010.e13

Petit, C., Lesbros, P., Ge, X. & Thompson, J.D. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79: 31--40.

Quantum Analysis 2014. FloMax: Software for multiparameter data analysis, instrument control, and data acquisition for flow cytometry for Windows. Münster: Quantum Analysis. http://quantumanalysis.com/

R development Core Team. 2017. A language and environment for statistical computing. Vienna: R Foundation for statistical computing. www.R-project.org.

Ramsey, J., Bradshaw, H.D. & Schemske, D.W. 2003. Components of reproductive isolations between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution. (Lancaster)* 57: 1520--1534. http://doi.org/10.1111/j.0014-3820.2003.tb00360.x

Rieseberg, L.H., Desrochers, A.M. & Youn, S.J. 1995. Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *Amer. J. Bot.* 82: 515--519. https://doi.org/10.1002/j.1537-2197.1995.tb15672.x

Soják, J. 2010. Origin of *Potentilla crantzii*, *P. verna* and *P. puberula* (Rosaceae) with a note on the nomenclature of *P. pusilla*. *Feddes Repert*. 121: 112--116. https://doi.org/10.1002/fedr.201000010

Soltis, P.S. & Soltis, D.E. 2016. Ancient WGD events as drivers of key innovations in angiosperms. *Curr. Opin. Plant Biol*. 30: 159--165. https://doi.org/10.1016/j.pbi.2016.03.015

Soltis, D.E., Soltis, P.S., Schemske, D.W., Hancock, J.F., Thompson, J.N., Husband,

B.C. & Judd, W.S. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon* 56: 13--30.

Sonnleitner, M., Flatscher, R., Garcıa, P.E., Rauchova, J., Suda, J., Schneeweiss, G.M., Hülber, K. & Schönswetter, P. 2010. Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps. *Ann. Bot. (Oxford)* 106: 967--977. http://doi.org/10.1093/aob/mcq192

Stebbins, G.L. 1947. Types of polyploids: Their classification and significance. *Advances Genet.* 1: 403--429. http://doi.org/10.1016/S0065-2660(08)60490-3

Sutherland, B.L. & Galloway, L.F. 2017. Postzygotic isolation varies by ploidy level within a polyploid complex. *New Phytol.* 213: 404–412. https://doi.org/10.1111/nph.14116

Takeuchi, H. & Higashiyama, T. 2012. A species-specific cluster of defensin-like genes encodes diffusible pollen tube attractants in *Arabidopsis*. *PLoS Biol.* 10:e1001449. http://doi.org/10.1371/journal.pbio.1001449

Tribsch, A. & Schönswetter, P. 2003. Patterns of endemism and comparative phylogeography confirm paleoenvironmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon* 52: 477--497. http://doi.org/10.2307/3647447

Trunschke, J. & Stöcklin, J. 2017. Plasticity of flower longevity in alpine plants is increased in populations from high elevation compared to low elevation populations. *Alpine Bot.* 127: 41–51. https://doi.org/10.1007/s00035-016-0176-4

Tovar-Méndez, A., Lu, L. & Mcclure, B. 2016. HT proteins contribute to S-RNAse-independent pollen rejection in *Solanum*. *Plant J.* 89: 718--729. https://doi.org/10.1111/tpj.13416

Wyatt, R., Boyles, S.B.& Derda, G.S. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *Amer. J. Bot*. 79: 636--642. https://doi.org/10.1002/j.1537-2197.1992.tb14605.

CONCLUSION

The questions proposed have been successfully answered within this thesis. Our results indicated that traits related to differences in reproductive mode rather than effects of ploidy drive ecological differentiation in *Potentilla puberula*. Sexual tetraploids prefer primary xeric habitats, in drier, steeper and more south-oriented slopes, while apomictic higher ploids occur predominantly in human-disturbed habitats, in more gentle slopes with higher annual precipitation. In contrast, the apomictic higher ploids did not present ecological divergences among themselves. These outcomes reflects evolutionary consequences for both reproductive modes, being the sexual tetraploids centred at the southern edges of the study area, a region considered as one glacial refugium in the Alps. Thus, one can argue that apomicts have managed to perform better in the margins of *P. puberula*'s distribution, in wetter and fragmented habitats that could have promote self-fertilization as a reproductive assurance in sites under poor weather conditions, with minor pollinator activity and with greater human impact.

Another important aspect to remark is the need to study ecological differentiation in areas of sympatry. Several ecological studies of amphi-apomictic taxa revealed mixed results. The certainty of the origin of the cytotypes, the mode of reproduction and the relationships of hybridization with other taxa are some of the factors that may lead to ambiguous interpretations. In our case, *P. puberula* presents both reproductive modes in sympatry, and the fact that the apomictic cytotypes are of autopolyploid origin, allowed us to exclude potential hybridization effects. In addition, having more than one ploidy level within the apomicts allowed to assess the impact of ploidy level versus differences in reproductive modes.

Sexual and apomictic cytotypes of *P. puberula* present ecological differences such that both reproductive modes can co-occur in sympatric conditions. However, the mode of reproduction also leaves a genetic fingerprint. To get a better understanding of the relationship among the cytotypes, it was necessary to identify the origins of the apomictic genotypes and the potential implication of other taxa. The AFLP and cpDNA results showed a clear genetic differentiation between sexuals and the higher apomictic ploidy levels. Three AFLP alleles were associated only with parthenogenetic genotypes. These fragments could open the door for further genetic mapping studies.

In addition, high ploidy individuals were found to be derived from spontaneous autopolyploidization of sexual tetraploids, which rule out any kind of hybridization with other *Potentilla* species. However, since the formation of a new apomict needs at least one apomictic parent, reproductive sexual-apomictic interactions are crucial in the dynamics of mixed-ploidy populations.

For this reason, experimental crossings with homo-, hetero- and mixed-ploidy pollen were performed in sexual tetraploid individuals. The results of reduced seed yield in heteroploid crossings were in line with previous studies of other species and *P. puberula* as well. Nevertheless, 13-15% of embryos from mixed-ploidy crossings experienced a change in ploidy compared to their mother plants due to the heteroploid pollen. This implies that the remaining embryos, i.e. 85-87%, were progeny of homoploid fertilizations. These results indicate, for the first time, homoploid pollen precedence in *Potentilla puberula*. Thus, pollen precedence plays a role as a reproductive barrier in heteroploid fertilizations, counteracting the potential losses of sexual embryos and promoting the co-existence of sexual and apomictic individuals. Under natural conditions, still, the effects of pollen precedence could decrease, since the probability of heteroploid or mixed-ploidy fertilizations would be reduced because of the cytotype tendency to cluster and the aforementioned differential ecological requirements.

Some limitations of the study, however, need to be mentioned. During the hand-pollination experiment in the common garden, some individuals used as pollen recipients did not produce as many flowers as expected. Besides, adverse weather conditions could influence both the flowering and the pollination processes. Moreover, it would be interesting to study the behaviour of pollen tubes during homo-, hetero- and mixed-ploidy fertilization, and test if there are interactions and at which level (pollen-pollen, pollen tube growth and speed, ovule signalling…). I did attempt a protocol for staining and visualizing pollen tubes in *Potentilla puberula* but I faced several setbacks due to technical reasons. Nevertheless, it could be a possibility worth continue exploring. Moreover, reciprocal transplantations of sexuals and apomicts, both in natural conditions and in a common garden, would be highly appreciated to verify the degree and strength of these ecological differences attributable to reproductive mode.

We have seen that reproductive mode and its associated traits are the key feature of *P. puberula* and the main reasons why sexual and apomictic cytotypes show ecological differences. This difference in reproduction implies evolutionary consequences for the cytotypes. In addition, although reproductive interactions can occur between sexuals and apomicts, homoploid pollen precedence acts as a reproductive barrier between them. Of course, other factors may have an effect in the dynamics of the populations and need to be taken into account, such as shifts in flowering time, assortative pollination and relative fertility or vigor of sexual and apomicts. Apart from reproductive interactions, competitive interactions also take place. All these parameters need to be studied together in a holistic approach of sexual-apomictic populations and their interactions projected in time.

Potentilla puberula, as an amphi-apomictic polyploid species, is a well-studied and suitable model for drawing conclusions on the ecological, genetic and reproductive interactions between cytotypes. The contributions of this thesis have both extended previous knowledge of polyploidy and apomixis, and helped to clarify other unknown aspects of sexual and apomictic relationships, such as the role of pollen precedence in heteroploid fertilizations and the autopolyploid origin of the apomictic cytotypes.

APPENDICES AND ELECTRONIC SUPPLEMENTS

Supplementary materials of CHAPTER 1 include ‘Appendix S1: Supplementary Tables A1–A5’ and ‘Appendix S2: Soil Analyses’.

CHAPTER 2 includes ‘Appendix 1’, ‘Appendix 2’, ‘Supplementary Tables S1–S6’ and ‘Supplementary Figures S1–S5’.

CHAPTER 3 includes the supplementary figure ‘Fig. S1’ and ‘Supplementary Tables 1–4’.

APPENDIX S1: SUPPLEMENTARY TABLES.

Table A1. Description of 238 populations of *Potentilla puberula* sampled between 1999 and 2015 in the Eastern European Alps. Geographical and collection information, number of sampled individuals and cytotype composition variables, as well as source of flow cytometric data (FCM) and populations from which soil analyses were conducted ("x") are given. Abbreviations: AT Andreas Tribsch; CD Christoph Dobeš; FDN Flavia Domizia Nardi; HAM Henar Alonso-Marcos; JAH Julian Ananda Haider; JP Juraj Paule; RS Roswitha Schmickl; SSc Susanne Scheffknecht; SSt Simon Stifter; TW Thomas Wilhalm.

Name	Longitude	Latitude	Country	Collection year	Collector	FCM	Soil subset	N	N 4*x*	N 5*x*	N 6*x*	N 7*x*	N 8*x*	N Sexuals	N Apomicts
Affenhausen	10.99608	47.30922	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	18	0	6	0	12	0	0	18
Ainet	12.68615	46.86663	Austria	2009	CD	present study		9	0	9	0	0	0	0	9
AlaE	11.02516	45.7554	Italy	2015	AT	Nardi et al. (2018)	x	18	18	0	0	0	0	18	0
Aldein	11.35436	46.37103	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	22	0	21	0	0	1	0	22
Ambach	10.86583	47.22028	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	19	0	1	0	0	19	0
Antholz	12.099	46.84659	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Arco Klettersteig	10.88811	45.92728	Italy	2010	CD	present study		18	18	0	0	0	0	18	0
Arco NE	10.89694	45.93125	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	19	19	0	0	0	0	19	0
Ardez	10.20403	46.77347	Switzerland	2015	HAM, FDN	Nardi et al. (2018)	x	10	10	0	0	0	0	10	0

Arnig N	12.6324	46.98423	Austria	2009	CD	present study		13	0	9	4	0	0	0	9
Arzler Alm	11.40576	47.29476	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	0	20	0	0	0	0	20
Aufler Klaunzerberg	12.55929	46.97456	Austria	2009	CD	present study		20	0	16	0	4	0	0	20
Avio	10.93042	45.73367	Italy	2015	AT	Nardi et al. (2018)	x	14	14	0	0	0	0	14	0
Avio 500	10.92178	45.71704	Italy	2013	CD	present study		5	0	0	0	5	0	0	5
Bagni Lusnizza	13.36923	46.5055	Italy	2015	CD	Nardi et al. (2018)	x	8	0	0	8	0	0	0	0
Beseno	11.10473	45.92957	Italy	2013	CD	present study		7	0	5	0	0	2	0	7
Bichl	12.539	46.98468	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	12	5	6	0	1	0	5	7
Birkach	10.55203	46.97808	Austria	2010	CD	present study		18	16	0	2	0	0	16	0
Bobojach	12.40425	47.01677	Austria	2009	CD	present study		14	12	2	0	0	0	12	2
Boedenalm	11.69811	46.92309	Italy	2015	HAM, FDN, SSt, JAH	Nardi et al. (2018)	x	18	12	6	0	0	0	12	6
Bozen Gries	11.33083	46.50664	Italy	2010	CD & SSc	present study		20	19	0	1	0	0	19	0
Brenito Belluno	10.87395	45.64632	Italy	2015	HAM, FDN, AT, CD	Nardi et al. (2018)	x	16	16	0	0	0	0	16	0
Bruneck	11.9404	46.79348	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Buchholz	11.24806	46.24878	Italy	2015	AT, FDN	Nardi et al. (2018)	x	20	0	7	0	7	6	0	20
Burg Berneck	10.70447	47.07828	Austria	2010	CD	present study		8	8	0	0	0	0	8	0
Burgfrieden	12.71358	46.79813	Austria	2009	CD	present study		12	0	8	4	0	0	0	8
Burgstall Kofler	11.19472	46.62228	Italy	2006	CD	present study		8	0	6	0	2	0	0	8
CampioloW	13.13437	46.39156	Italy	2015	CD	Nardi et al. (2018)	x	7	4	2	0	1	0	4	3
Canale	10.83288	45.59015	Italy	2013	CD	present study		15	15	0	0	0	0	15	0
Casalicolo	10.44345	45.60779	Italy	2013	CD	present study		8	7	0	1	0	0	7	0
Castelfeder	11.29108	46.33633	Italy	2010	CD & SSc	present study		20	18	0	2	0	0	18	0

Castellano	11.43325	46.27797	Italy	2015	AT, FDN	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0
Ceraino	10.82795	45.57418	Italy	2013	CD	present study		13	13	0	0	0	0	13	0
Dabaklamm	12.63125	47.02445	Austria	2009	CD	present study		27	0	26	1	0	0	0	26
Eigenhofen	11.19972	47.28222	Austria	2008	CD	present study		13	8	1	0	4	0	8	5
Elvas	11.66684	46.72881	Italy	2011	CD	present study		25	25	0	0	0	0	25	0
Erlach Forststrafle	12.3389	46.92442	Austria	2009	CD	present study		17	0	17	0	0	0	0	17
Erlach Gehoeft	12.33858	46.92237	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	11	0	11	0	0	0	0	11
Erlbach	12.36958	46.74653	Austria	2009	CD	present study		25	0	6	0	15	4	0	25
Eyrs	10.63164	46.63105	Italy	2010	CD & SSc	present study		20	20	0	0	0	0	20	0
Faggen1	10.67747	47.0765	Austria	2010	CD	present study		8	8	0	0	0	0	8	0
Faggen2	10.67508	47.07758	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Feld SSE	12.57247	46.95087	Austria	2009	CD	present study		8	4	4	0	0	0	4	4
Feldthurns	11.60049	46.66362	Italy	2015	CD	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0
Fineil	10.82583	46.74197	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	8	0	4	0	4	0	0	8
Finele	11.16506	46.6992	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	14	14	0	0	0	0	14	0
Fischleinbach	12.35239	46.66342	Italy	2011	CD	present study		15	0	0	0	15	0	0	15
Fiss	10.62539	47.05064	Austria	2010	CD	present study		9	9	0	0	0	0	9	0
Flans	11.46227	46.91411	Italy	2015	CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Flathalpe	10.55844	47.13292	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	11	0	11	0	0	0	0	11
Fliefl	10.63917	47.11611	Austria	2008	CD	present study		19	18	0	1	0	0	18	0
Flirsch	10.41042	47.15181	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	11	0	11	0	0	0	0	11
Forte Masua	10.87572	45.59223	Italy	2015	HAM, CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0

Fosse	10.9124	45.64068	Italy	2013	CD	present study		20	6	5	1	6	2	6	13
Franzensfeste	11.62985	46.77655	Italy	2015	CD	Nardi et al. (2018)	x	20	0	6	0	14	0	0	20
Frosnitztal	12.50262	47.04543	Austria	2009	CD	present study		17	0	17	0	0	0	0	17
Gaid	11.21383	46.50864	Italy	2010	CD & SSc	present study		20	0	0	0	11	9	0	20
Gaium Croce	10.82355	45.55506	Italy	2015	HAM, FDN, AT, CD	Nardi et al. (2018)	x	14	14	0	0	0	0	14	0
Garniga Therme	11.08814	46.00511	Italy	2011	CD	present study		24	0	1	6	6	11	0	18
Garniga Vecchio	11.08031	46.01447	Italy	2011	CD	present study		15	0	10	0	5	0	0	15
Gemona	13.14602	46.28551	Italy	2015	CD	Nardi et al. (2018)	x	19	0	4	2	13	0	0	17
Gonzach	12.66217	46.87545	Austria	2009	CD	present study		22	0	19	3	0	0	0	19
Graun	10.54503	46.80778	Italy	2010	CD & SSc	present study		20	20	0	0	0	0	20	0
Greit	10.56528	46.95917	Austria	2008	CD	present study		26	26	0	0	0	0	26	0
Grezzana	11.01033	45.5348	Italy	2015	CD	Nardi et al. (2018)	x	20	19	0	1	0	0	19	0
Grins2	10.53644	47.14308	Austria	2010	CD	present study		9	0	3	0	6	0	0	9
Grizzo	12.64385	46.15783	Italy	2015	CD	Nardi et al. (2018)	x	9	0	0	0	9	0	0	9
Groder	12.33223	47.01812	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	10	5	4	1	0	0	5	4
Gruben	12.51435	47.04818	Austria	2009	CD	present study		28	0	25	0	3	0	0	28
Grumes	11.29131	46.22039	Italy	2015	AT, FDN	Nardi et al. (2018)	x	18	0	6	9	3	0	0	9
Guarda	10.15072	46.77442	Switzerland	2010	CD	present study		12	12	0	0	0	0	12	0
Guardia	11.13076	45.90604	Italy	2013	CD	present study		11	0	3	2	6	0	0	9
Hafling Oberdorf	11.2195	46.65114	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	19	0	7	0	11	1	0	19
Heinfels Schloss	12.43778	46.75024	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	16	11	0	0	5	0	11	5
Hinterbichl N	12.34003	47.0202	Austria	2009	CD	present study		23	23	0	0	0	0	23	0

Hochmuth	11.12447	46.70395	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Hof	12.49742	46.92583	Austria	2009	CD	present study		13	0	6	7	0	0	0	6
Hopfgarten	12.52588	46.92562	Austria	2009	CD	present study		18	0	17	1	0	0	0	17
Huben	10.97861	47.04167	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	0	12	0	0	0	0	12
Innervillgraten	12.36117	46.81235	Austria	2009	CD	present study		15	4	3	5	3	0	4	6
Innichen	12.28697	46.73611	Italy	2011	CD	present study		24	24	0	0	0	0	24	0
Kaltern	11.28192	46.38514	Italy	2010	CD & SSc	present study		18	18	0	0	0	0	18	0
Katalalm	12.49057	47.05703	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	12	0	12	0	0	0	0	12
Kauderle	11.20222	46.30764	Italy	2010	CD & SSc	present study		7	0	2	0	5	0	0	7
Kaunertal Maut	10.73756	47.01928	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	0	12	0	0	0	0	12
Kauns	10.69806	47.08028	Austria	2008	CD	present study		14	13	0	0	1	0	13	1
Kematen	11.54327	46.96069	Italy	2015	CD	Nardi et al. (2018)	x	19	0	8	6	3	2	0	13
Kiens	11.85225	46.79899	Italy	2011	CD	present study		18	18	0	0	0	0	18	0
Kortsch	10.76367	46.63458	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Kosten	12.60242	46.78637	Austria	2009	CD	present study		20	0	18	0	0	2	0	20
Ladis	10.65247	47.07605	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Ladner	10.39094	47.06797	Austria	2010	CD	present study		16	0	13	0	3	0	0	16
Laengenfeld	10.96583	47.08083	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	20	0	0	0	0	20	0
Lafairs	10.565	46.99	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	11	11	0	0	0	0	11	0
Lago di Lago	12.21845	45.98977	Italy	2015	CD	Nardi et al. (2018)	x	17	0	6	3	3	5	0	14
Lana S	12.63151	46.98602	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	10	0	5	4	0	1	0	6
Lasino	10.97567	46.02592	Italy	2015	AT, FDN	Nardi et al. (2018)	x	21	19	0	2	0	0	19	0

Latzfons	11.55543	46.67389	Italy	2015	CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Lavin	10.11092	46.77053	Switzerland	2010	CD	present study		15	15	0	0	0	0	15	0
Luttach	11.91475	46.94756	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	19	16	3	0	0	0	16	3
M Locherboden	10.96294	47.28171	Austria	2011	CD	present study		21	20	1	0	0	0	20	1
Maria Hilf	12.29258	46.91262	Austria	2009	CD	present study		9	0	8	0	1	0	0	9
Martell	10.76136	46.5655	Italy	2011	TW	present study		19	13	0	6	0	0	13	0
Matrei B	11.45006	47.12288	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	16	0	1	0	15	0	0	16
Matrei NE	12.54415	47.00783	Austria	2009	CD	present study		23	5	15	2	1	0	5	16
Mattersberger	12.56735	46.962	Austria	2009	CD	present study		10	0	10	0	0	0	0	10
Mauls	11.5227	46.85529	Italy	2011	CD	present study		18	16	0	0	2	0	16	2
Maxer	12.33606	46.81352	Austria	2009	CD	present study		11	0	11	0	0	0	0	11
Melag	10.65644	46.83958	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	13	13	0	0	0	0	13	0
Mellitz	12.45663	46.9291	Austria	2009	CD	present study		29	0	29	0	0	0	0	29
Mezzocorona	11.12591	46.22478	Italy	2011	CD	present study		7	5	0	0	2	0	5	2
Mezzomonte	11.13336	45.92031	Italy	2015	HAM, CD	Nardi et al. (2018)	x	19	0	9	0	9	1	0	19
Mieders	11.37576	47.1694	Austria	2011	CD	present study		5	0	5	0	0	0	0	5
Mignano	11.70737	45.82835	Italy	2015	CD	Nardi et al. (2018)	x	20	15	2	0	2	1	15	5
Monreale	11.15578	46.20694	Italy	2015	HAM, CD	Nardi et al. (2018)	x	19	18	0	1	0	0	18	0
Moos Passeier	11.16847	46.83247	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	22	0	16	0	6	0	0	22
Mosson	11.43974	45.78665	Italy	2015	CD	Nardi et al. (2018)	x	21	14	4	0	3	0	14	7
Muehlbach	11.97746	46.84848	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	10	10	0	0	0	0	10	0
Nals	11.18589	46.53036	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	19	0	10	0	6	3	0	19

Nals Wehrburg	11.18753	46.55028	Italy	2010	CD & SSc	present study		18	3	6	1	2	6	3	14
Nauders	10.51028	46.89417	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	20	0	0	0	0	20	0
Niedermauern	12.43375	47.00242	Austria	2009	CD	present study		12	12	0	0	0	0	12	0
Oberassling	12.63775	46.78937	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	19	0	9	4	6	0	0	15
Oberbergtal	11.26232	47.11823	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	21	0	19	1	0	1	0	20
Oberbichl	12.36163	47.0224	Austria	2009	CD	present study		9	9	0	0	0	0	9	0
Oberdrauburg	12.96362	46.75371	Austria	2015	CD	Nardi et al. (2018)	x	15	0	0	14	0	1	0	1
Oberegg	13.112694	47.78117	Austria	2015	AT, FDN	Nardi et al. (2018)		20	0	6	0	14	0	0	20
Obergaimberg	12.7822	46.84623	Austria	2009	CD	present study		16	0	4	0	12	0	0	16
Obermauern	12.43528	47.00477	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0
Oberpeischlach	12.59413	46.93587	Austria	2009	CD	present study		30	0	26	0	4	0	0	30
Obersteiner	12.40872	47.01787	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	18	11	4	1	2	0	11	6
Olang	12.04316	46.77096	Italy	2011	CD	present study		23	21	0	1	1	0	21	1
Ossenigo	10.90949	45.67443	Italy	2015	HAM, FDN, AT, CD	Nardi et al. (2018)	x	15	9	4	0	1	1	9	6
Passeier Spath	11.20694	46.76222	Italy	2010	CD & SSc	present study		6	0	6	0	0	0	0	6
Passeier-Moos	11.19567	46.82269	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	14	0	12	2	0	0	0	12
Patsch	11.40512	47.20982	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	0	7	0	13	0	0	20
Pavone	10.43776	45.65391	Italy	2015	HAM, FDN, CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Perdross	10.57831	46.81395	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	14	12	2	0	0	0	12	2
Pettnau	11.1253	47.30593	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	11	11	0	0	0	0	11	0
Pflersch Ende	11.33019	46.96975	Italy	2015	CD	Nardi et al. (2018)	x	11	0	10	0	0	1	0	11
Pfossertal Abzw	10.91764	46.706	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0

Pfunders	11.69771	46.90367	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Pians	10.50642	47.13172	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	11	0	11	0	0	0	0	11
Planeil	10.57711	46.72214	Italy	2010	CD & SSc	present study		17	17	0	0	0	0	17	0
Plenten	11.53583	47.04639	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	0	17	0	1	2	0	20
Pontebba	13.31276	46.51041	Italy	2015	CD	Nardi et al. (2018)	x	21	0	1	20	0	0	0	1
Praegraten N	12.37452	47.02117	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Praemajur	10.50964	46.70414	Italy	2010	CD & SSc	present study		20	18	0	2	0	0	18	0
Prags	12.13593	46.72472	Italy	2011	CD	present study		25	25	0	0	0	0	25	0
Punt de la Resgia	10.40986	46.83493	Switzerland	2010	CD	present study		13	13	0	0	0	0	13	0
Raas W	11.6535	46.74708	Italy	2015	CD	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0
Rabenstein	12.4664	47.00887	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	12	0	12	0	0	0	0	12
Ramosch	10.3739	46.83735	Switzerland	2010	CD	present study		13	9	0	4	0	0	9	0
Raneburg	12.52795	47.0673	Austria	2009	CD	present study		25	0	25	0	0	0	0	25
Ratzell	12.53903	46.92542	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	12	0	12	0	0	0	0	12
Raut	12.5748	46.78128	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	9	5	4	0	0	0	5	4
Reifenstein	11.44342	46.87856	Italy	2015	CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Ridnaun	11.3093	46.91546	Italy	2015	CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Rodenegg	11.69085	46.77489	Italy	2011	CD	present study		16	16	0	0	0	0	16	0
Ronchi	11.06998	45.74151	Italy	2015	HAM, FDN, CD	Nardi et al. (2018)	x	12	0	0	0	12	0	0	12
Roppen	10.81667	47.22083	Austria	2008	CD	present study		28	26	2	0	0	0	26	2
Rossbach	10.87171	47.31492	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	0	12	0	0	0	0	12
Roveredo	13.24515	46.39924	Italy	2015	CD	Nardi et al. (2018)	x	10	0	0	10	0	0	0	0

S. Valentino	10.907799	45.77776	Italy	2015	AT, FDN	Nardi et al. (2018)		6	0	4	0	2	0	0	6
Saeben Burg	11.56974	46.64646	Italy	2011	CD	present study		19	18	0	1	0	0	18	0
Saeben N	11.58138	46.65169	Italy	2015	CD	Nardi et al. (2018)	x	18	3	4	0	11	0	3	15
San Eusebio	10.36253	45.61144	Italy	2015	HAM, FDN, CD	Nardi et al. (2018)	x	20	20	0	0	0	0	20	0
Santantonio	10.93782	45.85773	Italy	2015	HAM, CD	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Santosso	11.38215	45.74177	Italy	2015	CD	Nardi et al. (2018)	x	20	20	0	0	0	0	20	0
Scaiola	10.36401	45.53424	Italy	2015	HAM, CD	Nardi et al. (2018)	x	20	8	4	0	8	0	8	12
Schlaneid	11.23403	46.57875	Italy	2010	CD & SSc	present study		11	11	0	0	0	0	11	0
Schluderns	10.58537	46.66813	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	14	14	0	0	0	0	14	0
Schmirn	11.54472	47.07333	Austria	2008	CD	present study		16	0	12	4	0	0	0	12
Schmuders	11.46227	46.91411	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Schnalstal	10.88031	46.72386	Italy	2011	TW	present study		24	22	0	2	0	0	22	0
Schnann	10.37955	47.15446	Austria	2010	CD	present study		16	0	16	0	0	0	0	16
Schrottendorf	12.67388	46.79195	Austria	2009	CD	present study		10	0	3	7	0	0	0	3
Scuol-Ftan	10.26639	46.79133	Switzerland	2010	CD	present study		8	8	0	0	0	0	8	0
Sent	10.3495	46.81742	Switzerland	2015	HAM, FDN	Nardi et al. (2018)	x	19	19	0	0	0	0	19	0
Serravalle	11.01438	45.84627	Italy	2013	CD	present study		20	14	3	0	3	0	14	6
Sigmundskron	11.30494	46.47972	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	14	14	0	0	0	0	14	0
Silz N	10.91909	47.27242	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	19	19	0	0	0	0	19	0
Sinsen	10.35383	47.05372	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	9	0	4	0	0	5	0	9
Soelden	11.01301	46.95997	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	18	0	18	0	0	0	0	18
Soell	11.243	46.35469	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	19	19	0	0	0	0	19	0

Soelles	10.56327	46.6535	Italy	2006	CD	present study		8	8	0	0	0	0	8	0
Sonneburg	11.89358	46.78889	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	10	10	0	0	0	0	10	0
Sprechenstein	11.45618	46.88377	Italy	2011	CD	present study		21	21	0	0	0	0	21	0
St. Catarina	13.40105	46.50338	Italy	2015	CD	Nardi et al. (2018)	x	6	0	0	2	0	4	0	4
St. Jakob	11.99053	47.00096	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	20	17	2	1	0	0	17	2
St. Justina	12.58503	46.78878	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	10	1	9	0	0	0	1	9
St. Magdalena	12.23682	46.83717	Italy	2011	CD	present study		23	0	14	9	0	0	0	14
St. Peter	12.05884	47.02325	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
St. Sigmund	11.78566	46.81947	Italy	2015	CD	Nardi et al. (2018)	x	20	18	2	0	0	0	18	2
St. Sigmund Sellrain	11.11333	47.20444	Austria	2008	CD	present study		6	0	6	0	0	0	0	6
St. Ulrich	11.63529	46.5842	Italy	2015	CD	Nardi et al. (2018)	x	20	0	8	1	11	0	0	19
St. Veit	12.4221	46.92665	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	11	0	11	0	0	0	0	11
Staben	10.96417	46.64806	Italy	2009	JP & RS	present study		5	5	0	0	0	0	5	0
Stein	12.5281	47.02602	Austria	2015	SSt, JAH	Nardi et al. (2018)	x	9	0	3	0	2	4	0	9
Stein S	10.58008	46.99653	Austria	2010	CD	present study		14	13	0	1	0	0	13	0
Stroeden	12.3183	47.01762	Austria	2009	CD	present study		14	0	14	0	0	0	0	14
Strumerhof	12.51758	47.01022	Austria	2009	CD	present study		9	0	3	3	3	0	0	6
Tappein	11.16036	46.67886	Italy	2010	CD	present study		15	15	0	0	0	0	15	0
Taufers	11.94939	46.9229	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Terlan Mendel	11.24233	46.43081	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	19	19	0	0	0	0	19	0
Terlan N	11.25383	46.5443	Italy	2010	CD & SSc	present study		13	12	0	1	0	0	12	0

Thaur	11.48701	47.29489	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Thurn Ruine	12.1135	46.76267	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0
Tisens	11.54825	46.57006	Italy	2010	CD & SSc	present study		10	0	0	0	0	10	0	10
Toblach	12.21366	46.74275	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	12	11	0	1	0	0	11	0
Toldern	11.57674	47.0916	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	0	15	0	4	1	0	20
Trostburg	11.53644	46.58533	Italy	2015	CD	Nardi et al. (2018)	x	20	16	0	0	4	0	16	4
Tschoetscher H	11.64574	46.70514	Italy	2015	CD	Nardi et al. (2018)	x	20	20	0	0	0	0	20	0
Unteralbkus	12.69247	46.87547	Austria	2009	CD	present study		13	0	7	4	2	0	0	9
Unterfederaun	13.8124	46.57027	Austria	2015	CD	Nardi et al. (2018)	x	8	0	0	0	5	3	0	8
Unterfennberg	11.18047	46.27308	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	19	0	15	0	4	0	0	19
Unterleibning	12.63598	46.90305	Austria	2009	CD	present study		26	0	24	1	0	1	0	25
Unterparggen	12.36419	46.74742	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	17	0	6	0	10	1	0	17
Unterwaldalm	12.53593	47.07657	Austria	2009	CD	present study		18	0	18	0	0	0	0	18
Uttenheim	11.9365	46.86932	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Vellau Leiteralm	11.11092	46.70644	Italy	2010	CD	present study		20	20	0	0	0	0	20	0
Vellau W	11.10275	46.69303	Italy	2010	CD	present study		14	14	0	0	0	0	14	0
Vergein	12.58432	46.79387	Austria	2009	CD	present study		8	0	8	0	0	0	0	8
Vernagt	10.85089	46.73864	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	12	10	0	1	1	0	10	1
Vill	11.40614	47.23673	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	12	12	0	0	0	0	12	0
Vinaders	11.47236	47.03329	Austria	2015	HAM, FDN, SSt, JAH	Nardi et al. (2018)	x	15	0	10	0	0	5	0	15
Virgen	12.45863	47.00554	Austria	1990	CD	present study		10	0	2	0	8	0	0	10
Voels N	11.49778	46.52592	Italy	2006	CD	present study		10	0	5	0	5	0	0	10

Wassermann	11.82914	46.89418	Italy	2011	CD	present study		20	20	0	0	0	0	20	0
Welschnofen	11.54412	46.43325	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	20	0	4	0	16	0	0	20
Zabernig	12.51747	47.00618	Austria	2009	CD	present study		16	0	10	0	6	0	0	16
Zaunhof	10.82177	47.10001	Austria	2010	CD	present study		17	15	0	2	0	0	15	0
Zedlach NW	12.4899	47.00378	Austria	2009	CD	present study		26	0	25	0	1	0	0	26
Zernez	10.10152	46.6981	Switzerland	2009	CD	present study		30	28	0	2	0	0	28	0
Zerzertal	10.51728	46.75	Italy	2015	HAM, FDN	Nardi et al. (2018)	x	22	12	9	0	1	0	12	10
Zirl Ruine	11.23942	47.27785	Austria	2015	HAM, FDN	Nardi et al. (2018)	x	20	20	0	0	0	0	20	0
Zoesen	11.77515	46.91885	Italy	2015	SSt, JAH	Nardi et al. (2018)	x	9	9	0	0	0	0	9	0
Zwischenwasser	11.89436	46.7234	Italy	2011	CD	present study	subset	26	16	9	0	1	0	16	10

Table A2. Spearman's correlation coefficients among ecological variables. Abbreviations: normalised difference vegetation index (NDVI), annual mean temperature (bio01), mean diurnal range (bio02), isothermality (bio03), temperature seasonality (bio04), max temperature of warmest month (bio05), min temperature of coldest month (bio06), temperature annual range (bio07), mean temperature of wettest quarter (bio08), mean temperature of driest quarter (bio09), mean temperature of warmest quarter (bio10), mean temperature of coldest quarter (bio11), annual precipitation (bio12), precipitation of wettest month (bio13), precipitation of driest month (bio14), precipitation seasonality (bio15), precipitation of wettest quarter (bio16), precipitation of driest quarter (bio17), precipitation of warmest quarter (bio18), precipitation of coldest quarter (bio19).

	Elevation	**Inclination**	**Aspect**	**NDVI**	**bio01**	**bio02**	**bio03**	**bio04**	**bio05**	**bio06**	**bio07**	**bio08**	**bio09**	**bio10**	**bio11**	**bio12**	**bio13**	**bio14**	**bio15**	**bio16**	**bio17**	**bio18**	**bio19**
Elev.	1.00	0.08	0.03	0.06	-0.96	0.27	0.65	-0.72	-0.96	-0.95	-0.57	-0.74	-0.80	-0.96	-0.96	-0.19	-0.05	-0.23	0.37	-0.03	-0.23	0.13	-0.18
Incl.	0.08	1.00	0.02	0.10	-0.09	0.00	0.13	-0.17	-0.10	-0.07	-0.14	0.05	-0.01	-0.09	-0.07	-0.13	-0.14	-0.05	-0.01	-0.14	-0.05	-0.13	-0.05
Aspect	0.03	0.02	1.00	0.02	-0.03	0.00	0.04	-0.04	-0.03	-0.04	-0.06	0.01	-0.05	-0.03	-0.03	0.06	0.10	0.05	0.05	0.11	0.04	0.14	0.04
NDVI	0.06	0.1	0.02	1.00	-0.07	0.20	0.06	0.08	-0.06	-0.10	0.09	-0.04	-0.11	-0.06	-0.09	0.22	0.23	0.14	0.03	0.23	0.14	0.24	0.13
bio01	-0.96	-0.09	-0.03	-0.07	1.00	-0.35	-0.72	0.73	1.00	0.99	0.57	0.77	0.84	1.00	1.00	0.15	0.00	0.19	-0.36	-0.03	0.19	-0.20	0.13
bio02	0.27	0.00	0.00	0.2	-0.35	1.00	0.52	0.07	-0.32	-0.44	0.27	-0.19	-0.51	-0.33	-0.40	0.19	0.23	-0.03	0.28	0.25	-0.03	0.33	0.03
bio03	0.65	0.13	0.04	0.06	-0.72	0.52	1.00	-0.77	-0.73	-0.70	-0.62	-0.41	-0.51	-0.74	-0.70	-0.12	0.08	-0.07	0.39	0.11	-0.05	0.29	0.02

bio04	-0.72	-0.17	-0.04	0.08	0.73	0.07	-0.77	1.00	0.76	0.64	0.95	0.43	0.38	0.75	0.67	0.36	0.16	0.17	-0.31	0.14	0.16	-0.03	0.12
bio05	-0.96	-0.1	-0.03	-0.06	1.00	-0.32	-0.73	0.76	1.00	0.98	0.60	0.76	0.82	1.00	0.99	0.16	0.01	0.19	-0.36	-0.02	0.19	-0.19	0.13
bio06	-0.95	-0.07	-0.04	-0.10	0.99	-0.44	-0.70	0.64	0.98	1.00	0.47	0.76	0.88	0.98	1.00	0.11	-0.03	0.20	-0.38	-0.05	0.20	-0.22	0.13
bio07	-0.57	-0.14	-0.06	0.09	0.57	0.27	-0.62	0.95	0.60	0.47	1.00	0.31	0.23	0.59	0.50	0.40	0.21	0.18	-0.27	0.20	0.17	0.05	0.15
bio08	-0.74	0.05	0.01	-0.04	0.77	-0.19	-0.41	0.43	0.76	0.76	0.31	1.00	0.68	0.76	0.77	-0.17	-0.29	-0.09	-0.09	-0.29	-0.09	-0.37	-0.15
bio09	-0.80	-0.01	-0.05	-0.11	0.84	-0.51	-0.51	0.38	0.82	0.88	0.23	0.68	1.00	0.82	0.86	0.03	-0.03	0.22	-0.34	-0.05	0.22	-0.19	0.20
bio10	-0.96	-0.09	-0.03	-0.06	1.00	-0.33	-0.74	0.75	1.00	0.98	0.59	0.76	0.82	1.00	0.99	0.16	0.00	0.19	-0.36	-0.02	0.19	-0.20	0.12
bio11	-0.96	-0.07	-0.03	-0.09	1.00	-0.40	-0.70	0.67	0.99	1.00	0.50	0.77	0.86	0.99	1.00	0.12	-0.02	0.19	-0.36	-0.05	0.19	-0.22	0.12
bio12	-0.19	-0.13	0.06	0.22	0.15	0.19	-0.12	0.36	0.16	0.11	0.40	-0.17	0.03	0.16	0.12	1.00	0.93	0.88	-0.50	0.92	0.88	0.83	0.86
bio13	-0.05	-0.14	0.10	0.23	0.00	0.23	0.08	0.16	0.01	-0.03	0.21	-0.29	-0.03	0.00	-0.02	0.93	1.00	0.83	-0.28	1.00	0.83	0.94	0.84
bio14	-0.23	-0.05	0.05	0.14	0.19	-0.03	-0.07	0.17	0.19	0.20	0.18	-0.09	0.22	0.19	0.19	0.88	0.83	1.00	-0.68	0.82	1.00	0.74	0.98
bio15	0.37	-0.01	0.05	0.03	-0.36	0.28	0.39	-0.31	-0.36	-0.38	-0.27	-0.09	-0.34	-0.36	-0.36	-0.50	-0.28	-0.68	1.00	-0.25	-0.68	-0.13	-0.66

bio16	-0.03	-0.14	0.11	0.23	-0.03	0.25	0.11	0.14	-0.02	-0.05	0.20	-0.29	-0.05	-0.02	-0.05	0.92	1.00	0.82	-0.25	1.00	0.83	0.96	0.83
bio17	-0.23	-0.05	0.04	0.14	0.19	-0.03	-0.05	0.16	0.19	0.20	0.17	-0.09	0.22	0.19	0.19	0.88	0.83	1.00	-0.68	0.83	1.00	0.74	0.98
bio18	0.13	-0.13	0.14	0.24	-0.20	0.33	0.29	-0.03	-0.19	-0.22	0.05	-0.37	-0.19	-0.20	-0.22	0.83	0.94	0.74	-0.13	0.96	0.74	1.00	0.75
bio19	-0.18	-0.05	0.04	0.13	0.13	0.03	0.02	0.12	0.13	0.13	0.15	-0.15	0.20	0.12	0.12	0.86	0.84	0.98	-0.66	0.83	0.98	0.75	1.00

Table A3. Spearman's correlation coefficients among soil variables. Abbreviations: C_{org} organic carbon; N_{tot} total nitrogen; CEC cation exchange capacity; BS base saturation.

	pH	**CO_3^{2-}**	**C_{org}**	**N_{tot}**	**C/N**	**K^+**	**Na^+**	**Ca^{2+}**	**Mg^{2+}**	**Mn^{3+}**	**Al^{3+}**	**Fe^{3+}**	**H^+**	**CEC**	**BS**
pH	1.00	0.83	0.18	0.11	0.15	-0.32	-0.17	0.54	0.24	-0.93	-0.75	-0.70	-0.65	0.51	0.79
CO_3^{2-}	0.83	1.00	0.29	0.18	0.22	-0.44	-0.17	0.36	0.21	-0.77	-0.45	-0.53	-0.33	0.35	0.51
C_{org}	0.18	0.29	1.00	0.95	-0.01	0.12	0.29	0.72	0.56	-0.08	0.01	0.13	-0.03	0.77	0.20
N_{tot}	0.11	0.18	0.95	1.00	-0.28	0.15	0.33	0.72	0.55	0.00	0.03	0.15	-0.02	0.77	0.14
C/N	0.15	0.22	-0.01	-0.28	1.00	-0.02	-0.17	-0.15	-0.02	-0.19	-0.02	-0.04	0.00	-0.15	0.09
K^+	-0.32	-0.44	0.12	0.15	-0.02	1.00	0.23	0.02	0.14	0.28	0.07	0.07	0.09	0.06	-0.09
Na^+	-0.17	-0.17	0.29	0.33	-0.17	0.23	1.00	0.17	0.21	0.15	0.07	0.12	0.20	0.21	-0.12
Ca^{2+}	0.54	0.36	0.72	0.72	-0.15	0.02	0.17	1.00	0.48	-0.41	-0.49	-0.23	-0.51	0.99	0.60
Mg^{2+}	0.24	0.21	0.56	0.55	-0.02	0.14	0.21	0.48	1.00	-0.19	-0.15	-0.12	-0.30	0.59	0.35
Mn^{3+}	-0.93	-0.77	-0.08	0.00	-0.19	0.28	0.15	-0.41	-0.19	1.00	0.69	0.67	0.58	-0.38	-0.81
Al^{3+}	-0.75	-0.45	0.01	0.03	-0.02	0.07	0.07	-0.49	-0.15	0.69	1.00	0.75	0.70	-0.45	-0.75
Fe^{3+}	-0.70	-0.53	0.13	0.15	-0.04	0.07	0.12	-0.23	-0.12	0.67	0.75	1.00	0.56	-0.21	-0.59
H^+	-0.65	-0.33	-0.03	-0.02	0.00	0.09	0.20	-0.51	-0.30	0.58	0.70	0.56	1.00	-0.48	-0.77
CEC	0.51	0.35	0.77	0.77	-0.15	0.06	0.21	0.99	0.59	-0.38	-0.45	-0.21	-0.48	1.00	0.58
BS	0.79	0.51	0.20	0.14	0.09	-0.09	-0.12	0.60	0.35	-0.81	-0.75	-0.59	-0.77	0.58	1.00

Table A4. Pairwise comparison of soil variables among sexual and apomictic cytotypes of *Potentilla puberula*. Coefficients of regression ± standard errors are given. Positive and negative coefficients indicate higher and lower values for the cytotypes given first in the column headings, respectively. No significant difference was found for any comparison. Abbreviations: N_{tot} total nitrogen.

Variable	Sexuals vs Apomicts			Apomicts vs Apomicts		
	Tetra- vs Pentaploids	**Tetra- vs Heptaploids**	**Tetra- vs Octoploids**	**Penta- vs Heptaploids**	**Penta- vs Octoploids**	**Hepta- vs Octoploids**
CO_3^{2-}	0.29 ± 0.20	0.15 ± 0.22	-0.04 ± 0.24	-0.16 ± 0.21	-0.33 ± 0.24	-0.21 ± 0.27
N_{tot}	0.06 ± 0.18	-0.26 ± 0.20	-0.02 ± 0.25	-0.30 ± 0.20	-0.09 ± 0.26	0.28 ± 0.32
C/N	0.12 ± 0.19	0.01 ± 0.21	0.00 ± 0.25	-0.10 ± 0.22	-0.10 ± 0.25	-0.01 ± 0.25
K^+	0.09 ± 0.18	-0.13 ± 0.19	0.28 ± 0.30	-0.19 ± 0.20	0.14 ± 0.27	0.32 ± 0.31
Na^+	-0.05 ± 0.18	-0.13 ± 0.20	0.20 ± 0.31	-0.09 ± 0.20	0.29 ± 0.30	0.42 ± 0.33
Ca^{2+}	0.07 ± 0.18	-0.24 ± 0.20	-0.03 ± 0.25	-0.34 ± 0.20	-0.11 ± 0.25	0.28 ± 0.31
Mg^{2+}	-0.12 ± 0.17	-0.33 ± 0.20	-0.25 ± 0.23	-0.21 ± 0.20	-0.14 ± 0.26	0.12 ± 0.30
Al^{3+}	-0.01 ± 0.18	0.91 ± 0.67	0.39 ± 0.44	1.02 ± 0.77	0.42 ± 0.49	-0.19 ± 0.31
Fe^{3+}	-0.14 ± 0.19	0.24 ± 0.23	0.03 ± 0.25	0.52 ± 0.35	0.20 ± 0.33	-0.23 ± 0.28
H^+	0.08 ± 0.18	0.73 ± 0.47	0.38 ± 0.39	0.67 ± 0.44	0.30 ± 0.35	-0.17 ± 0.30

Table A5. Survey of published studies on ecological parthenogenesis of sexual-apomictic taxa. "Ploidy of sexuals" and "apomicts" determined within the cited study are given (in brackets, the ploidy of known but not assessed cytotypes is reported). We distinguish between an auto- and allopolyploid "origin of apomicts", and the question mark indicates a possible allopolyploid origin of apomicts. "Taxonomic relationship" indicates whether sexual-apomictic contrasts occur at the species (intraspecific) or generic level (interspecific). The geographic distribution of reproductive modes relative to each other is classified as sympatry, parapatry, allopatry, and "parasympatry", the latter here defined as the condition in which apomicts have a wider geographic distribution, which totally includes that of the sexuals. "Ecological differentiation" between sexual and apomict cytotypes ("yes" or "no") and whether an "immediate role of ecology as a driver of the observed distribution pattern" can be told apart from effects related to differential migration abilities of reproductive modes ("yes", "no" or "not applicable", in case of no ecological differentiation) are also given. We further conclude on whether the ecological distribution pattern can be attributed to effects of reproductive mode, ploidy level, or hybridisation. The reasons for "uncertainty" are provided according to the following scheme: evolutionary origin of apomictic cytotypes unknown (A), ploidy level was not assessed within the study (B), evolutionary divergence or hybridity (C), reproductive mode was not assessed within the study (D), sexual contrast missing (E), existence of only one cytotype linked to one reproductive mode (F), relationship among apomictic ploidy levels not studied (G). The "main result" of the study is also reported.

Taxon	**Ploidy of sexuals**	**Ploidy of apomicts**	**Origin of apomicts**	**Taxonomic relationship of sexuals and apomicts**	**Geographical relationship of sampled sexual and apomictic populations**	**Ecological differentiation**	**Can an immediate role of ecology as a driver of the observed differentiation be told apart from spatial effects?**	**Ecological differentiation pattern attributable to ploidy level, reproductive mode or other factors?**	**Main result**	**Reference**
Antennaria parlinii Fernald	6x	6x	?	Intraspecific	Common garden (individuals originally sampled from a sympatric area)	Yes	Yes	Uncertainty: A	Apomicts live in more disturbed habitats and sexuals in open wooded sites. Apomicts increase reproductive	Michaels and Bazzaz (1989)

									allocation when more light and nutrients are available, while sexuals do not.	
Antennaria rosea Greene and sexual progenitors[1]	(2x, 3x, 4x, 6x, 7x, 8x, 10x)[2]	(3x) (4x) (5x)[3]	Allo-polyploid	Interspecific	Parasympatry	Yes	No	Uncertainty: B, C, D	Apomicts occupy intermediate or similar niches compared to parental sexual species	Bayer et al. (1991)
Arnica cordifolia Hook.		3x 4x	Auto-polyploid[4]	Intraspecific	Mixed populations	No	Not applicable	Uncertainty: E	No habitat differentiation among apomictic cytotypes.	Kao (2008); Kao and Parker (2010)
Boechera Á. Löve & D. Löve	2x 3x	2x 3x	Species- and ploidy-specific[5]	Inter- and intraspecific	Mostly sympatry Correction for geographical distances	Yes	Yes	Uncertainty: C	Niche differentiation between sexual cytotypes but not between apomictic cytotypes. Niche differentiation between sexual and apomictic diploids.	Mau et al. (2015)

[1] *Antennaria parlinii* is an exclusively apomictic allopolyploid which was compared to its sexual progenitor species: *A. aromatica* Evert, *A. corymbosa* Nelson, *A. marginata* Greene, *A. media* Greene, *A. microphylla* Rydb., *A. racemosa* Hook., *A. rosulata* Rydb. and *A. umbrinella* Rydb.
[2] Bayer and Stebbins (1987).
[3] Bayer and Chandler (2007).
[4] Kao (2008).
[5] Hybridisation is known to play an important role in the diversification of the genus (Dobeš et al., 2007). In three species, apomictic diploids have been proven to be conspecific with sexuals, while apomictic triploids to derive by hybridisation (Lovell et al., 2013).

Crataegus subgen. *Sanguineae* Ufimov	2x	3x 4x 5x	Auto- and allo-polyploid	Inter- and intraspecific	Allopatry	Yes	No	Uncertainty: C	Allopolyploids inhabit cooler and more seasonally variable habitats than sexuals and autotriploids, and their niches are intermediate to progenitors. Niche broadening is associated with allopolyploidy.	Coughlan et al. (2017)
Erigeron strigosus Muhl. ex Willd.	(2x)	(3x) (4x) (6x)[6]	?	Intraspecific	Parasympatry	Yes	No	Uncertainty: A, B	Apomicts have wider niches in terms of precipitation and soil properties.	Noyes et al. (2006)
Hieracium alpinum L.	2x	3x	Auto-polyploid	Intraspecific	Allopatry	No	Not applicable	Uncertainty: F	No differences in eco-physiological plant traits among cytotypes.	Hartmann et al. (2018)
						No	Not applicable	Uncertainty: F	No differences among cytotypes in biotic interactions (Red Queen).	Hartmann et al. (2017)
Limonium Mill.	2x	3x (?) 4x[7]	?	Interspecific	Sympatry	Yes	Yes	Uncertainty: A, D	Apomicts show broader niches than sexuals, which	Caperta et al. (2017)

[6] Apomictic individuals were determined as such when they produced a high proportion of aborted pollen grains, but ploidy was not directly addressed.
[7] Three species complexes were sampled in this study, each representing a single ploidy level. The reproductive mode of the triploids, *L. algarvense* Erben, is unknown (Caperta et al., 2017).

									inhabit drier and warmer sites.	
Paspalum intermedium Munro ex Morong	2x	4x	Auto-polyploid	Intraspecific	Parapatry	Yes	No	Uncertainty: F	Apomicts inhabit colder and drier habitats than sexuals and have broader ecological niches.	Karunarathne et al. (2018)
Pilosella officinarum Vaill.	6x	5x 6x	?	Intraspecific	Allopatry	Yes[8]	No	Uncertainty: A, D	Pentaploids occur at higher elevation than hexaploids.	Mráz et al. (2008)
Poa cusickii Vasey subsp. *pallida* Soreng *P. fendleriana* (Steud.) Vasey subsp. *fendleriana* and subsp. *longiligula* (Scribn. & T.A.Williams) Soreng	(8x)[9]	(8x)[9]	Likely auto-polyploid[10]	Intraspecific	Parasympatry	Yes	No[11]	Uncertainty: B	Apomicts have broader ecological niches than sexuals and are more successful in cooler habitats with short frost-free season	Soreng (2000)

[8] In this case, a differentiation between pentaploids and hexaploids was made, without taking into consideration reproductive mode

[9] Ploidy level was not assessed within the study but was assumed on the basis of known chromosome counts (Soreng, 1991, 1986). Since these *Poa* species are dioecious, reproductive mode was estimated at the population level by the male: female plants ratios.

[10] R. J. Soreng, personal communication.

[11] Actually, (Soreng, 2000) distinguished between pistillate plants from exclusively apomictic regions and pistillate plants from regions where male plants occurred as well. However, no distinction in the reproductive mode of the sympatric pistillate plants was made.

Poa nervosa (Hook.) Vasey and *P. wheeleri* Vasey	(4x)[9]	(8-13x)[9]	Allo-polyploid[12]	Interspecific	Allopatry	Yes	No	Uncertainty: B, C	Apomicts have broader ecological niches than sexuals and are more successful in cooler habitats with short frost-free season	Soreng (2000)
Potentilla puberula Krašan	4x	5x, 7x, 8x	Auto-polyploid[13]	Intraspecific	Sympatry	Yes	Yes	Reproductive mode	Apomicts are more frequent in human-disturbed and wetter habitats, while sexuals occur in steeper and more south-oriented sites.	Present study
Ranunculus auricomus complex	2x 4x (autopoly-ploid)	3-8x	Allo-polyploid[14]	Mostly interspecific	Parasympatry	Yes	No	Uncertainty: C, D, G	Apomicts inhabit cooler and drier sites.	Paule et al. (2018)
Ranunculus carpaticola Soó	2x	6x	Allo-polyploid[15]	Intraspecific[16]	Allopatric	Yes	No	Uncertainty: C, F	Apomicts inhabit a higher variety of habitats than sexuals.	Paun et al. (2006a)
Ranunculus kuepferi	2x	4x	Auto-polyploid[17]	Intraspecific	Parapatry	Yes	No	Uncertainty: F[18]	4x apomicts occur at higher elevation and	Schinkel et al. (2016)

[12] Soreng (2007).
[13] Nardi et al. (2018).
[14] Hörandl et al. (2009).
[15] Paun et al. (2006b).
[16] The apomictic cytotype is currently treated as the hybrid form *R. carpaticola* × *cassubicifolius* (Hörandl et al., 2009).
[17] Cosendai et al. (2013).
[18] Schinkel et al. (2016) actually found rare apomictic diploids within the sexual geographical and ecological range. We are not considering them here, as their numbers were too low to draw conclusions on the role of ploidy and reproductive mode in determining the ecological differentiation.

Greuter & Burdet									under the related climatic factors.	
					Sympatry	Partial	Yes	Uncertainty: F	Incomplete niche divergence between apomicts and sexuals.	Kirchheimer et al. (2016)
					Allopatry	Yes	No	Uncertainty: F	Niche shift between apomicts and sexuals.	Kirchheimer et al. (2016)
Taraxacum sect. *Ruderalia* Kirschner, H.Øllg. & Štěpánek	2x	3x	Autopolyploid[19]	Intraspecific	Mixed populations	Yes	Yes	Uncertainty: F	Apomicts inhabit all microhabitats, while sexuals live in warmer and more southern slopes.	Verduijn et al. (2004)
					Mixed populations	Yes	Yes	Uncertainty: F	Apomicts are associated with human-disturbed areas (at lower elevation), sexuals with more stable sites at higher elevation.	Meirmans et al. (1999)

[19] P. Meirmans (personal communication).

References Table A5

Bayer, R.J., Chandler, G.T., 2007. Evolution of polyploid agamic complexes: a case study using the *Catipes* group of *Antennaria*, including the *A. rosea* complex (Asteraceae: Gnaphalieae), in: Hörandl, E., Grossniklaus, U., van Dijk, P.J., Sharbel, T.F. (Eds.), Apomixis: Evolution, Mechanisms and Perspectives. A. R. G. Gantner Verlag, Rugell, Ruggell, Liechtenstein, pp. 317–336.

Bayer, R.J., Purdy, B.G., Lebedyk, D.G., 1991. Niche differentiation among eight sexual species of *Antennaria* Gaertner (Asteraceae: Inuleae) and *A. rosea*, their allopolyploid derivative. Evol. Trends Plants 5, 109–123.

Bayer, R.J., Stebbins, G.L., 1987. Chromosome numbers, patterns of distribution, and apomixis in *Antennaria* (Asteraceae: Inuleae). Syst. Bot. 12, 305–319. https://doi.org/10.2307/2419326

Caperta, A.D., Castro, S., Loureiro, J., Róis, A.S., Conceição, S., Costa, J., Rhazi, L., Espírito Santo, D., Arsénio, P., 2017. Biogeographical, ecological and ploidy variation in related asexual and sexual *Limonium* taxa (*Plumbaginaceae*). Bot. J. Linn. Soc. 183, 75–93. https://doi.org/10.1111/boj.12498

Cosendai, A.-C., Wagner, J., Ladinig, U., Rosche, C., Hörandl, E., 2013. Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). Heredity (Edinb). 110, 560–569. https://doi.org/10.1038/hdy.2013.1

Coughlan, J.M., Han, S., Stefanović, S., Dickinson, T.A., 2017. Widespread generalist clones are associated with range and niche expansion in allopolyploids of Pacific Northwest Hawthorns (*Crataegus* L.). Mol. Ecol. 26, 5484–5499. https://doi.org/10.1111/mec.14331

Dobeš, C., Sharbel, T.F., Koch, M., 2007. Towards understanding the dynamics of hybridization and apomixis in the evolution of the genus *Boechera* (Brassicaceae). Syst. Biodivers. 5, 321–331. https://doi.org/10.1017/S1477200007002423

Hartmann, M., Jandová, K., Chrtek, J., Štefánek, M., Mráz, P., 2018. Effects of latitudinal and elevational gradients exceed the effects of between-cytotype differences in eco-physiological leaf traits in diploid and triploid *Hieracium alpinum*. Alp. Bot. 128, 133–147. https://doi.org/10.1007/s00035-018-0210-9

Hartmann, M., Štefánek, M., Zdvořák, P., Heřman, P., Chrtek, J., Mráz, P., 2017. The Red Queen hypothesis and geographical parthenogenesis in the alpine hawkweed *Hieracium alpinum* (Asteraceae). Biol. J. Linn. Soc. 122, 681–696. https://doi.org/10.1093/biolinnean/blx105

Hörandl, E., Greilhuber, J., Klímová, K., Paun, O., Temsch, E., Emadzade, K., 2009. Reticulate evolution and taxonomic concepts in the *Ranunculus auricomus* complex (Ranunculaceae): insights from analysis of morphological, karyological and molecular data. Taxon 58, 1194–1216.

Kao, R.H., 2008. Origins and widespread distribution of co-existing polyploids in *Arnica cordifolia*

(Asteraceae). Ann. Bot. 101, 145–52. https://doi.org/10.1093/aob/mcm271

Kao, R.H., Parker, I.M., 2010. Coexisting cytotypes of *Arnica cordifolia*: morphological differentiation and local-scale distribution. Int. J. Plant Sci. 171, 81–89. https://doi.org/10.1086/647924

Karunarathne, P., Schedler, M., Martínez, E.J., Honfi, A.I., Novichkova, A., Hojsgaard, D., 2018. Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. Ann. Bot. 121, 1183–1196. https://doi.org/10.1093/aob/mcy004

Kirchheimer, B., Schinkel, C.C.F., Dellinger, A.S., Klatt, S., Moser, D., Winkler, M., Lenoir, J., Caccianiga, M., Guisan, A., Nieto-Lugilde, D., Svenning, J.-C., Thuiller, W., Vittoz, P., Willner, W., Zimmermann, N.E., Hörandl, E., Dullinger, S., 2016. A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. J. Biogeogr. 43, 716–726. https://doi.org/10.1111/jbi.12663

Lovell, J.T., Aliyu, O.M., Mau, M., Schranz, M.E., Koch, M.A., Kiefer, C., Song, B.-H., Mitchell-Olds, T., Sharbel, T.F., 2013. On the origin and evolution of apomixis in *Boechera*. Plant Reprod. 26, 309–315. https://doi.org/10.1007/s00497-013-0218-7

Mau, M., Lovell, J.T., Corral, J.M., Kiefer, C., Koch, M.A., Aliyu, O.M., Sharbel, T.F., 2015. Hybrid apomicts trapped in the ecological niches of their sexual ancestors. PNAS 112, E2357–E2365. https://doi.org/10.1073/pnas.1423447112

Meirmans, P.G., Calame, F.G., Bretagnolle, F., Felber, F., den Nijs, J.C.M., 1999. Anthropogenic disturbance and habitat differentiation between sexual diploid and apomictic triploid *Taraxacum* sect. *Ruderalia*. Folia Geobot. 34, 451–469. https://doi.org/10.1007/BF02914922

Michaels, H.J., Bazzaz, F.A., 1989. Individual and population responses of sexual and apomictic plants to environmental gradients. Am. Nat. 134, 190–207. https://doi.org/10.1086/284975

Mráz, P., Šingliarová, B., Urfus, T., Krahulec, F., 2008. Cytogeography of *Pilosella officinarum* (Compositae): altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. Ann. Bot. 101, 59–71. https://doi.org/10.1093/aob/mcm282

Nardi, F.D., Dobeš, C., Müller, D., Grasegger, T., Myllynen, T., Alonso-Marcos, H., Tribsch, A., 2018. Sexual intraspecific recombination but not de novo origin governs the genesis of new apomictic genotypes in Potentilla puberula (Rosaceae). Taxon 67, 1108–1131. https://doi.org/10.12705/676.8.

Noyes, R.D., Gerling, H., Vandervoort, C., 2006. Sexual and apomictic prairie fleabane (*Erigeron strigosus*) in Texas: geographic analysis and a new combination (*Erigeron strigosus* var. *traversii*, Asteraceae). SIDA, Contrib. to Bot. 22, 265–276.

Paule, J., Dunkel, F.G., Schmidt, M., Gregor, T., 2018. Climatic differentiation in polyploid apomictic *Ranunculus auricomus* complex in Europe. BMC Ecol. 18, 16. https://doi.org/10.1186/s12898-018-0172-1

Paun, O., Greilhuber, J., Temsch, E.M., Hörandl, E., 2006a. Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). Mol. Ecol. 15, 897–910. https://doi.org/10.1111/j.1365-294X.2006.02800.x

Paun, O., Stuessy, T.F., Hörandl, E., 2006b. The role of hybridization, polyploidization and glaciation in the origin and evolution of the apomictic *Ranunculus cassubicus* complex. New Phytol. 171, 223–236. https://doi.org/10.1111/j.1469-8137.2006.01738.x

Schinkel, C.C.F., Kirchheimer, B., Dellinger, A.S., Klatt, S., Winkler, M., Dullinger, S., Hörandl, E., 2016. Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. AoB Plants 8, plw064. https://doi.org/10.1093/aobpla/plw064

Soreng, R.J., 2007. *Poa* L., in: Barkworth, M.E., Capels, K.M., Long, S., Anderton, L.K., Piep, M.B. (Eds.), Magnoliophyta: Commelinidae (in Part): Poaceae, Part 1. Flora of North America North of Mexico, 24. Oxford University Press, New York, Oxford, pp. 486–601.

Soreng, R.J., 2000. Apomixis and amphimixis comparative biogeography: a study in *Poa* (Poaceae), in: Jacobs, S.W.L., Everett, J. (Eds.), Grasses: Systematics and Evolution. CSIRO, Collingwood, Australia, pp. 294–306.

Soreng, R.J., 1991. Systematics of the “Epiles” group of *Poa* (Poaceae). Syst. Bot. 16, 507–528. https://doi.org/10.2307/2419340

Soreng, R.J., 1986. Distribution and evolutionary significance of apomixis in diclinous *Poa* of western North America. New Mexico State University, Las Cruces.

Verduijn, M.H., Van Dijk, P.J., Van Damme, J.M.M., 2004. Distribution, phenology and demography of sympatric sexual and asexual dandelions (*Taraxacum officinale s.l.*): geographic parthenogenesis on a small scale. Biol. J. Linn. Soc. 82, 205–218. https://doi.org/10.1111/j.1095-8312.2004.00325.x

APPENDIX S2: SOIL ANALYSES

Material and Methods

We sampled soil of the upper 10 cm (including the humus layer and the first mineral horizon) from 121 populations (Appendix 1) to analyse the following soil parameters: soil acidity (pH), carbonate content (CO_3^{2-}), organic carbon (C_{org}) and total nitrogen content (N_{tot}), cation content and cation exchange capacity (CEC). Soil samples varied from approximately 200 g to 1050 g. We determined values for pH with 5 ml spoon of soil in 25 ml $CaCl_2$ 0.01 M solution, stirred with a glass rod and incubated for at least two hours at room temperature for pH meter measurement (pH meter 713, Metrohm, Switzerland).

For measuring CO_3^{2-} we filled the Schleiber Unit with sealing fluid (deionized water acidified with H_2SO_4) up to the zero point. We added diluted HCl in deionized water to 2g of soil and measured the released CO_2 displacing the sealing fluid.
We compared the obtained values to 6–16 control samples of different Austrian soils: values below or above 6 indicate absence or presence of CO_3^{2-}, respectively. We calculated C_{org} and N_{tot} with the elemental analyser (TruMac CN, LECO, United States) by dry combustion of 400 mg of soil burned in a pure oxygen atmosphere at 1,250 °C, converting the C in CO_2 and the N in N_2 and NO_x, NO_x gases are reduced to N_2 through a copper catalyst. We determined C_{org} as the difference of total carbon and CO_3^{2-}.

Subsequently, we estimated the CEC using an Inductively Coupled Plasma Optical Emission Spectrometry Optima 8300 (ICP-OES, Perkin Elmer, United States). We kept 5 g of soil overnight in 100 ml $BaCl_2$ 0.1 M to equilibrate the samples and we shook them for two hours and filtrating them afterwards. In the filtrate we determined the H^+-ion by pH meter measurement and all the other cations (K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Mn^{3+}) by ICP-OES. For soils with CO_3^{2-} content, as the Ca^{2+}-ion and Mg^{2+}-ion were also dissolved, 100 ml HCl 0.2 M were added to replace the Ba^{2+} ions and make a mathematical correction for the dissolved cations. We used four mix solutions containing all the cations (K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Mn^{3+}) as calibration standards for ICP-OES measurements. We introduced the samples into inductively coupled argon plasma via a pneumatic atomisation system and at 9,726.85 °C the elements in the solution were excited to light emission. The emitted light was split via an echelle-based polychromator into element-specific wavelengths and detected for simultaneous analysis. The CEC is the sum of all cations: Fe^{3+}, Al^{3+}, Mn^{3+} and H^+ have an acid effect, whereas K^+, Na^+, Ca^{2+} and Mg^{2+} have a basic acting. Therefore, we calculated the base saturation by dividing the sum of $K^+ + Na^+ + Ca^{2+} + Mg^{2+}$ through the CEC and multiplied per 100, which gave us the result in percentages.

We removed highly inter-correlating soil variables (i.e., |Spearman correlation coefficient| > 0.75) from the analyses (Table A3). The then regressed the probability of occurrence of a cytotype (i.e., 0 or 1 in single-cytotype populations and 0.5 in mixed populations) onto the remaining soil variables (CO_3^{2-}, N_{tot}, C/N, K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+} and H^+) by means of logistic regressions as described in the main text for the other ecological variables.

Results

We found no significant differences among cytotypes for any of the studied soil variable (Table A4).

CHAPTER 2

Appendix 1. Studied populations, ploidy levels and reproductive modes of *Potentilla puberula* Krašan and seven closely-related species plus one putative hybrid.

ID	Taxon	Lat. (°N)	Long. (°E)	Population	Country	Elevation (ma.s.l.)	Collectors[b]	Ploidy level	N	Ng	N_s	A	HP	S_{reg}	S_{irreg}	N_i
5[c]	*P. puberula*	47.00887	12.4664	Rabenstein	A	1380	JAH & SS	5*x*	12	4	2	2				1
6[c]	*P. puberula*	47.00477	12.43528	Obermauern	A	1300	JAH & SS	4*x*	11	4	4			4		
								6*x*	1	1	1			1		
7[c]	*P. puberula*	46.75024	12.43778	Heinfels Schloss	A	1100	JAH & SS	4*x*	11	4	1			1		
								7*x*	5	4						
9[c,d]	*P. puberula*	46.78128	12.5748	Raut	A	1450	JAH & SS	4*x*	5	3	41			41		
								5*x*	4	4	31	29		2		
13[c,d]	*P. puberula*	47.01812	12.33223	Groder	A	1520	JAH & SS	4*x*	5	2	14			13	1	1
								5*x*	4	3	31	30			1	
								6*x*	1	1						1
15[c,d]	*P. puberula*	46.98602	12.63151	Lana S	A	1295	JAH & SS	5*x*	5	3	1	1				
								6*x*	4	3	52	49	1	1	1	
								8*x*	1	1						1
16[c]	*P. puberula*	46.92665	12.4221	St. Veit	A	1480	JAH & SS	5*x*	11	4	5	5				
17[c,d]	*P. puberula*	47.02602	12.5281	Stein	A	1300	JAH & SS	5*x*	3	3	8	7			1	
								7*x*	2	2	10	9		1		
								8*x*	4	4	13	12		1		
22[c]	*P. puberula*	47.05703	12.49057	Katalalm	A	1720	JAH & SS	5*x*	12	4	3	3				
26[c]	*P. puberula*	47.01067	12.3059	Pebellalm	A	1580	JAH & SS	5*x*	11	4	6	5		1		1
29[c]	*P. puberula*	46.98468	12.539	Bichl	A	940	JAH & SS	4*x*	5	4	3			2	1	4
								5*x*	6	4	2	2				2
								7*x*	1	1	1	1				1
31[c]	*P. puberula*	47.02117	12.37452	Prägraten N	A	1400	JAH & SS	4*x*	12	4	4			4		
39[c]	*P. puberula*	46.92542	12.53903	Ratzell	A	1190	JAH & SS	5*x*	12	3	1	1				
40[c]	*P. puberula*	46.78937	12.63775	Oberassling	A	1220	JAH & SS	5*x*	9	4	4	3			1	
								6*x*	4	4	3	3				
								7*x*	6	4	4	4				
43[c]	*P. puberula*	46.92237	12.33858	Erlach Gehöft	A	1610	JAH & SS	5*x*	11	3	4	4				
45[c]	*P. puberula*	47.01787	12.40872	Obersteiner	A	1460	JAH & SS	4*x*	11	4	2			2		2
								5*x*	4	4	4	4				1
								6*x*	1							
								7*x*	2		2	2				
54[c]	*P. puberula*	46.78878	12.58503	St. Justina	A	1200	JAH & SS	4*x*	1	1	1	1				1
								5*x*	8	4	1	1				2
61	*P. puberula*	45.93125	10.89694	Arco NE	I	110	HAM & FDN	4*x*	19	4	2			2		
62	*P. puberula*	45.55506	10.82355	Gaium Croce	I	180	HAM, CD, FDN & AT	4*x*	14	4	4			4		
63	*P. puberula*	45.64632	10.87395	Brenito Belluno	I	230	HAM, CD, FDN & AT	4*x*	16	4	3			3		
64	*P. puberula*	45.67443	10.90949	Ossenigo	I	250	HAM, CD, FDN & AT	4*x*	9	5	4			4		1
								5*x*	4	4	4	4				1
								7*x*	1	1	3	1		2		1
								8*x*	1	1	1		1			1
65	*P. puberula*	45.86729	11.18823	Zoreri	I	900	HAM & CD	5*x*	7	4	4	4				2
								7*x*	4	4	3	3				1
66	*P. puberula*	45.92031	11.13336	Mezzomonte	I	610	HAM & CD	5*x*	9	6	2	2				
								7*x*	9	4	4	4				
								8*x*	1	1	1	1				
67	*P. puberula*	46.20694	11.15578	Monreale	I	460	HAM & CD	4*x*	18	3	1			1		
								6*x*	1	1						
68	*P. puberula*	45.53424	10.36401	Scaiola	I	200	HAM & CD	4*x*	8	2	2			2		1
								5*x*	4	3	2	2				
								7*x*	8	5	3	3				1
69	*P. puberula*	45.74933	10.87946	Avio 1210	I	1210	FDN & AT	5*x*	12	3	1	1				3
								7*x*	1	1						1
70	*P. puberula*	46.02592	10.97567	Lasino	I	490	FDN & AT	4*x*	19	4	4			4		
								6*x*	2	2						
71	*P. puberula*	45.61144	10.36253	San Eusebio	I	630	HAM, CD & FDN	4*x*	20	4	2			2		
72	*P. puberula*	45.65391	10.43776	Pavone	I	330	HAM, CD & FDN	4*x*	12	4	3			3		

ID	Taxon	Lat. (°N)	Long. (°E)	Population	Country	Elevation (m a.s.l.)	Collectors[b]	Ploidy level	N	Ng	N_s	A	HP	S_{reg}	S_{irreg}	N_i
73	*P. puberula*	45.59223	10.87572	Forte Masua	I	960	HAM & CD	4*x*	12	4	4			4		
74	*P. puberula*	45.77776	10.907799	S. Valentino	I	1270	FDN & AT	5*x*	4	4	2	2				
								7*x*	2	2	1			1		
75	*P. puberula*	45.74151	11.06998	Ronchi	I	800	HAM, CD & FDN	7*x*	12	7	9	6		1	2	
76	*P. puberula*	45.73367	10.93042	Avio	I	180	AT	4*x*	14	4	3			3		4
77	*P. puberula*	45.7554	11.02516	AlaE	I	350	AT	4*x*	18	3	2			2		
78	*P. puberula*	46.27797	11.43325	Castellano	I	950	FDN & AT	4*x*	11	4	2			2		3
								6*x*	1	1	3			3		1
79	*P. puberula*	45.85773	10.93782	Santantonio	I	360	HAM & CD	4*x*	12	3	2			2		
80	*P. puberula*	45.80342	10.9103	S. Giacomo	I	1360	HAM & CD	5*x*	6	3	1	1				3
								6*x*	1	1	1	1				1
								7*x*	4	2	2	2				2
								8*x*	3	2	6	5	1			2
81	*P. puberula*	46.43081	11.24233	Terlan Mendel	I	600	HAM & FDN	4*x*	19	2	2			2		
82	*P. puberula*	46.35469	11.243	Söll	I	360	HAM & FDN	4*x*	19	3	1			1		
83	*P. puberula*	46.27308	11.18047	Unterfennberg	I	1050	HAM & FDN	5*x*	15	4	1	1				
								7*x*	4	4	4	4				
84	*P. puberula*	46.22039	11.29131	Grumes	I	890	FDN & AT	5*x*	6	4	2	2				2
								6*x*	9	4	2	2				1
								7*x*	3	3	1	1				2
85	*P. puberula*	46.24878	11.24806	Buchholz	I	550	FDN & AT	5*x*	7	4	1			1		2
								7*x*	7	4	3	3				2
								8*x*	6	4	7	4	3			2
86	*P. puberula*	46.65114	11.2195	Hafling Oberdorf	I	1340	HAM & FDN	5*x*	7	4	1	1				2
								7*x*	11	4	3	3				3
								8*x*	1	1	1	1				1
87	*P. puberula*	46.83247	11.16847	Moos Passeier	I	1050	HAM & FDN	5*x*	16	3	3	3				2
								7*x*	6	4	2	2				2
88	*P. puberula*	46.82269	11.19567	Passeier-Moos	I	890	HAM & FDN	5*x*	12	4	3	3				1
								6*x*	2	2	1	1				1
89	*P. puberula*	46.6992	11.16506	Finele	I	570	HAM & FDN	4*x*	14	4	4			4		
90	*P. puberula*	46.74197	10.82583	Fineil	I	1975	HAM & FDN	5*x*	4	4	1	1				2
								7*x*	4	4	2	1		1		
91	*P. puberula*	46.73864	10.85089	Vernagt	I	1760	HAM & FDN	4*x*	10	3	2			2		3
								6*x*	1	1						1
92	*P. puberula*	46.706	10.91764	Pfossertal Abzw	I	1250	HAM & FDN	4*x*	11	4	3			3		4
								6*x*	1	1	3			3		1
93	*P. puberula*	46.83958	10.65644	Melag	I	1940	HAM & FDN	4*x*	13	4	4			4		
94	*P. puberula*	46.66813	10.58537	Schluderns	I	1010	HAM & FDN	4*x*	14	4	3			3		
95	*P. puberula*	46.81395	10.57831	Perdross	I	1700	HAM & FDN	4*x*	12	4	2			2		4
								5*x*	2	2	3			3		2
96	*P. puberula*	46.43325	11.54412	Welschnofen	I	1200	HAM & FDN	5*x*	4	3	2	2				
								7*x*	16	4	3	3				
97	*P. puberula*	46.47972	11.30494	Sigmundskron	I	340	HAM & FDN	4*x*	14	4	4			4		
98	*P. puberula*	46.40467	11.45053	Schwarzenbach	I	1410	HAM & FDN	5*x*	2	2	2	2				
								7*x*	11	2	1	1				1
								8*x*	2	2	2	2				
99	*P. puberula*	46.37103	11.35436	Aldein	I	1225	HAM & FDN	5*x*	21	3	3	3				
								8*x*	1	1	1		1			
100	*P. puberula*	46.75	10.51728	Zerzertal	I	1810	HAM & FDN	4*x*	12	3	4			4		3
								5*x*	9	4	1		1			2
								7*x*	1	1						1
101	*P. puberula*	46.63458	10.76367	Kortsch	I	970	HAM & FDN	4*x*	12	4	2			2		
102	*P. puberula*	46.70395	11.12447	Hochmuth	I	1450	HAM & FDN	4*x*	12	2	2			2		
103	*P. puberula*	46.53036	11.18589	Nals	I	950	HAM & FDN	5*x*	10	4	2	1	1			
								7*x*	6	4	4	3		1		
								8*x*	3	2	2	2				
104	*P. puberula*	46.65972	10.45611	Muenstertal	I	1750	HAM & FDN	4*x*	16	4	1			1		4
				Tellahöfe				6*x*	1	1						1

ID	Taxon	Lat. (°N)	Long. (°E)	Population	Country	Elevation (ma..s.l.)	Collectors[b]	Ploidy level	N	Ng	N_s	A	HP	S_{reg}	S_{irreg}	N_i
105	*P. puberula*	46.81742	10.3495	Sent	CH	1330	HAM & FDN	4*x*	19	4	3			3		
106	*P. puberula*	46.89417	10.51028	Nauders	A	1500	HAM & FDN	4*x*	20	3	3			3		
107	*P. puberula*	46.77347	10.20403	Ardez	CH	1460	HAM & FDN	4*x*	10	4	4			4		
108	*P. puberula*	46.99	10.565	Lafairs	A	1100	HAM & FDN	4*x*	11	4	4			4		2
109	*P. puberula*	46.91546	11.3093	Ridnaun	I	1390	CD	4*x*	12	3	4			4		
110	*P. puberula*	46.96975	11.33019	Pflersch Ende	I	1450	CD	5*x*	10	3	1	1				
								8*x*	1	1	1	1				
111	*P. puberula*	46.96069	11.54327	Kematen	I	1500	CD	5*x*	8	2	2	2				1
								6*x*	6	2	2	2				2
								7*x*	3	3	2	2				2
								8*x*	2	2						1
112	*P. puberula*	46.87856	11.44342	Reifenstein	I	960	CD	4*x*	12	3	4			4		3
113	*P. puberula*	46.91411	11.46227	Flans	I	1430	CD	4*x*	12	4	4			4		
114	*P. puberula*	46.58533	11.53644	Trostburg	I	1010	CD	4*x*	16	4	4			4		
								7*x*	4	3	2	2				
115	*P. puberula*	46.5842	11.63529	St. Ulrich	I	1130	CD	5*x*	8	3	2	2				
								6*x*	1	1	1	1				
								7*x*	11	4	5	4		1		
116	*P. puberula*	46.67389	11.55543	Latzfons	I	1280	CD	4*x*	12	3	3			2	1	
117	*P. puberula*	46.65169	11.58138	Saeben N	I	730	CD	4*x*	3	3	3			3		3
								5*x*	4	4	1	1				2
								7*x*	11	4	2	2				2
118	*P. puberula*	46.66362	11.60049	Feldthurns	I	810	CD	4*x*	11	4	4			4		
								6*x*	1	1	2			2		
119	*P. puberula*	46.77655	11.62985	Franzensfeste	I	730	CD	5*x*	6	4	1	1				
								7*x*	14	4	1	1				
120	*P. puberula*	46.70514	11.64574	Tschoetscher H	I	680	CD	4*x*	20	4	1			1		
121	*P. puberula*	46.74708	11.6535	Raas W	I	750	CD	4*x*	11	4	2			2		
								6*x*	1	1	1			1		
122	*P. puberula*	46.84502	11.62849	Vals	I	1410	CD	4*x*	8	3	2			2		3
								5*x*	9	1	1	1				1
								6*x*	1	1	3	3				
								8*x*	1							
123	*P. puberula*	46.81947	11.78566	St. Sigmund	I	1160	CD	4*x*	18	4	4			4		
								5*x*	2	2	3	3				
125	*P. puberula*	47.05372	10.35383	Sinsen	A	1290	HAM & FDN	5*x*	4	4	1	1				2
								8*x*	5	4	5	3		2		1
126	*P. puberula*	47.15181	10.41042	Flirsch	A	1190	HAM & FDN	5*x*	11	4	1	1				
127	*P. puberula*	47.13172	10.50642	Pians	A	910	HAM & FDN	5*x*	11	2	2	2				
128	*P. puberula*	47.07605	10.65247	Ladis	A	1150	HAM & FDN	4*x*	12	4	4			4		
129	*P. puberula*	47.13292	10.55844	Flathalpe	A	1100	HAM & FDN	5*x*	11	3	1	1				2
130	*P. puberula*	47.27242	10.91909	Silz N	A	690	HAM & FDN	4*x*	19	4	2			2		
131	*P. puberula*	47.07758	10.67508	Faggen2	A	920	HAM & FDN	4*x*	12	3	3			3		
132	*P. puberula*	47.05669	10.75383	Nufels S	A	1250	HAM & FDN	5*x*	11	3	2	2				1
133	*P. puberula*	47.01928	10.73756	Kaunertal Maut	A	1375	HAM & FDN	5*x*	12	4	2	2				
134	*P. puberula*	47.22028	10.86583	Ambach	A	850	HAM & FDN	4*x*	19	4	4			3	1	2
								6*x*	1	1	1			1		
135	*P. puberula*	47.08083	10.96583	Längenfeld	A	1180	HAM & FDN	4*x*	20	4	4			4		
136	*P. puberula*	46.95997	11.01301	Sölden	A	1400	HAM & FDN	5*x*	18	4	2	2				
137	*P. puberula*	47.04167	10.97861	Huben	A	1250	HAM & FDN	5*x*	12	3	3	3				
138	*P. puberula*	47.30922	10.99608	Affenhausen	A	870	HAM & FDN	5*x*	6	4	2	2				
								7*x*	12	3	1	1				
139	*P. puberula*	47.02547	10.30531	Versahl	A	1360	HAM & FDN	5*x*	2	1						
								7*x*	1							
140	*P. puberula*	47.31492	10.87171	Rossbach	A	1000	HAM & FDN	5*x*	12	2	1	1				
141	*P. puberula*	47.27785	11.23942	Zirl Ruine	A	720	HAM & FDN	4*x*	20	4	4			4		
142	*P. puberula*	47.30593	11.1253	Pettnau	A	690	HAM & FDN	4*x*	11	2	2			2		
143	*P. puberula*	47.03329	11.47236	Vinaders	A	1210	HAM, J.A.H, FDN & SS	5*x*	10	4	2	2				1
								8*x*	5	3	2	2				2

ID	Taxon	Lat. (°N)	Long. (°E)	Population	Country	Elevation (ma..s.l.)	Collectors[b]	Ploidy level	N	Ng	N_s	A	HP	S_{reg}	S_{irreg}	N_i
144	*P. puberula*	46.92309	11.69811	Boedenalm	I	1700	HAM, J.A.H, FDN & SS	4*x*	12	4	4			4		
								5*x*	6	3	2	2				
145	*P. puberula*	46.91885	11.77515	Zoesen	I	1720	JAH & SS	4*x*	9	2	4			4		
146	*P. puberula*	47.00096	11.99053	St. Jakob	I	1110	JAH & SS	4*x*	17	3	3			3		
								5*x*	2	2	2	2				
								6*x*	1	1	2			2		
147	*P. puberula*	47.03851	12.10801	Prettau	I	1540	JAH & SS	5*x*	16	3	2	2				2
148	*P. puberula*	47.02325	12.05884	St. Peter	I	1350	JAH & SS	4*x*	12	3	4			3	1	
149	*P. puberula*	46.94756	11.91475	Luttach	I	1070	JAH & SS	4*x*	16	2	1			1		
								5*x*	3	3	2	2				
150	*P. puberula*	46.74927	11.86294	Onach	I	1230	JAH & SS	5*x*	12	1						
								6*x*	1	1	1	1				1
								7*x*	1	1						1
								8*x*	1	1	1		1			1
151	*P. puberula*	46.69656	11.89933	Pikolein	I	1370	JAH & SS	4*x*	13	3	2			2		3
								5*x*	1	1						
152	*P. puberula*	46.78889	11.89358	Sonneburg	I	860	JAH & SS	4*x*	10	2	1			1		
153	*P. puberula*	46.84848	11.97746	Muehlbach	I	1540	JAH & SS	4*x*	10	4	3			3		
154	*P. puberula*	46.88095	11.62192	Fanealm	I	1700	JAH & SS	5*x*	11	1	1	1				1
155[c]	*P. puberula*	46.76267	12.1135	Thurn Ruine	I	1180	JAH & SS	4*x*	11	4	3			3		
								6*x*	1	1						
156	*P. puberula*	47.0916	11.57674	Toldern	A	1600	HAM & FDN	5*x*	15	4	3	3				
								7*x*	4	3	3	3				
								8*x*	1	1	1	1				
157	*P. puberula*	47.04639	11.53583	Plenten	A	1350	HAM & FDN	5*x*	17	4	3	3				
								7*x*	1	1	1	1				
								8*x*	2	1	1	1				
158	*P. puberula*	47.12288	11.45006	Matrei B	A	1050	HAM & FDN	5*x*	1							
								7*x*	15	3	2	1	1			
159	*P. puberula*	47.11823	11.26232	Oberbergtal	A	1350	HAM & FDN	5*x*	19	4	2	2				
								6*x*	1	1	1	1				
								8*x*	1	1	1	1				
160	*P. puberula*	47.20982	11.40512	Patsch	A	960	HAM & FDN	5*x*	7	4	1	1				
								7*x*	13	3	3	3				
161	*P. puberula*	47.29489	11.48701	Thaur	A	620	HAM & FDN	4*x*	12	4	4			4		
162	*P. puberula*	47.23673	11.40614	Vill	A	850	HAM & FDN	4*x*	12	3	3			3		
163	*P. puberula*	47.29476	11.40576	Arzler Alm	A	1000	HAM & FDN	5*x*	20	4	3	3				
164[c]	*P. puberula*	46.74275	12.21366	Toblach	I	1330	JAH & SS	4*x*	11	4	3			3		
								6*x*	1	1	1			1		
165[c]	*P. puberula*	46.74742	12.36419	Unterparggen	I	1410	JAH & SS	5*x*	6	4	1	1				
								7*x*	10	4	3	3				
								8*x*	1		1	1				
169	*P. puberula*	45.5348	11.01033	Grezzana	I	250	CD	4*x*	19	4	3			3		4
								6*x*	1	1						
170	*P. puberula*	45.78665	11.43974	Mosson	I	500	CD	4*x*	14	1	1			1		
								5*x*	4	2	2	2				
								7*x*	3	2	2	2				
171	*P. puberula*	45.74177	11.38215	Santosso	I	400	CD	4*x*	20	3	3			3		
172	*P. puberula*	45.82835	11.70737	Mignano	I	200	CD	4*x*	15	4	3			3		
								5*x*	2	2	1	1				
								7*x*	2	2	1	1				
								8*x*	1	1	1	1				
173	*P. puberula*	45.98977	12.21845	Lago di Lago	I	330	CD	5*x*	6	3	1	1				
								6*x*	3	2						
								7*x*	3	3	2	2				1
								8*x*	5	4	1	1				1
174	*P. puberula*	46.28463	12.36928	Erto	I	1030	CD	5*x*	9	4	3	3				
								7*x*	10	2	2	2				
175	*P. puberula*	46.15783	12.64385	Grizzo	I	150	CD	7*x*	9	4	2	2				2

ID	Taxon	Lat. (°N)	Long. (°E)	Population	Country	Elevation (ma..s.l.)	Collectors[b]	Ploidy level	N	Ng	N_s	A	HP	S_{reg}	S_{irreg}	N_i
176[c]	*P. puberula*	46.78551	13.14602	Gemona	I	400	CD	5*x*	4	1						
								6*x*	2	2						
								7*x*	13	3						
179	*P. puberula*	46.57027	13.8124	Unterfederaun	A	540	CD	7*x*	5	4	3	3				
								8*x*	3	3	5	5				
180	*P. puberula*	46.50338	13.40105	St. Catarina	I	690	CD	6*x*	2	2	2	2				1
								8*x*	4	2	2	2				1
181	*P. puberula*	46.5055	13.36923	Bagni Lusnizza	I	650	CD	6*x*	8	4	1	1				
182	*P. puberula*	46.39924	13.24515	Roveredo	I	410	CD	6*x*	10	4	1	1				1
183	*P. puberula*	46.51041	13.31276	Pontebba	I	600	CD	5*x*	1	1	1	1				
								6*x*	20	1						
184	*P. puberula*	46.45248	13.37211	Chiut	I	870	CD	5*x*	3	1	1	1				
								6*x*	10	3	4	4				1
								7*x*	3		1	1				
								8*x*	2	1	1	1				1
185	*P. puberula*	46.39156	13.13437	CampioloW	I	300	CD	4*x*	4	4	3			3		2
								5*x*	2	2	4	1			3	1
								7*x*	1	1	1	1				1
186[c]	*P. puberula*	46.75371	12.96362	Oberdrauburg	A	730	CD	6*x*	14	4	1	1				2
								8*x*	1	1	1	1				1
189	*P. puberula*	47.78117	13.1126944	Oberegg	A	890	FDN & AT	5*x*	6	5						1
								7*x*	8	4						1
195	*P. argentea*	46.27797	11.43325	Castellano	I	950	AT & FDN	2*x*	6	4						
								6*x*	2	1						
198	*P. argentea*	46.65114	11.2195	Hafling Oberdorf	I	1340	FDN & HAM	2*x*	8	5						
203	*P. argentea*	48.109146	17,009172	Edelsthal	A	270	FDN	6*x*	7	5						
204	*P. argentea*	47.941762	16,714377	Breitenbrunn	A	190	FDN	6*x*	1	1						
192	*P. aurea*	46.30302	12.2754	Forca Sesarola	I	700	CD	2*x*[e]	4	4						
194	*P. aurea*	46.74197	10.82583	Fineil	I	2305	FDN & HAM	2*x*	4	5						
196	*P. aurea*	46.88095	11.62192	Vals	I	2305	CD	2*x*[e]	4	4						
199	*P. aurea*	47.13292	10.55844	Flathalpe	A	2350	FDN & HAM	2*x*[e]	4	4						
216	*P. aurea*	47.055833	12.825	Forschungstation Glocknerstraße	A	2305	AT	2*x*[e]	4	4						
211_aur	*P. aurea*	46.838333	11.034167	Rotmoostal	A	2310	KH	2*x*	2	2						
205	*P. brauneana*	47.31363889	11.39544444	Gleirschspitze	A	2206	EH, FH & SH	2*x*[e]	4	3						
206	*P. brauneana*	46.51683333	10.56008333	Sulden	I	2740	EH, FH & SH	2*x*[e]	5	5						
214	*P. brauneana*	47.311944	11.220833	Haferlekar	A	1630	KH	2*x*[e]	4	4						
220	*P. brauneana*	47.055833	12.825	Forschungstation Glocknerstraße	A	2305	AT	2*x*[e]	5	5						
193	*P. crantzii*	46.29547	12.27181	Cima dell'Albero	I	1920	CD	4*x*	5	4						
207	*P. crantzii*	46.51802778	10.56825	Sulden	I	2680	EH, FH & SH	7*x*	5	5						
211	*P. crantzii*	46.838333	11.034167	Rotmoostal	A	2310	KH	6*x*	3	3						
222	*P. crantzii*	47.036944	12.356111	Sajathütte	A	2475	AT	6*x*	1	1						
								7*x*	3	3						
223	*P. crantzii*	47.055833	12.825	Forschungstation Glocknerstraße	A	2305	AT	6*x*	3	5						
224	*P. crantzii*	45.74888889	10.88027778	Avio 1210	I	1210	AT & FDN	5*x*	2	2						
								6*x*	1	1						
hyb_104	*P. crantzii* × *P. puberula*	46.65972	10.45611	Münstertal Tellahöfe	I	1750	FDN & HAM	5*x*?[f]	4	4	2	2				
hyb_122	*P. crantzii* × *P. puberula*	46.84502	11.62849	Vals	I	1410	CD	5*x*?[f]	1	1						
hyb_132	*P. crantzii* × *P. puberula*	47.05669	10.75383	Nufels S	A	1250	FDN & HAM	5*x*?[f]	1	1						
hyb_139	*P. crantzii* × *P. puberula*	47.02547	10.30531	Versahl	A	1360	FDN & HAM	7*x*?[f]	4	4	1	1				
hyb_147	*P. crantzii* × *P. puberula*	47.03851	12.10801	Prettau	I	1540	JAH & SS	5*x*?[f]	1	1	1	1				
								8*x*?[f]	3	3	1	1				

ID	Taxon	Lat. (°N)	Long. (°E)	Population	Country	Elevation (ma.s.l.)	Collectors[b]	Ploidy level	N	Ng	Ns	A	HP	Sreg	Sirreg	Ni
hyb_150	*P. crantzii* × *P. puberula*	46.74927	11.86294	Onach	I	1230	JAH & SS	5*x*?[f]		3	3	1	1			
hyb_151	*P. crantzii* × *P. puberula*	46.69656	11.89933	Pikolein	I	1370	JAH & SS	5*x*?[f]		2	2	1	1			
hyb_154	*P. crantzii* × *P. puberula*	46.88095	11.62192	Fanealm	I	1700	JAH & SS	5*x*?[f]		1	1	1	1			
hyb_184	*P. crantzii* × *P. puberula*	46.45248	13.37211	Chiut	I	870	CD	5*x*?[f]		2	2	1			1	
hyb_224	*P. crantzii* × *P. puberula*	45.74888889	10.88027778	Avio 1210	I	1210	AT & FDN			1	1					
hyb_26	*P. crantzii* × *P. puberula*	47.01067	12.3059	Pebellalm	A	1580	JAH & SS	6*x*?[f]		1	1	1	1			
hyb_65	*P. crantzii* × *P. puberula*	45.86729	11.18823	Zoreri	I	900	CD & HAM	6*x*?[f]		3	3	3	3			
hyb_69	*P. crantzii* × *P. puberula*	45.74933	10.87946	Avio 1210	I	1210	AT & FDN	5*x*?[f]		3	3	1	1			
								7*x*?[f]		2	2	3	2		1	
hyb_80	*P. crantzii* × *P. puberula*	45.80342	10.9103	S. Giacomo	I	1360	CD & HAM	5*x*?[f]		1	1					
								7*x*?[f]		2	2	1	1			
								8*x*?[f]		1	1					
209	*P. frigida*	46.54577778	10.42786111	Piz Umbrail	CH	2660	EH, FH & SH	4*x*[e]		4	4					
210	*P. frigida*	46.7565	10.48316667	St. Valentin	I	2825	EH, FH & SH	4*x*[e]		5	5					
213	*P. frigida*	46.84889	11.030833	Hohe Mut	A	2590	KH	4*x*[e]		4	4					
225	*P. grandiflora*	47.0325	12.37222222	Sajatmähder	A	2324	AT	4*x*[e]		3	3					
197	*P. incana*	45.52858	10.28635	Sant'Eufemia	I	1640	FDN	4*x*		3	3					
200	*P. incana*	48.127348	16,252334	Perchtoldsdorfer Heide	A	310	FDN	4*x*		5	5					
201	*P. incana*	48.120765	16,931566	Hainburg an der Donau	A	215	FDN	4*x*		5	5					
202	*P. incana*	47.941762	16,714377	Breitenbrunn	A	190	FDN	4*x*		5	5					

N: total number of individuals collected; Ng: number of individuals successfully genotyped for the AFLP analysis; Ns: number of seeds screened by FCSS; A: number of seeds derived by apomixis; HP: number of seeds derived by haploid parthenogenesis; Sreg: number of seeds derived by regular sexuality; S_{irreg}: number of seeds derived by irregular sexuality (B_{III} hybrids formation); N_i: number of individuals selected for the interspecific analysis. Hybridity status of *P. crantzii* × *P. puberula* individuals was determined in this study.

a A: Austria; CH: Switzerland; I: Italy

b AT: Andreas Tribsch; CD: Christoph Dobeš; EH: Elvira Hörandl; FDN: Flavia Domizia Nardi; FH: Franz Hadacek; HAM: Henar Alonso-Marcos; JAH: Julian Ananda Haider; KH: Karl Hülber; SH: Stephan Hörandl; SS: Simon Stifter

c Population from Eastern Tyrol, Austria.

d Ploidy and reproductive mode determined by Dobeš & al. (2018).

e Ploidy levels taken from the literature (Dobeš & Vitek, 2000; Kurtto & al., 2004)

f The exact ploidies of *P. crantzii* × *P. puberula* remained uncertain.

Appendix 2. Herbarium vouchers representing studied taxa and deposited in W and GOET (an extended tabular version is available as Table S1 in Electronic Suppl. 1).

Taxon, population, country, province, collection number, barcode

Potentilla argentea L., **195**, Italy, Trentino-Alto Adige, *Nardi, F.D. 195_03* (W 2018-0002034); **195**, Italy, Trentino-Alto Adige, *Nardi, F.D. 195_04* (W 2018-0002036); **195**, Italy, Trentino-Alto Adige, *Nardi, F.D. 195_05* (W 2018-0002033); **198**, Italy, Trentino-Alto Adige, *Nardi, F.D. 198_08* (W 2018- 0002032); **198**, Italy, Trentino-Alto Adige, *Nardi, F.D. 198_10* (W 2018-0002031); ***P. aurea*** L., **194**, Italy, Trentino-Alto Adige, *Nardi, F.D. 194_08* (W 2018-0002038); **199**, Italy, Trentino-Alto Adige, *Nardi, F.D. 199_04* (W 2018-0002035); ***P. brauneana*** Hoppe, **205**, Austria, Tyrol, *Hörandl, E., Hörandl, S., Hadacek, F. 10247* (GOET); **206**, Italy, Trentino-Alto Adige, *Hörandl, E., Hörandl, S., Hadacek, F. 10267* (GOET); ***P. crantzii*** (Crantz) Beck ex Fritsch, **207**, Italy, Trentino-Alto Adige, *Hörandl, E., Hörandl, S., Hadacek, F. 10266* (GOET); **208**, Switzerland, Grisons, *Hörandl, E., Hörandl, S., Hadacek, F. 10278* (GOET); ***P. crantzii*** (Crantz) Beck ex Fritsch × ***P. puberula*** Krašan, **026**, Austria, Tyrol, *Nardi, F.D. 026_31* (W 2018-0002240); **065**, Italy, Trentino- Alto Adige, *Nardi, F.D. 065_12* (W 2018-0002224); **069**, Italy, Trentino-Alto Adige, *Nardi, F.D. 069_02* (W 2018-0002214); **069**, Italy, Trentino-Alto Adige, *Nardi, F.D. 069_03* (W 2018-0002247); **098**, Italy, Trentino-Alto Adige, *Nardi, F.D. 098_01* (W 2018-0002148); **104**, Italy, Trentino-Alto Adige, *Nardi, F.D. 104_07* (W 2018-0002167); **139**, Austria, Tyrol, *Nardi, F.D. 139_01* (W 2018-0002125); **147**, Italy, Trentino-Alto Adige, *Nardi, F.D. 147_07* (W 2018- 0002075); **147**, Italy, Trentino-Alto Adige, *Nardi, F.D. 147_15* (W 2018-0002116); **150**, Italy, Trentino-Alto Adige, *Nardi, F.D. 150_17* (W 2018-0002112); **151**, Italy, Trentino-Alto Adige, *Nardi, F.D. 151_13* (W 2018-0002109); **174**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 174_07* (W 2018-0002084); **184**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 184_01* (W 2018-0002076); ***P. frigida*** Vill., **209**, Switzerland, Grisons, *Hörandl, E., Hörandl, S., Hadacek, F. 10277* (GOET); **210**, Italy, Trentino-Alto Adige, *Hörandl, E., Hörandl, S., Hadacek, F. 10283* (GOET); ***P. incana*** G.Gaertn., B.Mey. & Scherb., **197**, Italy, Lombardy,*Nardi, F.D. 197_06* (W 2018-0002030); **197**, Italy, Lombardy, *Nardi, F.D. 197_10* (W 2018-0002029); ***P. puberula*** Krašan, **005**, Austria, Tyrol, *Nardi, F.D. 005_ 54* (W 2018-0002246); **006**, Austria, Tyrol, *Nardi, F.D. 006_ 54* (W 2018-0002245); **006**, Austria, Tyrol, *Nardi, F.D. 006_ 55* (W 2018-0002244); **007**, Austria, Tyrol, *Nardi, F.D. 007_ 54* (W 2018-0002243); **007**, Austria, Tyrol, *Nardi, F.D. 007_62* (W 2018-0002242); **016**, Austria, Tyrol, *Nardi, F.D. 016_52* (W 2018-0002241); **022**, Austria, Tyrol, *Nardi, F.D. 022_60* (W 2018-0002236); **026**, Austria, Tyrol, *Nardi, F.D. 026_42* (W 2018-0002222); **029**, Austria, Tyrol, *Nardi, F.D. 029_44* (W 2018-0002239); **029**, Austria, Tyrol, *Nardi, F.D. 029_47* (W 2018-0002237); **029**, Austria, Tyrol, *Nardi, F.D. 029_48* (W 2018- 0002238); **031**, Austria, Tyrol, *Nardi, F.D. 031_41* (W 2018-0002235); **039**, Austria, Tyrol, *Nardi, F.D. 039_45* (W 2018-0002233); **040**, Austria, Tyrol, *Nardi, F.D. 040_46* (W 2018-0002234); **040**, Austria, Tyrol, *Nardi, F.D. 040_52* (W 2018-0002232); **043**, Austria, Tyrol, *Nardi, F.D. 043_24* (W 2018-0002231); **045**, Austria, Tyrol, *Nardi, F.D. 045_34* (W 2018-0002230); **054**, Austria, Tyrol, *Nardi, F.D. 054_19* (W 2018-0002221); **061**, Italy, Trentino-Alto Adige, *Nardi, F.D. 061_07* (W 2018-0002229); **062**, Italy, Veneto, *Nardi, F.D. 062_06* (W 2018-0002228); **063**, Italy, Veneto, *Nardi, F.D. 063_06* (W 2018-0002227); **064**, Italy, Veneto, *Nardi, F.D. 064_01* (W 2018-0002223); **064**, Italy, Veneto, *Nardi, F.D. 064_09* (W 2018-0002220); **065**, Italy, Trentino-Alto Adige, *Nardi, F.D. 065_04* (W 2018-0002225); **065**, Italy, Trentino-Alto Adige, *Nardi, F.D. 065_07* (W 2018-0002226); **066**, Italy, Trentino-Alto Adige, *Nardi, F.D. 066_01* (W 2018-0002217); **066**, Italy, Trentino-Alto Adige, *Nardi, F.D. 066_06* (W 2018-0002219); **066**, Italy, Trentino-Alto Adige, *Nardi, F.D. 066_09* (W 2018-0002218); **067**, Italy, Trentino-Alto Adige, *Nardi, F.D. 067_01* (W 2018-0002216); **067**, Italy, Trentino-Alto Adige, *Nardi, F.D. 067_09* (W 2018-0002215); **068**, Italy, Lombardy, *Nardi, F.D. 068_15* (W 2018-0002213); **070**, Italy, Trentino-Alto Adige, *Nardi, F.D. 070_09* (W 2018-0002212); **071**, Italy, Lombardy, *Nardi, F.D. 071_04* (W 2018-0002048); **072**, Italy, Lombardy, *Nardi, F.D. 072_03* (W 2018-0002210); **073**, Italy, Veneto, *Nardi, F.D. 073_06* (W 2018-0002050); **074**, Italy, Trentino-Alto Adige, *Nardi, F.D. 074_02* (W 2018-0002211); **074**, Italy, Trentino-Alto Adige, *Nardi, F.D. 074_03* (W 2018-0002208); **075**, Italy, Trentino-Alto Adige, *Nardi, F.D. 075_13* (W 2018-0002069); **076**, Italy, Trentino-Alto Adige, *Nardi, F.D. 076_08* (W 2018- 0002209); **078**, Italy, Trentino-Alto Adige, *Nardi, F.D. 078_08* (W 2018-0002206); **078**, Italy, Trentino-Alto Adige, *Nardi, F.D. 078_10* (W 2018-0002207); **079**, Italy, Trentino-Alto Adige, *Nardi, F.D. 079_02* (W 2018-0002049); **080**, Italy, Trentino-Alto Adige, *Nardi, F.D. 080_02* (W 2018-0002205); **080**, Italy, Trentino-Alto Adige, *Nardi, F.D. 080_03* (W 2018-0002204); **080**, Italy, Trentino-Alto Adige, *Nardi, F.D. 080_06* (W 2018-0002070); **080**, Italy, Trentino- Alto Adige, *Nardi, F.D. 080_12* (W 2018-0002202); **082**, Italy, Trentino-Alto Adige, *Nardi, F.D. 082_17* (W 2018-0002203); **083**, Italy, Trentino-Alto Adige, *Nardi, F.D. 083_02* (W 2018-0002201); **083**, Italy, Trentino-Alto Adige, *Nardi, F.D. 083_20* (W 2018-0002200); **084**, Italy, Trentino-Alto Adige, *Nardi, F.D. 084_01* (W 2018-0002199); **084**, Italy, Trentino-Alto Adige, *Nardi, F.D. 084_12* (W 2018-0002198); **084**, Italy, Trentino-Alto Adige, *Nardi, F.D. 084_16* (W 2018-0002197); **085**, Italy, Trentino-Alto Adige, *Nardi, F.D. 085_03* (W 2018-0002196); **085**, Italy, Trentino-Alto Adige, *Nardi, F.D. 085_08* (W 2018-0002195); **085**, Italy, Trentino-Alto Adige, *Nardi, F.D. 085_12* (W 2018-0002146); **086**, Italy, Trentino-Alto Adige, *Nardi, F.D. 086_19* (W 2018- 0002194); **086**, Italy, Trentino-Alto Adige, *Nardi, F.D. 086_*

20 (W 2018-0002193); **086**, Italy, Trentino-Alto Adige, *Nardi, F.D. 086_ 22* (W 2018- 0002192); **087**, Italy, Trentino-Alto Adige, *Nardi, F.D. 087_01* (W 2018-0002191); **087**, Italy, Trentino-Alto Adige, *Nardi, F.D. 087_20* (W 2018-0002189); **088**, Italy, Trentino-Alto Adige, *Nardi, F.D. 088_08* (W 2018-0002190); **088**, Italy, Trentino-Alto Adige, *Nardi, F.D. 088_14* (W 2018-0002188); **089**, Italy, Trentino- Alto Adige, *Nardi, F.D. 089_01* (W 2018-0002186); **090**, Italy, Trentino-Alto Adige, *Nardi, F.D. 090_02* (W 2018-0002187); **090**, Italy, Trentino-Alto Adige, *Nardi, F.D. 090_06* (W 2018-0002185); **091**, Italy, Trentino-Alto Adige, *Nardi, F.D. 091_07* (W 2018-0002184); **092**, Italy, Trentino-Alto Adige, *Nardi, F.D. 092_05* (W 2018-0002182); **092**, Italy, Trentino-Alto Adige, *Nardi, F.D. 092_10* (W 2018-0002181); **093**, Italy, Trentino-Alto Adige, *Nardi, F.D. 093_02* (W 2018-0002183); **094**, Italy, Trentino-Alto Adige, *Nardi, F.D. 094_03* (W 2018-0002180); **095**, Italy, Trentino-Alto Adige, *Nardi, F.D. 095_10* (W 2018- 0002179); **096**, Italy, Trentino-Alto Adige, *Nardi, F.D. 096_02* (W 2018-0002177); **096**, Italy, Trentino-Alto Adige, *Nardi, F.D. 096_10* (W 2018-0002147); **097**, Italy, Trentino-Alto Adige, *Nardi, F.D. 097_03* (W 2018-0002178); **098**, Italy, Trentino-Alto Adige, *Nardi, F.D. 098_02* (W 2018-0002175); **098**, Italy, Trentino-Alto Adige, *Nardi, F.D. 098_07* (W 2018-0002176); **099**, Italy, Trentino-Alto Adige, *Nardi, F.D. 099_09* (W 2018-0002173); **099**, Italy, Trentino- Alto Adige, *Nardi, F.D. 099_18* (W 2018-0002174); **100**, Italy, Trentino-Alto Adige, *Nardi, F.D. 100_01* (W 2018-0002170); **100**, Italy, Trentino-Alto Adige, *Nardi, F.D. 100_02* (W 2018-0002172); **100**, Italy, Trentino-Alto Adige, *Nardi, F.D. 100_17* (W 2018-0002171); **101**, Italy, Trentino-Alto Adige, *Nardi, F.D. 101_09* (W 2018-0002169); **102**, Italy, Trentino-Alto Adige, *Nardi, F.D. 102_06* (W 2018-0002168); **103**, Italy, Trentino-Alto Adige, *Nardi, F.D. 103_06* (W 2018-0002166); **103**, Italy, Trentino-Alto Adige, *Nardi, F.D. 103_07* (W 2018-0002165); **103**, Italy, Trentino-Alto Adige, *Nardi, F.D. 103_10* (W 2018- 0002068); **104**, Italy, Trentino-Alto Adige, *Nardi, F.D. 104_11* (W 2018-0002066); **104**, Italy, Trentino-Alto Adige, *Nardi, F.D. 104_13* (W 2018-0002046); **105**, Switzerland, Grisons, *Nardi, F.D. 105_08* (W 2018-0002047); **109**, Italy, Trentino-Alto Adige, *Nardi, F.D. 109_03* (W 2018-0002164); **110**, Italy, Trentino-Alto Adige, *Nardi, F.D. 110_01* (W 2018-0002163); **111**, Italy, Trentino-Alto Adige, *Nardi, F.D. 111_01* (W 2018-0002067); **111**, Italy, Trentino- Alto Adige, *Nardi, F.D. 111_04* (W 2018-0002161); **111**, Italy, Trentino-Alto Adige, *Nardi, F.D. 111_06* (W 2018-0002162); **111**, Italy, Trentino-Alto Adige, *Nardi, F.D. 111_15* (W 2018-0002160); **112**, Italy, Trentino-Alto Adige, *Nardi, F.D. 112_06* (W 2018-0002158); **113**, Italy, Trentino-Alto Adige, *Nardi, F.D. 113_04* (W 2018-0002159); **115**, Italy, Trentino-Alto Adige, *Nardi, F.D. 115_01* (W 2018-0002157); **115**, Italy, Trentino-Alto Adige, *Nardi, F.D. 115_03* (W 2018-0002156); **115**, Italy, Trentino-Alto Adige, *Nardi, F.D. 115_04* (W 2018-0002155); **116**, Italy, Trentino-Alto Adige, *Nardi, F.D. 116_03* (W 2018-0002154); **117**, Italy, Trentino-Alto Adige, *Nardi, F.D. 117_03* (W 2018-0002151); **117**, Italy, Trentino-Alto Adige, *Nardi, F.D. 117_04* (W 2018-0002153); **117**, Italy, Trentino-Alto Adige, *Nardi, F.D. 117_07* (W 2018-0002152); **118**, Italy, Trentino-Alto Adige, *Nardi, F.D. 118_01* (W 2018-0002150); **118**, Italy, Trentino-Alto Adige, *Nardi, F.D. 118_02* (W 2018-0002149); **119**, Italy, Trentino-Alto Adige, *Nardi, F.D. 119_15* (W 2018-0002111); **120**, Italy, Trentino- Alto Adige, *Nardi, F.D. 120_14* (W 2018-0002145); **121**, Italy, Trentino-Alto Adige, *Nardi, F.D. 121_01* (W 2018-0002065); **121**, Italy, Trentino-Alto Adige, *Nardi, F.D. 121_04* (W 2018-0002144); **122**, Italy, Trentino-Alto Adige, *Nardi, F.D. 122_05* (W 2018-0002062); **122**, Italy, Trentino-Alto Adige, *Nardi, F.D. 122_08* (W 2018-0002107); **122**, Italy, Trentino-Alto Adige, *Nardi, F.D. 122_10* (W 2018-0002143); **122**, Italy, Trentino-Alto Adige, *Nardi, F.D. 122_12* (W 2018-0002142); **123**, Italy, Trentino-Alto Adige, *Nardi, F.D. 123_08* (W 2018-0002141); **123**, Italy, Trentino-Alto Adige, *Nardi, F.D. 123_10* (W 2018-0002140); **125**, Austria, Tyrol, *Nardi, F.D. 125_11* (W 2018-0002045); **126**, Austria, Tyrol, *Nardi, F.D. 126_09* (W 2018-0002139); **127**, Austria, Tyrol, *Nardi, F.D. 127_07* (W 2018-0002138); **128**, Austria, Tyrol, *Nardi, F.D. 128_06* (W 2018-0002137); **129**, Austria, Tyrol, *Nardi, F.D. 129_11* (W 2018- 0002136); **130**, Austria, Tyrol, *Nardi, F.D. 130_09* (W 2018-0002134); **131**, Austria, Tyrol, *Nardi, F.D. 131_01* (W 2018-0002135); **132**, Austria, Tyrol, *Nardi, F.D. 132_02* (W 2018-0002132); **133**, Austria, Tyrol, *Nardi, F.D. 133_09* (W 2018-0002133); **134**, Austria, Tyrol, *Nardi, F.D. 134_13* (W 2018- 0002130); **134**, Austria, Tyrol, *Nardi, F.D. 134_15* (W 2018-0002131); **135**, Austria, Tyrol, *Nardi, F.D. 135_19* (W 2018-0002128); **136**, Austria, Tyrol, *Nardi, F.D. 136_06* (W 2018-0002064); **137**, Austria, Tyrol, *Nardi, F.D. 137_02* (W 2018-0002129); **138**, Austria, Tyrol, *Nardi, F.D. 138_10* (W 2018- 0002126); **138**, Austria, Tyrol, *Nardi, F.D. 138_11* (W 2018-0002127); **139**, Austria, Tyrol, *Nardi, F.D. 139_03* (W 2018-0002124); **140**, Austria, Tyrol, *Nardi, F.D. 140_12* (W 2018-0002122); **141**, Austria, Tyrol, *Nardi, F.D. 141_01* (W 2018-0002123); **142**, Austria, Tyrol, *Nardi, F.D. 142_06* (W 2018-0002121); **143**, Austria, Tyrol, *Nardi, F.D. 143_05* (W 2018-0002110); **143**, Austria, Tyrol, *Nardi, F.D. 143_06* (W 2018-0002061); **145**, Italy, Trentino-Alto Adige, *Nardi, F.D. 145_01* (W 2018-0002119); **146**, Italy, Trentino-Alto Adige, *Nardi, F.D. 146_03* (W 2018-0002118); **146**, Italy, Trentino-Alto Adige, *Nardi, F.D. 146_07* (W 2018-0002063); **146**, Italy, Trentino-Alto Adige, *Nardi, F.D. 146_20* (W 2018-0002120); **148**, Italy, Trentino-Alto Adige, *Nardi, F.D. 148_05* (W 2018-0002117); **149**, Italy, Trentino-Alto Adige, *Nardi, F.D. 149_13* (W 2018-0002060); **149**, Italy, Trentino-Alto Adige, *Nardi, F.D. 149_14* (W 2018- 0002058); **150**, Italy, Trentino-Alto Adige, *Nardi, F.D. 150_05* (W 2018-0002115); **150**, Italy, Trentino-Alto Adige, *Nardi, F.D. 150_13* (W 2018-0002114); **150**, Italy, Trentino-Alto Adige, *Nardi, F.D. 150_18* (W 2018-0002113); **151**, Italy, Trentino-Alto Adige, *Nardi, F.D. 151_03* (W 2018-0002059); **152**, Italy, Trentino-Alto Adige, *Nardi, F.D. 152_03* (W 2018-0002044); **153**, Italy, Trentino-Alto Adige, *Nardi, F.D. 153_08* (W 2018-0002108); **154**, Italy, Trentino- Alto Adige, *Nardi, F.D. 154_07* (W 2018-0002105); **156**, Austria, Tyrol, *Nardi, F.D. 156_01* (W 2018-0002104); **156**, Austria, Tyrol, *Nardi, F.D. 156_02* (W 2018-0002103); **157**, Austria, Tyrol, *Nardi, F.D. 157_02* (W 2018-

0002106); **157**, Austria, Tyrol, *Nardi, F.D. 157_12* (W 2018-0002101); **158**, Austria, Tyrol, *Nardi, F.D. 158_07* (W 2018-0002102); **159**, Austria, Tyrol, *Nardi, F.D. 159_04* (W 2018- 0002100); **159**, Austria, Tyrol, *Nardi, F.D. 159_16* (W 2018- 0002099); **159**, Austria, Tyrol, *Nardi, F.D. 159_18* (W 2018-0002096); **160**, Austria, Tyrol, *Nardi, F.D. 160_07* (W 2018-0002097); **160**, Austria, Tyrol, *Nardi, F.D. 160_13* (W 2018-0002094); **161**, Austria, Tyrol, *Nardi, F.D. 161_02* (W 2018-0002042); **162**, Austria, Tyrol, *Nardi, F.D. 162_07* (W 2018-0002053); **163**, Austria, Tyrol, *Nardi, F.D. 163_01* (W 2018-0002095); **164**, Italy, Trentino-Alto Adige, *Nardi, F.D. 164_01* (W 2018-0002098); **164**, Italy, Trentino-Alto Adige, *Nardi, F.D. 164_04* (W 2018-0002057); **165**, Italy, Trentino-Alto Adige, *Nardi, F.D. 165_03* (W 2018-0002093); **165**, Italy, Trentino- Alto Adige, *Nardi, F.D. 165_11* (W 2018-0002091); **165**, Italy, Trentino-Alto Adige, *Nardi, F.D. 165_18* (W 2018-0002092); **169**, Italy, Veneto, *Nardi, F.D. 169_02* (W 2018-0002056); **169**, Italy, Veneto, *Nardi, F.D. 169_15* (W 2018-0002090); **170**, Italy, Veneto, *Nardi, F.D. 170_02* (W 2018-0002089); **170**, Italy, Veneto, *Nardi, F.D. 170_05* (W 2018-0002055); **170**, Italy, Veneto, *Nardi, F.D. 170_14* (W 2018-0002054); **171**, Italy, Veneto, *Nardi, F.D. 171_01* (W 2018-0002088); **172**, Italy, Veneto, *Nardi, F.D. 172_04* (W 2018-0002043); **172**, Italy, Veneto, *Nardi, F.D. 172_13* (W 2018-0002041); **172**, Italy, Veneto, *Nardi, F.D. 172_18* (W 2018-0002087); **172**, Italy, Veneto, *Nardi, F.D. 172_20* (W 2018-0002040); **173**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 173_03* (W 2018- 0002086); **173**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 173_08* (W 2018-0002039); **173**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 173_14* (W 2018-0002037); **174**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 174_01* (W 2018-0002085); **175**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 175_06* (W 2018-0002083); **179**, Austria, Carinthia, *Nardi, F.D. 179_02* (W 2018-0002082); **179**, Austria, Carinthia, *Nardi, F.D. 179_04* (W 2018-0002081); **180**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 180_02* (W 2018-0002078); **180**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 180_06* (W 2018-0002080); **181**, Italy, Friuli-Venezia Giulia, *Nardi,F.D. 181_04* (W 2018-0002079); **183**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 183_09* (W 2018-0002052); **183**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 183_22* (W 2018-0002077); **184**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 184_06* (W 2018-0002074); **185**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 185_01* (W 2018-0002073); **185**, Italy, Friuli-Venezia Giulia, *Nardi, F.D. 185_05* (W 2018-0002072); **186**, Austria, Carinthia, *Nardi, F.D. 186_03* (W 2018-0002071); **186**, Austria, Carinthia, *Nardi, F.D. 186_05* (W 2018-0002051)

Table S1. Herbarium vouchers representing studied taxa and deposited in W and GOET.

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla argentea L.	195	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 195_03*	(W 2018-0002034)
Potentilla argentea L.	195	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 195_04*	(W 2018-0002036)
Potentilla argentea L.	195	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 195_05*	(W 2018-0002033)
Potentilla argentea L.	198	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 198_08*	(W 2018-0002032)
Potentilla argentea L.	198	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 198_10*	(W 2018-0002031)
Potentilla aurea L.	194	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 194_08*	(W 2018-0002038)
Potentilla aurea L.	199	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 199_04*	(W 2018-0002035)
Potentilla brauneana Hoppe	205	Austria	Tyrol	*Hörandl, E., Hörandl, S., Hadacek, F.*		*Hörandl, E., Hörandl, S., Hadacek, F. 10247*	(GOET)
Potentilla brauneana Hoppe	206	Italy	Trentino-Alto Adige	*Hörandl, E., Hörandl, S., Hadacek, F.*		*Hörandl, E., Hörandl, S., Hadacek, F. 10267*	(GOET)
Potentilla crantzii (Crantz) Beck ex Fritsch	207	Italy	Trentino-Alto Adige	*Hörandl, E., Hörandl, S., Hadacek, F.*		*Hörandl, E., Hörandl, S., Hadacek, F. 10266*	(GOET)
Potentilla crantzii (Crantz) Beck ex Fritsch	208	Switzerland	Grisons	*Hörandl, E., Hörandl, S., Hadacek, F.*		*Hörandl, E., Hörandl, S., Hadacek, F. 10278*	(GOET)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	026	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 026_31*	(W 2018-0002240)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	065	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 065_12*	(W 2018-0002224)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	069	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 069_02*	(W 2018-0002214)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	069	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 069_03*	(W 2018-0002247)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	098	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 098_01*	(W 2018-0002148)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	104	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 104_07*	(W 2018-0002167)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	139	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 139_01*	(W 2018-0002125)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	147	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 147_07*	(W 2018-0002075)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	147	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 147_15*	(W 2018-0002116)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	150	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 150_17*	(W 2018-0002112)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	151	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 151_13*	(W 2018-0002109)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	174	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 174_07*	(W 2018-0002084)
Potentilla crantzii (Crantz) Beck ex Fritsch × *P. puberula* Krašan	184	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 184_01*	(W 2018-0002076)
Potentilla frigida Vill.	209	Switzerland	Grisons	*Hörandl, E., Hörandl, S., Hadacek, F.*		*Hörandl, E., Hörandl, S., Hadacek, F. 10277*	(GOET)
Potentilla frigida Vill.	210	Italy	Trentino-Alto Adige	*Hörandl, E., Hörandl, S., Hadacek, F.*		*Hörandl, E., Hörandl, S., Hadacek, F. 10283*	(GOET)
Potentilla incana G.Gaertn., B.Mey. & Scherb.	197	Italy	Lombardy	*Alonso-Marcos, H., Nardi, F.D., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 197_06*	(W 2018-0002030)
Potentilla incana G.Gaertn., B.Mey. & Scherb.	197	Italy	Lombardy	*Alonso-Marcos, H., Nardi, F.D., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 197_10*	(W 2018-0002029)
Potentilla puberula Krašan	005	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 005_54*	(W 2018-0002246)
Potentilla puberula Krašan	006	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 006_54*	(W 2018-0002245)
Potentilla puberula Krašan	006	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 006_55*	(W 2018-0002244)
Potentilla puberula Krašan	007	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 007_54*	(W 2018-0002243)
Potentilla puberula Krašan	007	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 007_62*	(W 2018-0002242)
Potentilla puberula Krašan	016	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 016_52*	(W 2018-0002241)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	022	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 022_60*	(W 2018-0002236)
Potentilla puberula Krašan	026	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 026_42*	(W 2018-0002222)
Potentilla puberula Krašan	029	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 029_44*	(W 2018-0002239)
Potentilla puberula Krašan	029	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 029_47*	(W 2018-0002237)
Potentilla puberula Krašan	029	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 029_48*	(W 2018-0002238)
Potentilla puberula Krašan	031	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 031_41*	(W 2018-0002235)
Potentilla puberula Krašan	039	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 039_45*	(W 2018-0002233)
Potentilla puberula Krašan	040	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 040_46*	(W 2018-0002234)
Potentilla puberula Krašan	040	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 040_52*	(W 2018-0002232)
Potentilla puberula Krašan	043	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 043_24*	(W 2018-0002231)
Potentilla puberula Krašan	045	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 045_34*	(W 2018-0002230)
Potentilla puberula Krašan	054	Austria	Tyrol	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 054_19*	(W 2018-0002221)
Potentilla puberula Krašan	061	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 061_07*	(W 2018-0002229)
Potentilla puberula Krašan	062	Italy	Veneto	*Alonso-Marcos, H., Nardi, F.D., Tribsch, A., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 062_06*	(W 2018-0002228)
Potentilla puberula Krašan	063	Italy	Veneto	*Alonso-Marcos, H., Nardi, F.D., Tribsch, A., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 063_06*	(W 2018-0002227)
Potentilla puberula Krašan	064	Italy	Veneto	*Alonso-Marcos, H., Nardi, F.D., Tribsch, A., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 064_01*	(W 2018-0002223)
Potentilla puberula Krašan	064	Italy	Veneto	*Alonso-Marcos, H., Nardi, F.D., Tribsch, A., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 064_09*	(W 2018-0002220)
Potentilla puberula Krašan	065	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 065_04*	(W 2018-0002225)
Potentilla puberula Krašan	065	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 065_07*	(W 2018-0002226)
Potentilla puberula Krašan	066	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 066_01*	(W 2018-0002217)
Potentilla puberula Krašan	066	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 066_06*	(W 2018-0002219)
Potentilla puberula Krašan	066	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 066_09*	(W 2018-0002218)
Potentilla puberula Krašan	067	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 067_01*	(W 2018-0002216)
Potentilla puberula Krašan	067	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 067_09*	(W 2018-0002215)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	068	Italy	Lombardy	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 068_15*	(W 2018-0002213)
Potentilla puberula Krašan	070	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 070_09*	(W 2018-0002212)
Potentilla puberula Krašan	071	Italy	Lombardy	*Alonso-Marcos, H., Nardi, F.D., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 071_04*	(W 2018-0002048)
Potentilla puberula Krašan	072	Italy	Lombardy	*Alonso-Marcos, H., Nardi, F.D., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 072_03*	(W 2018-0002210)
Potentilla puberula Krašan	073	Italy	Veneto	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 073_06*	(W 2018-0002050)
Potentilla puberula Krašan	074	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 074_02*	(W 2018-0002211)
Potentilla puberula Krašan	074	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 074_03*	(W 2018-0002208)
Potentilla puberula Krašan	075	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 075_13*	(W 2018-0002069)
Potentilla puberula Krašan	076	Italy	Trentino-Alto Adige	*Tribsch, A.*	*Nardi, F.D.*	*Nardi, F.D. 076_08*	(W 2018-0002209)
Potentilla puberula Krašan	078	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 078_08*	(W 2018-0002206)
Potentilla puberula Krašan	078	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 078_10*	(W 2018-0002207)
Potentilla puberula Krašan	079	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 079_02*	(W 2018-0002049)
Potentilla puberula Krašan	080	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 080_02*	(W 2018-0002205)
Potentilla puberula Krašan	080	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 080_03*	(W 2018-0002204)
Potentilla puberula Krašan	080	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 080_06*	(W 2018-0002070)
Potentilla puberula Krašan	080	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 080_12*	(W 2018-0002202)
Potentilla puberula Krašan	082	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 082_17*	(W 2018-0002203)
Potentilla puberula Krašan	083	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 083_02*	(W 2018-0002201)
Potentilla puberula Krašan	083	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 083_20*	(W 2018-0002200)
Potentilla puberula Krašan	084	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 084_01*	(W 2018-0002199)
Potentilla puberula Krašan	084	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 084_12*	(W 2018-0002198)
Potentilla puberula Krašan	084	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 084_16*	(W 2018-0002197)
Potentilla puberula Krašan	085	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 085_03*	(W 2018-0002196)
Potentilla puberula Krašan	085	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 085_08*	(W 2018-0002195)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	085	Italy	Trentino-Alto Adige	*Tribsch, A., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 085_12*	(W 2018-0002146)
Potentilla puberula Krašan	086	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 086_19*	(W 2018-0002194)
Potentilla puberula Krašan	086	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 086_20*	(W 2018-0002193)
Potentilla puberula Krašan	086	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 086_22*	(W 2018-0002192)
Potentilla puberula Krašan	087	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 087_01*	(W 2018-0002191)
Potentilla puberula Krašan	087	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 087_20*	(W 2018-0002189)
Potentilla puberula Krašan	088	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 088_08*	(W 2018-0002190)
Potentilla puberula Krašan	088	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 088_14*	(W 2018-0002188)
Potentilla puberula Krašan	089	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 089_01*	(W 2018-0002186)
Potentilla puberula Krašan	090	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 090_02*	(W 2018-0002187)
Potentilla puberula Krašan	090	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 090_06*	(W 2018-0002185)
Potentilla puberula Krašan	091	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 091_07*	(W 2018-0002184)
Potentilla puberula Krašan	092	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 092_05*	(W 2018-0002182)
Potentilla puberula Krašan	092	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 092_10*	(W 2018-0002181)
Potentilla puberula Krašan	093	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 093_02*	(W 2018-0002183)
Potentilla puberula Krašan	094	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 094_03*	(W 2018-0002180)
Potentilla puberula Krašan	095	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 095_10*	(W 2018-0002179)
Potentilla puberula Krašan	096	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 096_02*	(W 2018-0002177)
Potentilla puberula Krašan	096	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 096_10*	(W 2018-0002147)
Potentilla puberula Krašan	097	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 097_03*	(W 2018-0002178)
Potentilla puberula Krašan	098	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 098_02*	(W 2018-0002175)
Potentilla puberula Krašan	098	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 098_07*	(W 2018-0002176)
Potentilla puberula Krašan	099	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 099_09*	(W 2018-0002173)
Potentilla puberula Krašan	099	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 099_18*	(W 2018-0002174)
Potentilla puberula Krašan	100	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 100_01*	(W 2018-0002170)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	100	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 100_02*	(W 2018-0002172)
Potentilla puberula Krašan	100	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 100_17*	(W 2018-0002171)
Potentilla puberula Krašan	101	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 101_09*	(W 2018-0002169)
Potentilla puberula Krašan	102	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 102_06*	(W 2018-0002168)
Potentilla puberula Krašan	103	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 103_06*	(W 2018-0002166)
Potentilla puberula Krašan	103	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 103_07*	(W 2018-0002165)
Potentilla puberula Krašan	103	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 103_10*	(W 2018-0002068)
Potentilla puberula Krašan	104	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 104_11*	(W 2018-0002066)
Potentilla puberula Krašan	104	Italy	Trentino-Alto Adige	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 104_13*	(W 2018-0002046)
Potentilla puberula Krašan	105	Switzerland	Grisons	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 105_08*	(W 2018-0002047)
Potentilla puberula Krašan	109	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 109_03*	(W 2018-0002164)
Potentilla puberula Krašan	110	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 110_01*	(W 2018-0002163)
Potentilla puberula Krašan	111	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 111_01*	(W 2018-0002067)
Potentilla puberula Krašan	111	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 111_04*	(W 2018-0002161)
Potentilla puberula Krašan	111	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 111_06*	(W 2018-0002162)
Potentilla puberula Krašan	111	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 111_15*	(W 2018-0002160)
Potentilla puberula Krašan	112	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 112_06*	(W 2018-0002158)
Potentilla puberula Krašan	113	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 113_04*	(W 2018-0002159)
Potentilla puberula Krašan	115	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 115_01*	(W 2018-0002157)
Potentilla puberula Krašan	115	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 115_03*	(W 2018-0002156)
Potentilla puberula Krašan	115	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 115_04*	(W 2018-0002155)
Potentilla puberula Krašan	116	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 116_03*	(W 2018-0002154)
Potentilla puberula Krašan	117	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 117_03*	(W 2018-0002151)
Potentilla puberula Krašan	117	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 117_04*	(W 2018-0002153)
Potentilla puberula Krašan	117	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 117_07*	(W 2018-0002152)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	118	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 118_01*	(W 2018-0002150)
Potentilla puberula Krašan	118	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 118_02*	(W 2018-0002149)
Potentilla puberula Krašan	119	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 119_15*	(W 2018-0002111)
Potentilla puberula Krašan	120	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 120_14*	(W 2018-0002145)
Potentilla puberula Krašan	121	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 121_01*	(W 2018-0002065)
Potentilla puberula Krašan	121	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 121_04*	(W 2018-0002144)
Potentilla puberula Krašan	122	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 122_05*	(W 2018-0002062)
Potentilla puberula Krašan	122	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 122_08*	(W 2018-0002107)
Potentilla puberula Krašan	122	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 122_10*	(W 2018-0002143)
Potentilla puberula Krašan	122	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 122_12*	(W 2018-0002142)
Potentilla puberula Krašan	123	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 123_08*	(W 2018-0002141)
Potentilla puberula Krašan	123	Italy	Trentino-Alto Adige	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 123_10*	(W 2018-0002140)
Potentilla puberula Krašan	125	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 125_11*	(W 2018-0002045)
Potentilla puberula Krašan	126	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 126_09*	(W 2018-0002139)
Potentilla puberula Krašan	127	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 127_07*	(W 2018-0002138)
Potentilla puberula Krašan	128	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 128_06*	(W 2018-0002137)
Potentilla puberula Krašan	129	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 129_11*	(W 2018-0002136)
Potentilla puberula Krašan	130	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 130_09*	(W 2018-0002134)
Potentilla puberula Krašan	131	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 131_01*	(W 2018-0002135)
Potentilla puberula Krašan	132	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 132_02*	(W 2018-0002132)
Potentilla puberula Krašan	133	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 133_09*	(W 2018-0002133)
Potentilla puberula Krašan	134	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 134_13*	(W 2018-0002130)
Potentilla puberula Krašan	134	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 134_15*	(W 2018-0002131)
Potentilla puberula Krašan	135	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 135_19*	(W 2018-0002128)
Potentilla puberula Krašan	136	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 136_06*	(W 2018-0002064)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	137	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 137_02*	(W 2018-0002129)
Potentilla puberula Krašan	138	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 138_10*	(W 2018-0002126)
Potentilla puberula Krašan	138	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 138_11*	(W 2018-0002127)
Potentilla puberula Krašan	139	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 139_03*	(W 2018-0002124)
Potentilla puberula Krašan	140	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 140_12*	(W 2018-0002122)
Potentilla puberula Krašan	141	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 141_01*	(W 2018-0002123)
Potentilla puberula Krašan	142	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 142_06*	(W 2018-0002121)
Potentilla puberula Krašan	143	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D., Stifter, S., Julian Ananda Hainer*	*Nardi, F.D.*	*Nardi, F.D. 143_05*	(W 2018-0002110)
Potentilla puberula Krašan	143	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D., Stifter, S., Julian Ananda Hainer*	*Nardi, F.D.*	*Nardi, F.D. 143_06*	(W 2018-0002061)
Potentilla puberula Krašan	145	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 145_01*	(W 2018-0002119)
Potentilla puberula Krašan	146	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 146_03*	(W 2018-0002118)
Potentilla puberula Krašan	146	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 146_07*	(W 2018-0002063)
Potentilla puberula Krašan	146	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 146_20*	(W 2018-0002120)
Potentilla puberula Krašan	148	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 148_05*	(W 2018-0002117)
Potentilla puberula Krašan	149	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 149_13*	(W 2018-0002060)
Potentilla puberula Krašan	149	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 149_14*	(W 2018-0002058)
Potentilla puberula Krašan	150	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 150_05*	(W 2018-0002115)
Potentilla puberula Krašan	150	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 150_13*	(W 2018-0002114)
Potentilla puberula Krašan	150	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 150_18*	(W 2018-0002113)
Potentilla puberula Krašan	151	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 151_03*	(W 2018-0002059)
Potentilla puberula Krašan	152	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 152_03*	(W 2018-0002044)
Potentilla puberula Krašan	153	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 153_08*	(W 2018-0002108)
Potentilla puberula Krašan	154	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 154_07*	(W 2018-0002105)
Potentilla puberula Krašan	156	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 156_01*	(W 2018-0002104)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	156	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 156_02*	(W 2018-0002103)
Potentilla puberula Krašan	157	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 157_02*	(W 2018-0002106)
Potentilla puberula Krašan	157	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 157_12*	(W 2018-0002101)
Potentilla puberula Krašan	158	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 158_07*	(W 2018-0002102)
Potentilla puberula Krašan	159	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 159_04*	(W 2018-0002100)
Potentilla puberula Krašan	159	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 159_16*	(W 2018-0002099)
Potentilla puberula Krašan	159	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 159_18*	(W 2018-0002096)
Potentilla puberula Krašan	160	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 160_07*	(W 2018-0002097)
Potentilla puberula Krašan	160	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 160_13*	(W 2018-0002094)
Potentilla puberula Krašan	161	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 161_02*	(W 2018-0002042)
Potentilla puberula Krašan	162	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 162_07*	(W 2018-0002053)
Potentilla puberula Krašan	163	Austria	Tyrol	*Alonso-Marcos, H., Nardi, F.D.*	*Nardi, F.D.*	*Nardi, F.D. 163_01*	(W 2018-0002095)
Potentilla puberula Krašan	164	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 164_01*	(W 2018-0002098)
Potentilla puberula Krašan	164	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 164_04*	(W 2018-0002057)
Potentilla puberula Krašan	165	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 165_03*	(W 2018-0002093)
Potentilla puberula Krašan	165	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 165_11*	(W 2018-0002091)
Potentilla puberula Krašan	165	Italy	Trentino-Alto Adige	*Stifter, S., Haider, J.A.*	*Nardi, F.D.*	*Nardi, F.D. 165_18*	(W 2018-0002092)
Potentilla puberula Krašan	169	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 169_02*	(W 2018-0002056)
Potentilla puberula Krašan	169	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 169_15*	(W 2018-0002090)
Potentilla puberula Krašan	170	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 170_02*	(W 2018-0002089)
Potentilla puberula Krašan	170	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 170_05*	(W 2018-0002055)
Potentilla puberula Krašan	170	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 170_14*	(W 2018-0002054)
Potentilla puberula Krašan	171	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 171_01*	(W 2018-0002088)
Potentilla puberula Krašan	172	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 172_04*	(W 2018-0002043)
Potentilla puberula Krašan	172	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 172_13*	(W 2018-0002041)

Taxon	Population	Country	Province	Original collectors	Collector ex cult.	Collection number	Herbarium
Potentilla puberula Krašan	172	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 172_18*	(W 2018-0002087)
Potentilla puberula Krašan	172	Italy	Veneto	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 172_20*	(W 2018-0002040)
Potentilla puberula Krašan	173	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 173_03*	(W 2018-0002086)
Potentilla puberula Krašan	173	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 173_08*	(W 2018-0002039)
Potentilla puberula Krašan	173	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 173_14*	(W 2018-0002037)
Potentilla puberula Krašan	174	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 174_01*	(W 2018-0002085)
Potentilla puberula Krašan	175	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 175_06*	(W 2018-0002083)
Potentilla puberula Krašan	179	Austria	Carinthia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 179_02*	(W 2018-0002082)
Potentilla puberula Krašan	179	Austria	Carinthia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 179_04*	(W 2018-0002081)
Potentilla puberula Krašan	180	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 180_02*	(W 2018-0002078)
Potentilla puberula Krašan	180	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 180_06*	(W 2018-0002080)
Potentilla puberula Krašan	181	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 181_04*	(W 2018-0002079)
Potentilla puberula Krašan	183	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 183_09*	(W 2018-0002052)
Potentilla puberula Krašan	183	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 183_22*	(W 2018-0002077)
Potentilla puberula Krašan	184	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 184_06*	(W 2018-0002074)
Potentilla puberula Krašan	185	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 185_01*	(W 2018-0002073)
Potentilla puberula Krašan	185	Italy	Friuli-Venezia Giulia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 185_05*	(W 2018-0002072)
Potentilla puberula Krašan	186	Austria	Carinthia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 186_03*	(W 2018-0002071)
Potentilla puberula Krašan	186	Austria	Carinthia	*Dobeš, C.*	*Nardi, F.D.*	*Nardi, F.D. 186_05*	(W 2018-0002051)

Table S2. GenBank (Benson & al., 2005) codes orf*rps16* and *trnH-psbA* cpDNA regions in *Potentilla puberula* Krašan reported by combined haplotype.

Haplotype code	*rps16*	*trnH-psbA*
H1	MG995594	MG995643
H2	MG995595	MG995644
H3	MG995596	MG995645
H4	MG995597	MG995646
H5	MG995598	MG995647
H6	MG995599	MG995648
H7	MG995600	MG995649
H8	MG995601	MG995650
H9	MG995602	MG995651
H10	MG995603	MG995652
H11	MG995604	MG995653
H12	MG995605	MG995654
H13	MG995606	MG995655
H14	MG995607	MG995656
H15	MG995608	MG995657
H16	MG995609	MG995658
H17	MG995610	MG995659
H18	MG995611	MG995660
H19	MG995612	MG995661
H20	MG995613	MG995662
H21	MG995614	MG995663
H22	MG995615	MG995664
H23	MG995616	MG995665
H24	MG995617	MG995666
H25	MG995618	MG995667
H26	MG995619	MG995668
H27	MG995620	MG995669
H28	MG995621	MG995670
H29	MG995622	MG995671
H30	MG995623	MG995672
H31	MG995624	MG995673
H32	MG995625	MG995674
H33	MG995626	MG995675
H34	MG995627	MG995676
H35	MG995628	MG995677
H36	MG995629	MG995678
H37	MG995630	MG995679
H38	MG995631	MG995680
H39	MG995632	MG995681
H40	MG995633	MG995682
H41	MG995634	MG995683
H42	MG995635	MG995684
H43	MG995636	MG995685
H44	MG995637	MG995686
H45	MG995638	MG995687
H46	MG995639	MG995688
H47	MG995640	MG995689
H48	MG995641	MG995690
H49	MG995642	MG995691

Table S3, **S4**, **S5** and **S6** can be found here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6382066/ named: ***NIHMS81441-supplement-Electronic_Supplement_2.xlsx*** (GUID: 4CB466F0-3BFC-4A16-B90D-4298A8FE9196).

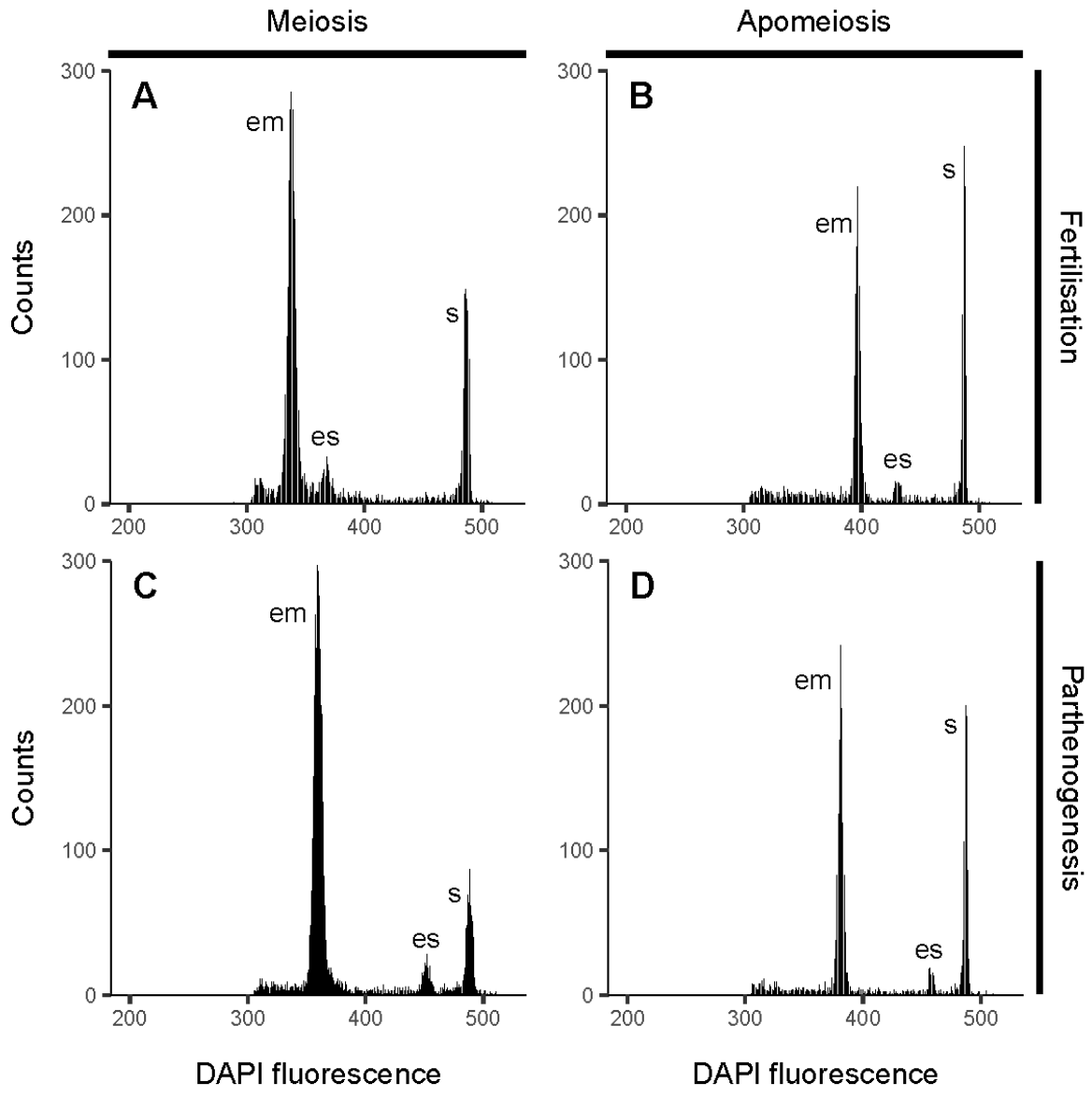


Fig. S1. Examples of reproductive modes of seed formation in *Potentilla puberula* Krašan as observed by flow cytometric seed screen. **A**, Regular sexuality in a tetraploid individual; **B**, Irregular sexuality (B_{III} hybrids formation) in a pentaploid individual; **C**, Haploid parthenogenesis in an octoploid individual; **D**, Apomixis in a heptaploid individual. – em: embryo, es: endosperm; s: standard

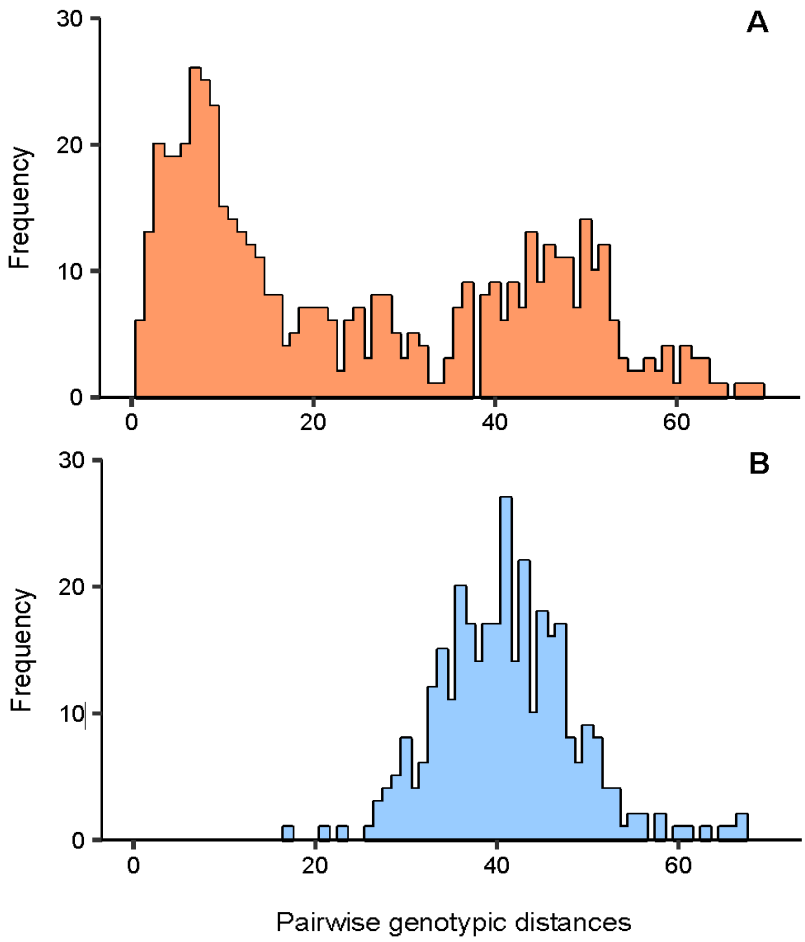


Fig. S2. Distribution of the pairwise genotypic distances (simple matching) among penta- to octoploid (**A**) and tetraploid (**B**) individuals of the same ploidy and same geographic population in *Potentilla puberula* Krašan.

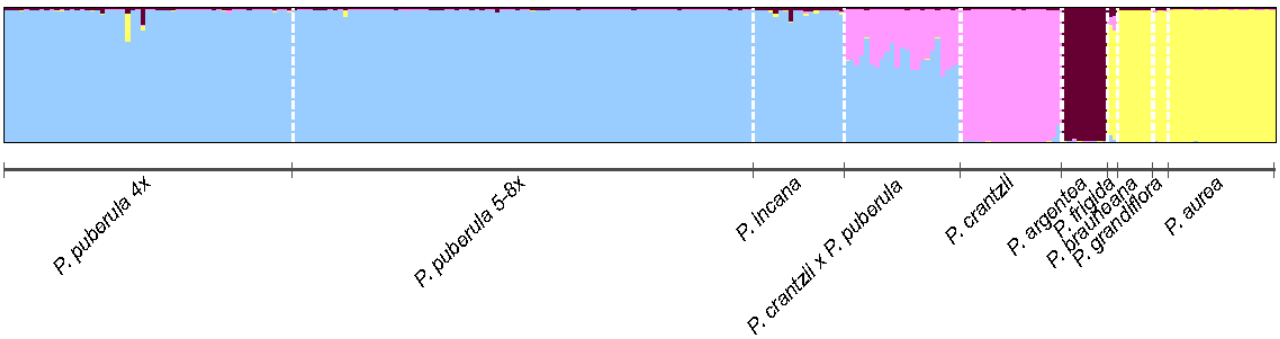


Fig. S3. Individual cluster assignment of 251 single genotypes of *Potentilla L.* taxa resulting from a STRUCTURE analysis based on 335 AFLP markers with K = 4, according to 8/10 runs with lower likelihood.

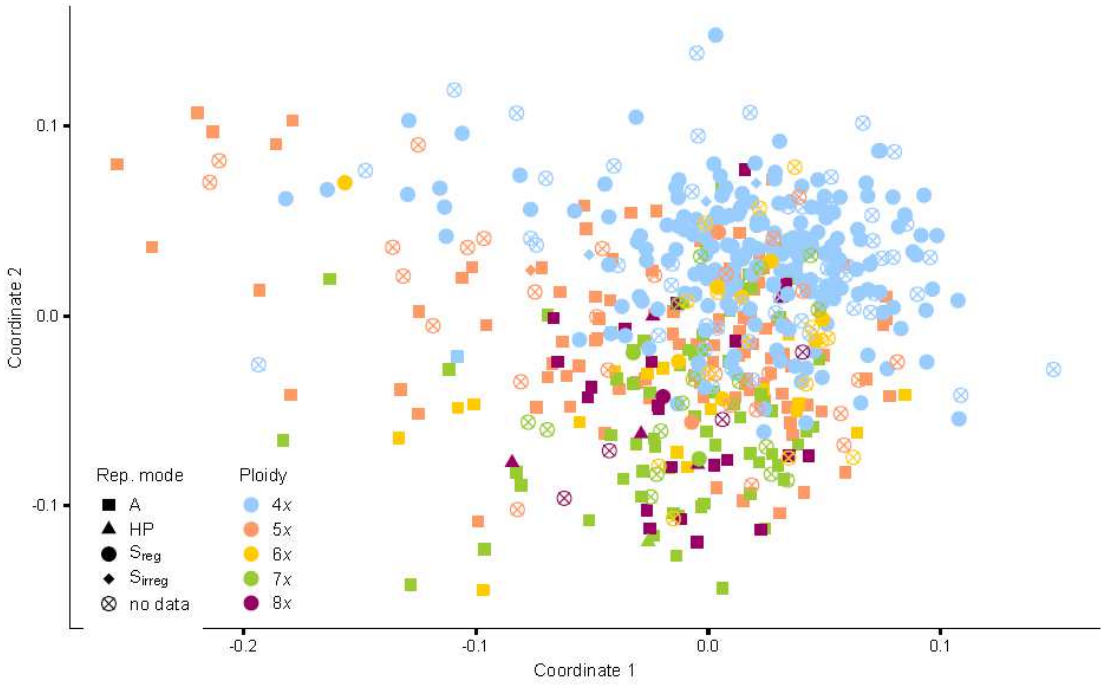


Fig. S4. Principal coordinate analysis of 554 unique genotypes of *Potentilla puberula* Krašan based on 367 AFLP markers, after removal of AFLP fragments 170 VIC, 219 FAM and 286 FAM. The coordinates 1 and 2 explain 2.81% and 2.16% of the total genetic variation, respectively. A: apomixes; HP: haploid parthenogenesis, S_{irreg}: irregular sexuality (B_{III} hybrids formation); S_{reg}: regular sexuality.

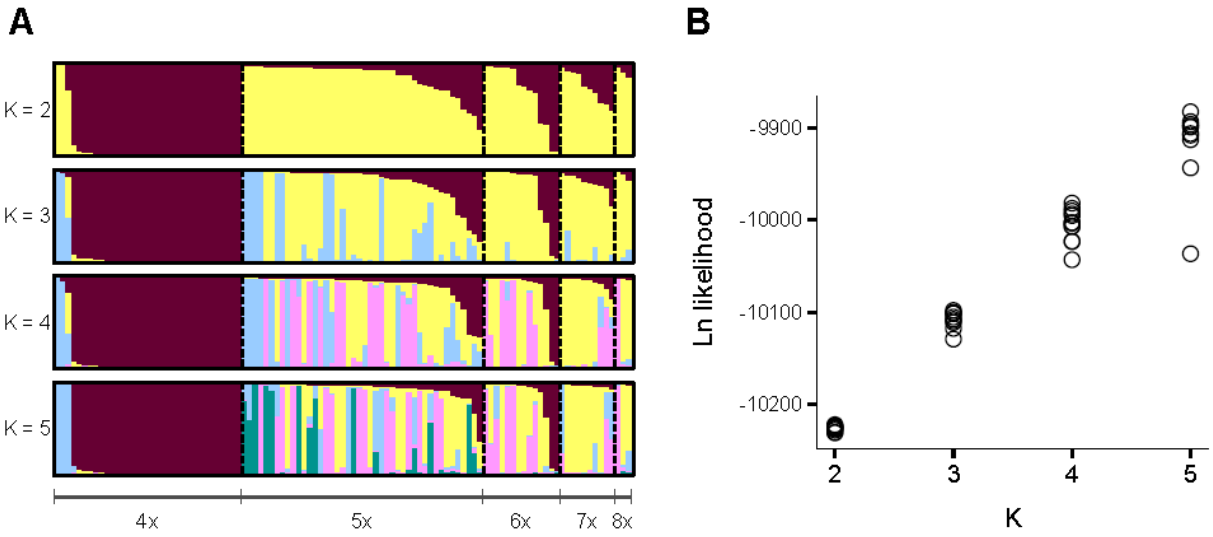


Fig. S5. Results of the STRUCTURE analysis conducted on 105 unique genotypes of *Potentilla puberula* Krašan from Eastern Tyrol populations and based on 252 polymorphic markers. **A**, Individual cluster membership to Ks from 2 to 5; **B**, Logarithmic likelihood of single runs per K value.

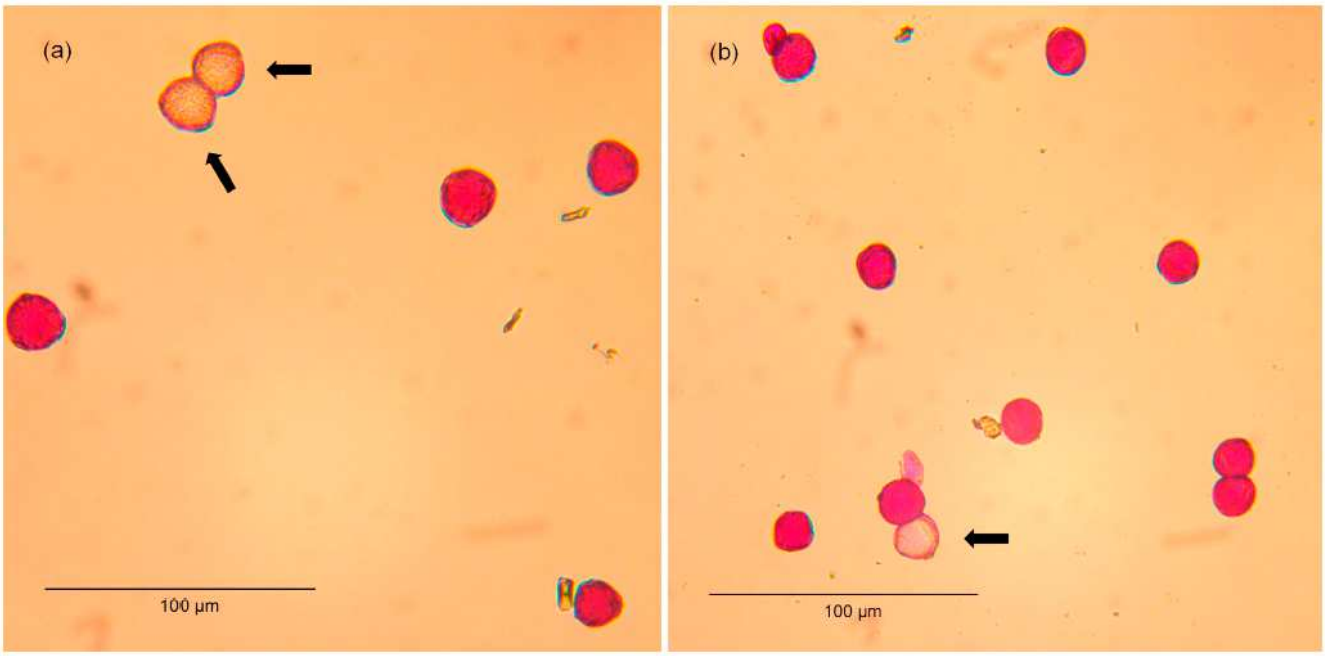


Fig. S1. Peterson's vitality stain used to differentiate vital (i.e., stained, red grains) and aborted pollen (non-stained grains, indicated by arrows) of *Potentilla puberula* Krašan exemplified with **(A)** a heptaploid individual from region Raas/Fortezza (119-72) and **(B)** a pentaploid individual from Bodenalm (144-25), Laborlux S microscope, 400x.

Supplementary Table 1. Descriptive statistics of a controlled *ex situ* crossing experiment carried out on sexual and apomictic individuals of *Potentilla puberula* Krašan from five local regions from East Tyrol, Austria, and South Tyrol and the Trentino/Lombardia, Italy. Fifty-five sexual tetraploid individuals served as pollen recipient which were pollinated by A) 72 sexual tetraploid ("Treatment" IA), 74 apomictic pentaploid (IE 5x) and additionally in two regions 44 apomictic heptaploid pollen donors (IE 7x), and B) mixtures of equivalent amounts of pollen from sexual and apomictic donors (MIX). In the regions Ossenigo/Scaiola and Raas/Fortezza, which comprise two populations, the first mentioned population furnished the sexual cytotypes, the second mentioned the apomictic pollen donors. The initial letter in front of each donor indicates from which of the two populations is the individual. For each manipulation, the pollen "Recipient", the "Donor" and the obtained "Seed yield" is provided.

A) Homo- and heteroploid crosses

Region	Recipient	Donor	Treatment	Seed yield
Zabernig	119	57	IE 5x	2
	119	61	IE 5x	0
	119	130	IA	0
	119	136	IA	1
	125	69	IE 5x	1
	125	105	IE 5x	0
	125	130	IA	6
	125	136	IA	0
	125	136	IA	6
	137	56	IE 5x	0
	137	71	IE 5x	1
	137	88	IE 5x	1
	137	119	IA	2
	137	130	IA	0
	137	136	IA	4
	140	56	IE 5x	3
	140	106	IE 5x	1
	140	111	IA	0
	142	57	IE 5x	0
	142	57	IE 5x	4
	142	110	IE 5x	0
	142	119	IA	5
	142	147	IA	2
	142	148	IA	1
	147	94	IE 5x	0
	147	120	IA	0
	148	60	IE 5x	3

	148	129	IA	10
Obersteiner	56	38	IE 5x	15
	56	43	IA	18
	56	48	IA	1
	56	57	IE 5x	3
	103	36	IE 5x	10
	103	43	IA	13
	103	44	IA	17
	103	46	IA	3
	103	145	IE 5x	11
	103	148	IE 5x	4
	104	44	IA	7
	104	54	IE 5x	0
	104	58	IE 5x	0
	104	127	IA	1
	104	140	IA	19
	104	145	IE 5x	13
	105	38	IE 5x	3
	105	41	IE 5x	12
	105	47	IA	17
	105	106	IA	0
	105	126	IA	23
	106	35	IA	5
	106	46	IA	10
	106	48	IA	1
	106	121	IE 5x	12
	109	41	IE 5x	13
	109	48	IA	5
	109	103	IA	13
	109	138	IE 5x	19
	109	139	IA	12
	110	36	IE 5x	1
	110	47	IA	6
	110	58	IE 5x	10
	110	106	IA	11
	113	48	IA	1
	113	96	IA	0
	113	106	IA	1
	113	125	IE 5x	0
	113	133	IE 5x	0
	116	35	IA	5
	116	45	IA	21
	116	58	IE 5x	8
	116	103	IA	9
	116	139	IA	13
	116	148	IE 5x	4
	126	45	IA	7

	126	140	IA	20
	126	140	IA	16
	143	43	IA	39
	143	45	IA	26
	143	48	IA	0
	143	48	IA	9
	143	121	IE 5x	16
	143	133	IE 5x	2
	146	31	IA	3
	146	36	IE 5x	14
	146	41	IE 5x	9
	146	104	IA	11
	146	111	IE 5x	9
	146	127	IA	14
	146	127	IA	24
Ossenigo/Scaiola	14	O 20	IA	32
	14	S 5	IE 7x	8
	14	S 5	IE 7x	7
	14	S 13	IE 7x	22
	14	S 30	IE 5x	9
	14	S 30	IE 5x	9
	14	S 37	IE 5x	17
	14	S 37	IE 5x	23
	14	S 71	IE 7x	16
	14	S 117	IE 5x	16
	15	O 4	IA	0
	15	O 14	IA	0
	15	O 20	IA	0
	15	O 55	IA	1
	15	S 10	IE 7x	0
	15	S 22	IE 5x	0
	15	S 37	IE 5x	0
	16	O 13	IA	0
	16	O 34	IA	0
	16	O 49	IA	0
	16	S 22	IE 5x	1
	16	S 36	IE 5x	3
	16	S 91	IE 5x	1
	17	O 12	IA	6
	17	O 49	IA	5
	17	O 55	IA	11
	17	S 6	IE 7x	2
	17	S 7	IE 5x	0
	17	S 29	IE 5x	1
	17	S 34	IE 5x	2
	17	S 34	IE 5x	6
	17	S 42	IE 7x	3

18	O 12	IA	12
18	O 14	IA	9
18	O 17	IA	10
18	S 35	IE 5x	4
18	S 41	IE 5x	3
18	S 48	IE 7x	1
18	S 80	IE 7x	7
18	S 84	IE 7x	5
20	O 18	IA	1
20	O 21	IA	5
20	O 55	IA	4
20	S 16	IE 7x	7
20	S 16	IE 7x	0
20	S 27	IE 5x	3
20	S 32	IE 5x	4
20	S 45	IE 7x	1
20	S 45	IE 7x	0
20	S 57	IE 7x	1
20	S 90	IE 5x	1
27	O 16	IA	0
27	O 20	IA	9
27	O 32	IA	15
27	S 16	IE 7x	8
27	S 21	IE 5x	2
27	S 31	IE 5x	2
27	S 33	IE 5x	0
27	S 100	IE 7x	3
27	S 100	IE 7x	8
29	O 4	IA	8
29	O 32	IA	5
29	S 8	IE 7x	2
36	O 12	IA	0
36	S 35	IE 5x	0
36	S 38	IE 5x	7
36	S 54	IE 7x	0
36	S 63	IE 7x	1
42	O 18	IA	0
42	S 57	IE 7x	0
42	S 90	IE 5x	0
44	O 13	IA	5
44	O 22	IA	0
44	O 34	IA	3
44	S 27	IE 5x	0
44	S 30	IE 5x	0
44	S 55	IE 7x	2
44	S 70	IE 7x	2
44	S 95	IE 5x	0

	48	O 13	IA	6
	48	O 55	IA	12
	48	S 29	IE 5x	3
	48	S 46	IE 7x	0
	48	S 65	IE 5x	0
	48	S 74	IE 7x	0
	48	S 78	IE 7x	2
	48	S 86	IE 5x	0
Raas/Fortezza	16	F 48	IE 5x	0
	16	F 59	IE 7x	0
	16	F 60	IE 5x	1
	18	F 12	IE 5x	6
	18	F 13	IE 5x	2
	18	F 19	IE 5x	0
	18	F 43	IE 7x	0
	18	F 59	IE 7x	7
	18	R 29	IA	7
	18	R 31	IA	15
	18	R 33	IA	3
	20	F 3	IE 5x	6
	20	F 18	IE 7x	24
	20	F 18	IE 7x	18
	20	F 24	IE 5x	16
	20	F 60	IE 5x	4
	20	R 3	IA	15
	20	R 8	IA	18
	20	R 8	IA	6
	20	R 10	IA	21
	22	F 14	IE 7x	2
	22	F 25	IE 5x	4
	22	F 27	IE 7x	0
	22	F 28	IE 5x	1
	22	F 33	IE 5x	2
	22	R 3	IA	9
	22	R 6	IA	9
	22	R 52	IA	11
	26	F 9	IE 7x	2
	26	F 11	IE 7x	2
	26	F 28	IE 5x	0
	26	F 41	IE 7x	3
	26	F 44	IE 5x	7
	26	F 47	IE 5x	4
	26	R 2	IA	10
	26	R 10	IA	12
	26	R 11	IA	9
	27	F 8	IE 5x	9
	27	F 16	IE 7x	3

27	F 48	IE 5x	5
27	F 49	IE 7x	5
27	F 60	IE 5x	3
27	R 2	IA	5
27	R 6	IA	22
27	R 9	IA	24
30	F 19	IE 5x	1
30	F 31	IE 5x	7
30	F 32	IE 5x	5
30	F 67	IE 7x	10
30	F 73	IE 7x	0
30	F 73	IE 7x	5
30	R 12	IA	20
30	R 13	IA	11
30	R 52	IA	13
36	F 1	IE 7x	4
36	F 12	IE 5x	10
36	F 23	IE 7x	12
36	F 26	IE 7x	6
36	F 29	IE 7x	8
36	F 33	IE 5x	5
36	F 47	IE 5x	3
36	R 5	IA	8
36	R 12	IA	11
36	R 29	IA	2
37	F 8	IE 5x	3
37	F 13	IE 5x	4
37	F 23	IE 7x	4
37	F 51	IE 7x	2
37	F 53	IE 7x	4
37	R 5	IA	11
37	R 11	IA	9
37	R 13	IA	7
38	F 8	IE 5x	0
38	F 12	IE 5x	0
40	F 25	IE 5x	13
40	F 32	IE 5x	8
40	F 50	IE 7x	6
40	F 63	IE 7x	1
40	F 65	IE 7x	5
40	F 66	IE 5x	5
40	R 3	IA	9
40	R 6	IA	18
40	R 8	IA	6
43	F 3	IE 5x	0
43	F 4	IE 7x	2
43	F 16	IE 7x	0

	43	F 31	IE 5x	11
	43	F 37	IE 7x	0
	43	F 66	IE 5x	9
	43	R 31	IA	8
	43	R 35	IA	4
	43	R 44	IA	10
Bodenalm	29	2	IE 5x	0
	29	8	IA	40
	29	28	IA	28
	29	28	IA	27
	31	4	IA	11
	31	5	IE 5x	1
	31	6	IA	2
	31	8	IA	4
	31	10	IE 5x	20
	31	26	IE 5x	1
	33	20	IA	15
	33	21	IA	31
	33	21	IA	9
	33	22	IA	18
	33	25	IE 5x	11
	33	27	IE 5x	0
	33	96	IE 5x	0
	37	2	IE 5x	22
	37	10	IE 5x	9
	37	30	IA	31
	37	50	IA	10
	37	77	IA	34
	37	99	IE 5x	24
	47	9	IA	30
	47	15	IE 5x	32
	47	18	IA	4
	47	27	IE 5x	17
	47	30	IA	13
	47	69	IE 5x	0
	49	15	IE 5x	2
	49	37	IA	8
	49	47	IA	1
	49	50	IA	12
	49	96	IE 5x	1
	49	100	IE 5x	3
	49	100	IE 5x	0
	50	25	IE 5x	8
	50	37	IA	12
	50	49	IA	23
	50	77	IA	6
	50	100	IE 5x	5

50	100	IE 5x	4
51	4	IA	14
51	6	IA	17
51	15	IE 5x	1
51	18	IA	5
51	26	IE 5x	1
51	97	IE 5x	9
51	100	IE 5x	2
56	2	IE 5x	0
56	2	IE 5x	0
56	2	IE 5x	6
56	28	IA	0
56	29	IA	13
56	47	IA	10
56	69	IE 5x	0
56	97	IE 5x	4
56	97	IE 5x	4
57	5	IE 5x	2
57	9	IA	4
57	26	IE 5x	2
57	27	IE 5x	2
57	36	IA	3
57	50	IA	5
58	29	IA	20
60	9	IA	1
60	22	IA	8
60	25	IE 5x	19
60	35	IE 5x	0
60	47	IA	1
60	97	IE 5x	0

B) Mixed crosses

Population	Recipient	Donor 1	Donor 2	Treatment	Seed yield
Zabernig	119	120	57	MIX 4x + 5x	0
	137	136	88	MIX 4x + 5x	0
	137	130	56	MIX 4x + 5x	0
	137	119	71	MIX 4x + 5x	0
	137	56	130	MIX 5x + 4x	0
	137	71	119	MIX 5x + 4x	0
	140	111	106	MIX 4x + 5x	4
	140	106	111	MIX 5x + 4x	0
	142	147	97	MIX 4x + 5x	0
	142	147	97	MIX 4x + 5x	2
	142	147	97	MIX 4x + 5x	11
	142	137	99	MIX 4x + 5x	0

	142	97	147	MIX 5x + 4x	0
	142	94	136	MIX 5x + 4x	6
	147	136	94	MIX 4x + 5x	0
	147	94	136	MIX 5x + 4x	0
	148	60	136	MIX 5x + 4x	0
Obersteiner	103	36	43	MIX 5x + 4x	5
	103	43	36	MIX 4x + 5x	7
	103	44	145	MIX 4x + 5x	12
	103	145	44	MIX 5x + 4x	17
	104	44	58	MIX 4x + 5x	13
	104	54	127	MIX 5x + 4x	8
	104	58	44	MIX 5x + 4x	3
	104	127	54	MIX 4x + 5x	14
	104	140	145	MIX 4x + 5x	15
	104	145	140	MIX 5x + 4x	9
	105	38	47	MIX 5x + 4x	7
	105	41	106	MIX 5x + 4x	13
	105	47	38	MIX 4x + 5x	2
	105	106	41	MIX 4x + 5x	18
	106	35	121	MIX 4x + 5x	3
	106	121	35	MIX 5x + 4x	5
	109	41	43	MIX 5x + 4x	4
	109	43	41	MIX 4x + 5x	11
	109	103	138	MIX 4x + 5x	14
	109	138	103	MIX 5x + 4x	16
	110	36	106	MIX 5x + 4x	12
	110	47	58	MIX 4x + 5x	4
	110	58	47	MIX 5x + 4x	6
	110	106	36	MIX 4x + 5x	1
	110	127	145	MIX 4x + 5x	3
	110	145	127	MIX 5x + 4x	1
	113	46	125	MIX 4x + 5x	4
	113	106	111	MIX 4x + 5x	1
	113	125	46	MIX 5x + 4x	3
	116	35	58	MIX 4x + 5x	1
	116	45	148	MIX 4x + 5x	17
	116	58	35	MIX 5x + 4x	0
	116	148	45	MIX 5x + 4x	10
	126	36	103	MIX 5x + 4x	17
	126	103	36	MIX 4x + 5x	18
	143	43	121	MIX 4x + 5x	18
	143	45	57	MIX 4x + 5x	14
	143	45	133	MIX 4x + 5x	3
	143	121	43	MIX 5x + 4x	18
	143	133	45	MIX 5x + 4x	5
	146	31	36	MIX 4x + 5x	9
	146	36	31	MIX 5x + 4x	9

	146	41	127	MIX 5x + 4x	0
	146	104	111	MIX 4x + 5x	12
	146	111	104	MIX 5x + 4x	23
	146	127	41	MIX 4x + 5x	6
Ossenigo/Scaiola	14	O 18	S 30	MIX 4x + 5x	12
	14	O 20	S 71	MIX 4x + 7x	20
	14	O 20	S 117	MIX 4x + 5x	16
	14	S 30	O 18	MIX 5x + 4x	24
	14	S 37	O 43	MIX 5x + 4x	38
	14	O 43	S 37	MIX 4x + 5x	2
	14	S 71	O 20	MIX 7x + 4x	14
	14	S 117	O 20	MIX 5x + 4x	13
	15	S 4	O 22	MIX 5x + 4x	0
	15	O 12	S 37	MIX 4x + 5x	0
	15	O 12	S 78	MIX 4x + 7x	0
	15	O 22	S 4	MIX 4x + 5x	0
	15	S 37	O 12	MIX 5x + 4x	0
	15	S 78	O 12	MIX 7x + 4x	0
	16	O 13	S 88	MIX 4x + 7x	0
	16	O 13	S 91	MIX 4x + 5x	0
	16	S 22	O 49	MIX 5x + 4x	0
	16	O 34	S 36	MIX 4x + 5x	0
	16	O 34	S 87	MIX 4x + 7x	0
	16	S 36	O 34	MIX 5x + 4x	2
	16	O 49	S 22	MIX 4x + 5x	0
	16	S 87	O 34	MIX 7x + 4x	0
	16	S 91	O 13	MIX 5x + 4x	0
	17	S 6	O 55	MIX 7x + 4x	6
	17	S 7	O 55	MIX 5x + 4x	0
	17	O 12	S 29	MIX 4x + 5x	3
	17	O 12	S 42	MIX 4x + 7x	0
	17	S 29	O 12	MIX 5x + 4x	0
	17	S 34	O 49	MIX 5x + 4x	4
	17	S 42	O 12	MIX 7x + 4x	0
	17	O 49	S 34	MIX 4x + 5x	5
	17	O 55	S 6	MIX 4x + 7x	8
	17	O 55	S 7	MIX 4x + 5x	2
	18	O 12	S 35	MIX 4x + 5x	0
	18	S 35	O 12	MIX 5x + 4x	0
	20	S 16	O 18	MIX 7x + 4x	3
	20	O 18	S 16	MIX 4x + 7x	10
	20	O 18	S 95	MIX 4x + 5x	2
	20	O 21	S 32	MIX 4x + 5x	1
	20	O 21	S 57	MIX 4x + 7x	0
	20	S 32	O 21	MIX 5x + 4x	2
	20	S 57	O 21	MIX 7x + 4x	0
	20	S 95	O 18	MIX 5x + 4x	2

	27	O 12	S 33	MIX 4x + 5x	11
	27	O 12	S 120	MIX 4x + 7x	16
	27	O 16	S 100	MIX 4x + 7x	0
	27	O 32	S 16	MIX 4x + 7x	14
	27	O 32	S 16	MIX 4x + 7x	16
	27	S 100	O 16	MIX 7x + 4x	2
	27	S 120	O 12	MIX 7x + 4x	11
	36	O 12	S 35	MIX 4x + 5x	3
	36	S 35	O 12	MIX 5x + 4x	0
	42	O 21	S 57	MIX 4x + 7x	0
	44	O 13	S 30	MIX 4x + 5x	0
	44	O 13	S 70	MIX 4x + 7x	0
	44	O 22	S 27	MIX 4x + 5x	1
	44	S 27	O 22	MIX 5x + 4x	2
	44	O 34	S 55	MIX 4x + 7x	10
	44	S 55	O 34	MIX 7x + 4x	3
	44	S 70	O 13	MIX 7x + 4x	0
	48	O 12	S 29	MIX 4x + 5x	0
	48	O 12	S 46	MIX 4x + 7x	1
	48	O 27	S 74	MIX 4x + 7x	6
	48	S 29	O12	MIX 5x + 4x	1
	48	S 29	O 43	MIX 5x + 4x	5
	48	O 43	S 29	MIX 4x + 5x	0
	48	S 46	O 12	MIX 7x + 4x	4
	48	S 74	O 27	MIX 7x + 4x	4
	16	31	48	MIX 4x + 5x	2
Raas/Fortezza	16	48	31	MIX 5x + 4x	1
	18	12	31	MIX 5x + 4x	10
	18	13	33	MIX 5x + 4x	4
	18	19	29	MIX 5x + 4x	11
	18	29	19	MIX 4x + 5x	0
	18	31	12	MIX 4x + 5x	0
	18	33	13	MIX 4x + 5x	3
	18	52	53	MIX 4x + 7x	3
	18	53	52	MIX 7x + 4x	11
	20	3	3	MIX 4x + 5x	5
	20	3	3	MIX 5x + 4x	1
	20	3	10	MIX 4x + 7x	14
	20	10	3	MIX 7x + 4x	14
	20	10	24	MIX 4x + 5x	14
	20	24	10	MIX 5x + 4x	1
	20	31	50	MIX 4x + 7x	19
	20	31	50	MIX 4x + 7x	20
	20	31	60	MIX 4x + 5x	2
	20	50	31	MIX 7x + 4x	5
	20	60	31	MIX 5x + 4x	16
	22	3	14	MIX 4x + 7x	9

22	6	27	MIX 4x + 7x	11
22	6	28	MIX 4x + 5x	1
22	10	52	MIX 7x + 4x	5
22	14	3	MIX 7x + 4x	6
22	25	52	MIX 5x + 4x	0
22	27	6	MIX 7x + 4x	3
22	28	6	MIX 5x + 4x	1
22	52	10	MIX 4x + 7x	5
22	52	25	MIX 4x + 5x	2
26	2	9	MIX 4x + 7x	24
26	2	47	MIX 4x + 5x	8
26	9	2	MIX 7x + 4x	25
26	10	11	MIX 4x + 7x	0
26	10	28	MIX 4x + 5x	7
26	11	10	MIX 7x + 4x	3
26	11	41	MIX 4x + 7x	0
26	11	44	MIX 4x + 5x	3
26	28	10	MIX 5x + 4x	0
26	41	11	MIX 7x + 4x	0
26	44	11	MIX 5x + 4x	7
26	47	2	MIX 5x + 4x	2
27	2	46	MIX 4x + 7x	0
27	2	48	MIX 4x + 5x	22
27	6	8	MIX 4x + 5x	10
27	6	49	MIX 4x + 7x	12
27	8	6	MIX 5x + 4x	7
27	8	6	MIX 5x + 4x	6
27	46	2	MIX 7x + 4x	13
27	49	6	MIX 7x + 4x	8
30	12	19	MIX 4x + 5x	3
30	12	22	MIX 4x + 7x	14
30	13	31	MIX 4x + 5x	12
30	13	67	MIX 4x + 7x	10
30	19	12	MIX 5x + 4x	0
30	31	52	MIX 5x + 4x	10
30	32	13	MIX 5x + 4x	8
30	52	31	MIX 4x + 5x	0
30	52	73	MIX 4x + 7x	16
30	67	13	MIX 7x + 4x	8
30	73	52	MIX 7x + 4x	8
36	2	64	MIX 4x + 7x	6
36	5	26	MIX 4x + 7x	10
36	12	12	MIX 4x + 5x	1
36	12	12	MIX 5x + 4x	4
36	26	5	MIX 7x + 4x	4
36	29	29	MIX 4x + 7x	1
36	29	29	MIX 7x + 4x	13

	36	29	47	MIX 4x + 5x	7
	36	47	29	MIX 5x + 4x	1
	36	64	12	MIX 7x + 4x	10
	37	5	8	MIX 4x + 5x	1
	37	5	52	MIX 7x + 4x	0
	37	5	53	MIX 4x + 7x	5
	37	8	5	MIX 5x + 4x	4
	37	11	24	MIX 4x + 5x	6
	37	13	13	MIX 4x + 5x	7
	37	13	13	MIX 5x + 4x	10
	37	13	51	MIX 4x + 7x	3
	37	24	11	MIX 5x + 4x	8
	37	51	13	MIX 7x + 4x	4
	37	52	5	MIX 4x + 7x	4
	37	53	5	MIX 7x + 4x	3
	40	3	66	MIX 4x + 5x	7
	40	6	25	MIX 4x + 5x	2
	40	6	32	MIX 4x + 5x	17
	40	6	32	MIX 4x + 5x	26
	40	32	6	MIX 5x + 4x	16
	40	66	3	MIX 5x + 4x	18
	43	3	33	MIX 5x + 4x	14
	43	4	33	MIX 7x + 4x	0
	43	12	16	MIX 4x + 7x	0
	43	12	16	MIX 4x + 7x	3
	43	16	12	MIX 7x + 4x	1
	43	16	12	MIX 7x + 4x	3
	43	31	31	MIX 4x + 5x	13
	43	31	31	MIX 5x + 4x	0
	43	33	3	MIX 4x + 5x	17
	43	33	4	MIX 4x + 7x	0
	43	44	66	MIX 4x + 5x	16
	43	66	44	MIX 5x + 4x	5
	29	2	8	MIX 5x + 4x	0
Bodenalm	29	8	2	MIX 4x + 5x	31
	29	99	20	MIX 5x + 4x	0
	31	4	5	MIX 4x + 5x	9
	31	5	4	MIX 5x + 4x	9
	31	6	10	MIX 4x + 5x	1
	31	8	26	MIX 4x + 5x	3
	31	10	6	MIX 5x + 4x	18
	31	26	8	MIX 5x + 4x	1
	33	20	25	MIX 4x + 5x	0
	33	21	96	MIX 4x + 5x	0
	33	22	27	MIX 4x + 5x	0
	33	25	20	MIX 5x + 4x	6
	33	27	22	MIX 5x + 4x	15

33	96	21	MIX 5x + 4x	11
37	2	77	MIX 5x + 4x	13
37	10	50	MIX 5x + 4x	22
37	30	99	MIX 4x + 5x	13
37	50	10	MIX 4x + 5x	30
37	77	2	MIX 4x + 5x	2
37	99	30	MIX 5x + 4x	10
47	9	27	MIX 4x + 5x	14
47	15	18	MIX 5x + 4x	28
47	18	15	MIX 4x + 5x	24
47	27	9	MIX 5x + 4x	23
47	30	69	MIX 4x + 5x	17
47	69	30	MIX 5x + 4x	0
49	15	47	MIX 5x + 4x	13
49	37	100	MIX 4x + 5x	2
49	47	15	MIX 4x + 5x	3
49	50	96	MIX 4x + 5x	4
49	96	50	MIX 5x + 4x	5
49	100	37	MIX 5x + 4x	3
50	25	49	MIX 5x + 4x	13
50	35	37	MIX 5x + 4x	9
50	37	35	MIX 4x + 5x	5
50	49	25	MIX 4x + 5x	25
50	77	100	MIX 4x + 5x	18
50	100	77	MIX 4x + 5x	17
51	4	26	MIX 4x + 5x	18
51	6	15	MIX 4x + 5x	12
51	18	97	MIX 4x + 5x	0
51	26	4	MIX 5x + 4x	13
51	97	18	MIX 5x + 4x	6
56	2	29	MIX 5x + 4x	19
56	28	97	MIX 4x + 5x	22
56	29	2	MIX 4x + 5x	18
56	47	99	MIX 4x + 5x	9
56	97	28	MIX 5x + 4x	11
56	99	47	MIX 5x + 4x	21
57	5	9	MIX 5x + 4x	4
57	9	5	MIX 4x + 5x	4
57	27	77	MIX 5x + 4x	3
57	50	26	MIX 4x + 5x	1
57	77	27	MIX 4x + 5x	6
58	21	100	MIX 4x + 5x	7
58	21	100	MIX 4x + 5x	0
58	29	96	MIX 4x + 5x	16
58	96	29	MIX 5x + 4x	10
58	100	21	MIX 5x + 4x	4
60	9	35	MIX 4x + 5x	8

60	22	25	MIX 4x + 5x	6
60	25	22	MIX 5x + 4x	9
60	35	9	MIX 5x + 4x	1
60	47	97	MIX 4x + 5x	14
60	97	47	MIX 5x + 4x	17

Supplementary Table 2. Fixed effect estimates of zero-inflated Poisson Generalized Linear Mixed Models relating the seed yield of sexual tetraploid *Potentilla puberula* individuals to three pollination treatments applied in *an ex situ* pollination experiment. The first treatment given in the comparison of treatments was used as baseline. Per each comparison, models were calculated both with the data pooled and unpooled. Significant differences ($\alpha = 0.05$) between the treatments are highlighted by bold p-values.

Comparison of treatments	Data	Estimate	±SE	z value	p value
Homoploid ↔ Heteroploid	pooled	-0.33	0.04	-7.827	**<0.001**
Homoploid ↔ Heteroploid pentaploid	unpooled	-0.6194	0.1001	-6.189	**<0.001**
Homoploid ↔ Heteroploid heptaploid	unpooled	-0.841	0.133	-6.324	**<0.001**
Mixed-ploidy ↔ Homoploid	pooled	0.31	0.08	3.73	**<0.001**
Mixed-ploidy pentaploid ↔ Homoploid	unpooled	0.33905	0.09901	3.424	**<0.001**
Mixed-ploidy heptaploid ↔ Homoploid	unpooled	0.3233	0.1212	2.668	**0.007**
Heteroploid pentaploid ↔ Heteroploid heptaploid	unpooled	-0.01182	0.17468	-0.068	0.946
Mixed-ploidy pentaploid ↔ Mixed-ploidy heptaploid	unpooled	-0.3062	0.1749	-1.751	0.08
Mixed-ploidy ↔ Heteroploid	pooled	-0.35	0.08	-4.34	**<0.001**
Mixed-ploidy pentaploid ↔ Heteroploid pentaploid	unpooled	-0.1842	0.1043	-1.766	0.077
Mixed-ploidy heptaploid ↔ Heteroploid heptaploid	unpooled	-0.4715	0.1558	-3.026,000	**0.002**
Mixed-ploidy ↔ (Homoploid, Heteroploid)	pooled	0.01	0.02	0.33	0.738
Mixed-ploidy pentaploid ↔ (Homoploid, Heteroploid pentaploid)	unpooled	-0.0175	0.0249	-0.703	0.482
Mixed-ploidy heptaploid ↔ (Homoploid, Heteroploid heptaploid)	unpooled	0.01788	0.0489	0.365	0.715

Supplementary Table 3. Descriptive statistics of pollen grains stained and counted. Four counts were made in 2µl aliquot of water for two anthers of the same individual. The values were averaged for the eight counts for the stained and non-stained pollen grains and calculated for the initial volume of 50µl. The number of stained and non-stained pollen grains per flower was then multiplied by the number of anthers of the same individual, and the pollen quality percentage calculated.

Population	Individual	Ploidy	Number anthers/ flower	Stained grains	Non-stained grains	Total counted grains	Average stained grains in 2µl	Average non-stained grains in 2µl	Stained grains per anther in 50µl	Non-stained grains per anther in 50µl	Stained grains per flower	Non-stained grains per flower	Pollen quality (%)
Zabernig	56	5	18	388	74	462	48.5	9.25	1,212.5	231.25	21,825	41,62.5	83.98
	57	5	18	392	10	402	49	1.25	1,225	31.25	22,050	562.5	97.51
	60	5	na	na	na	na	na	na	na	na	na	na	na
	61	5	18	107	6	113	13.375	0.75	334.375	18.75	6,018.75	337.5	94.69
	69	5	20	268	36	304	33.5	4.5	837.5	112.5	16,750	2,250	88.16
	71	5	na	na	na	na	na	na	na	na	na	na	na
	88	5	18	693	17	710	86.625	2.125	2,165.625	53.125	38,981.25	956.25	97.61
	94	5	na	na	na	na	na	na	na	na	na	na	na
	105	5	18	184	17	201	23	2.125	575	53.125	10,350	956.25	91.54
	106	5	na	na	na	na	na	na	na	na	na	na	na
	110	5	20	89	24	113	11.125	3	278.125	75	5,562.5	1500	78.76
	111	4	na	na	na	na	na	na	na	na	na	na	na
	119	4	20	590	89	679	73.75	11.125	1,843.75	278.125	36,875	5,562.5	86.89
	120	4	na	na	na	na	na	na	na	na	na	na	na
	129	4	na	na	na	na	na	na	na	na	na	na	na
	130	4	24	483	30	513	60.375	3.75	1,509.375	93.75	36,225	2,250	94.15
	136	4	18	798	13	811	99.75	1.625	2,493.75	40.625	44,887.5	731.25	98.40
	147	4	18	798	5	803	99.75	0.625	2,493.75	15.625	44,887.5	281.25	99.38
	148	4	18	1,085	330	1415	135.625	41.25	3,390.625	1031.25	61,031.25	18,562.5	76.68
Obersteiner	31	4	na	na	na	na	na	na	na	na	na	na	na
	35	4	18	211	3	214	26.375	0.375	659.375	9.375	11,868.75	168.75	98.60
	36	5	20	268	71	339	33.5	8.875	837.5	221.875	16,750	4,437.5	79.06
	38	5	16	139	25	164	17.375	3.125	434.375	78.125	6,950	1,250	84.76
	41	5	18	379	11	390	47.375	1.375	1,184.375	34.375	21,318.75	618.75	97.18

	43	4	16	265	6	271	33.125	0.75	828.125	18.75	13,250	300	97.79
	44	4	18	502	2	504	62.75	0.25	1,568.75	6.25	28,237.5	112.5	99.60
	45	4	18	342	19	361	42.75	2.375	1,068.75	59.375	19,237.5	1,068.75	94.74
	46	4	20	740	10	750	92.5	1.25	2,312.5	31.25	46,250	625	98.67
	47	4	16	576	1	577	72	0.125	1,800	3.125	28,800	50	99.83
	48	4	20	802	17	819	100.25	2.125	2,506.25	53.125	50,125	1,062.5	97.92
	54	5	16	139	0	139	17.375	0	434.375	0	6,950	0	100.00
	57	5	na	na	na	na	na	na	na	na	na	na	na
	58	5	16	289	11	300	36.125	1.375	903.125	34.375	14,450	550	96.33
	96	4	na	na	na	na	na	na	na	na	na	na	na
	103	4	16	408	32	440	51	4	1,275	100	20,400	1,600	92.73
	104	4	na	na	na	na	na	na	na	na	na	na	na
	106	4	20	326	9	335	40.75	1.125	1,018.75	28.125	20,375	562.5	97.31
	111	5	22	358	40	398	44.75	5	1,118.75	125	24,612.5	2,750	89.95
	121	5	20	153	2	155	19.125	0.25	478.125	6.25	9,562.5	125	98.71
	125	5	18	212	17	229	26.5	2.125	662.5	53.125	11,925	956.25	92.58
	126	4	14	234	9	243	29.25	1.125	731.25	28.125	10,237.5	393.75	96.30
	127	4	20	408	7	415	51	0.875	1,275	21.875	25,500	437.5	98.31
	133	5	14	294	14	308	36.75	1.75	918.75	43.75	12,862.5	612.5	95.45
	138	5	na	na	na	na	na	na	na	na	na	na	na
	139	4	22	106	0	106	13.25	0	331.25	0	7,287.5	0	100.00
	140	4	16	1,030	6	1,036	128.75	0.75	3,218.75	18.75	51,500	300	99.42
	145	5	18	501	6	507	62.625	0.75	1,565.625	18.75	28,181.25	337.5	98.82
	148	5	16	246	9	255	30.75	1.125	768.75	28.125	12,300	450	96.47
Ossenigo/	4	4	20	347	3	350	43.375	0.375	1,084.375	9.375	21,687.5	187.5	99.14
Scaiola	12	4	20	485	113	598	60.625	14.125	1,515.625	353.125	30,312.5	7,062.5	81.10
	13	4	20	699	16	715	87.375	2	2,184.375	50	43,687.5	1,000	97.76
	14	4	na	na	na	na	na	na	na	na	na	na	na
	16	4	20	712	100	812	89	12.5	2,225	312.5	44,500	6,250	87.68
	17	4	na	na	na	na	na	na	na	na	na	na	na
	18	4	20	1,334	1	1,335	166.75	0.125	4,168.75	3.125	83,375	62.5	99.93
	20	4	20	309	30	339	38.625	3.75	965.625	93.75	19,312.5	1,875	91.15
	21	4	16	483	193	676	60.375	24.125	1,509.375	603.125	24,150	9,650	71.45
	22	4	na	na	na	na	na	na	na	na	na	na	na
	32	4	18	593	1	594	74.125	0.125	1,853.125	3.125	33,356.25	56.25	99.83

34	4	18	610	120	730	76.25	15	1,906.25	375	34,312.5	6,750	83.56
49	4	20	207	3	210	43.125	0.375	1,078.125	9.375	21,562.5	187.5	99.14
55	4	na	na	na	na	na	na	na	na	na	na	na
5	7	20	196	15	211	24.5	1.875	612.5	46.875	12,250	937.5	92.89
6	7	18	20	3	23	2.5	0.375	62.5	9.375	1,125	168.75	86.96
7	5	18	135	104	239	16.875	13	421.875	325	7,593.75	5,850	56.49
8	7	20	888	33	921	111	4.125	2,775	103.125	55,500	2,062.5	96.42
10	7	18	122	25	147	15.25	3.125	381.25	78.125	6,862.5	1,406.25	82.99
13	7	20	96	11	107	12	1.375	300	34.375	6,000	687.5	89.72
16	7	18	318	4	322	39.75	0.5	993.75	12.5	17,887.5	225	98.76
21	5	20	134	14	148	16.75	1.75	418.75	43.75	8,375	875	90.54
22	5	18	478	13	491	59.75	1.625	1,493.75	40.625	26,887.5	731.25	97.35
27	5	18	84	10	94	10.5	1.25	262.5	31.25	4,725	562.5	89.36
29	5	16	65	22	87	8.125	2.75	203.125	68.75	3,250	1,100	74.71
30	5	18	169	1	170	21.125	0.125	528.125	3.125	9,506.25	56.25	99.41
31	5	18	203	1	204	25.375	0.125	634.375	3.125	11,418.75	56.25	99.51
32	5	na	na	na	na	na	na	na	na	na	na	na
33	5	20	240	13	253	30	1.625	750	40.625	15,000	812.5	94.86
34	5	18	223	11	234	27.875	1.375	696.875	34.375	12,543.75	618.75	95.30
35	5	18	168	15	183	21	1.875	525	46.875	9,450	843.75	91.80
36	5	na	na	na	na	na	na	na	na	na	na	na
37	5	20	356	8	364	44.5	1	1,112.5	25	22,250	500	97.80
38	5	18	99	9	108	12.375	1.125	309.375	28.125	5,568.75	506.25	91.67
41	5	18	108	5	113	13.5	0.625	337.5	15.625	6,075	281.25	95.58
42	7	na	na	na	na	na	na	na	na	na	na	na
45	7	18	287	56	343	35.875	7	896.875	175	16,143.75	3,150	83.67
46	7	18	354	3	357	44.25	0.375	1,106.25	9.375	19,912.5	168.75	99.16
48	7	na	na	na	na	na	na	na	na	na	na	na
54	7	18	329	11	340	41.125	1.375	1,028.125	34.375	18,506.25	618.75	96.76
55	7	22	490	18	508	61.25	2.25	1,531.25	56.25	33,687.5	1,237.5	96.46
57	7	20	527	28	555	65.875	3.5	1,646.875	87.5	32,937.5	1,750	94.95
63	7	na	na	na	na	na	na	na	na	na	na	na
65	5	na	na	na	na	na	na	na	na	na	na	na
70	7	22	190	5	195	23.75	0.625	593.75	15.625	13,062.5	343.75	97.44
71	7	18	24	6	30	3	0.75	75	18.75	1,350	337.5	80.00

	74	7	16	77	28	105	9.625	3.5	240.625	87.5	3,850	1,400	73.33
	78	7	20	546	13	559	68.25	1.625	1,706.25	40.625	34,125	812.5	97.67
	80	7	22	87	30	117	10.875	3.75	271.875	93.75	5,981.25	2,062.5	74.36
	84	7	22	808	24	832	101	3	2,525	75	55,550	1,650	97.12
	86	5	16	219	1	220	27.375	0.125	684.375	3.125	10,950	50	99.55
	90	5	16	337	39	376	42.125	4.875	1,053.125	121.875	16,850	1,950	89.63
	91	5	16	210	53	263	26.25	6.625	656.25	165.625	10,500	2,650	79.85
	95	5	na	na	na	na	na	na	na	na	na	na	na
	100	7	20	120	2	122	15	0.25	375	6.25	7,500	125	98.36
	117	5	18	252	55	307	31.5	6.875	787.5	171.875	14,175	3,093.75	82.08
	120	5	na	na	na	na	na	na	na	na	na	na	na
Raas/	1	7	21	1	0	1	0.125	0	3.125	0	65.625	0	100.00
Fortezza	3	5	17	142	102	244	17.75	12.75	443.75	318.75	7,543.75	5,418.75	58.20
	4	7	18	113	40	153	14.125	5	353.125	125	6,356.25	2,250	73.86
	8	5	16	122	34	156	15.25	4.25	381.25	106.25	6,100	1,700	78.21
	9	7	21	52	95	147	6.5	11.875	162.5	296.875	3,412.5	6,234.375	35.37
	11	7	14	45	13	58	5.625	1.625	140.625	40.625	1,968.75	568.75	77.59
	12	5	19	448	120	568	56	15	1,400	375	26,600	7,125	78.87
	13	5	18	657	30	687	82.125	3.75	2,053.125	93.75	36,956.25	1,687.5	95.63
	14	7	20	186	66	252	23.25	8.25	581.25	206.25	11,625	4,125	73.81
	16	7	19	46	8	54	5.75	1	143.75	25	2,731.25	475	85.19
	18	7	na	na	na	na	na	na	na	na	na	na	na
	19	5	17	312	132	444	39	16.5	975	412.5	16,575	7,012.5	70.27
	23	7	19	498	8	506	62.25	1	1,556.25	25	29,568.75	475	98.42
	24	5	18	270	85	355	33.75	10.625	843.75	265.625	15,187.5	4,781.25	76.06
	25	5	20	486	28	514	60.75	3.5	1,518.75	87.5	30,375	1,750	94.55
	26	7	18	411	3	414	51.375	0.375	1,284.375	9.375	23,118.75	168.75	99.28
	27	7	18	108	20	128	13.5	2.5	337.5	62.5	6,075	1,125	84.38
	28	5	19	51	13	64	6.375	1.625	159.375	40.625	3,028.125	771.875	79.69
	29	7	18	199	90	289	24.875	11.25	621.875	281.25	11,193.75	5,062.5	68.86
	31	5	20	133	8	141	16.625	1	415.625	25	8,312.5	500	94.33
	32	5	19	435	44	479	54.375	5.5	1,359.375	137.5	25,828.125	2,612.5	90.81
	33	5	18	461	12	473	57.625	1.5	1,440.625	37.5	25,931.25	675	97.46
	37	7	18	75	2	77	9.375	0.25	234.375	6.25	4,218.75	112.5	97.40
	41	7	20	69	14	83	8.625	1.75	215.625	43.75	4,312.5	875	83.13

	43	7	23	124	50	174	15.5	6.25	387.5	156.25	8,912.5	3,593.75	71.26
	44	5	20	575	75	650	71.875	9.375	1,796.875	234.375	35,937.5	4,687.5	88.46
	47	5	20	769	16	785	96.125	2	2,403.125	50	48,062.5	1,000	97.96
	48	5	20	755	24	779	94.375	3	2,359.375	75	47,187.5	1,500	96.92
	49	7	20	11	0	11	1.375	0	34.375	0	687.5	0	100.00
	50	7	18	86	74	160	10.75	9.25	268.75	231.25	4,837.5	4,162.5	53.75
	51	7	16	128	2	130	16	0.25	400	6.25	6,400	100	98.46
	53	7	22	73	30	103	9.125	3.75	228.125	93.75	5,018.75	2,062.5	70.87
	59	7	20	106	32	138	13.25	4	331.25	100	6,625	2,000	76.81
	60	5	19	420	62	482	52.5	7.75	1,312.5	193.75	24,937.5	3,681.25	87.14
	63	7	18	205	36	241	25.625	4.5	640.625	112.5	11,531.25	2,025	85.06
	65	7	16	100	13	113	12.5	1.625	312.5	40.625	5,000	650	88.50
	66	5	20	627	34	661	78.375	4.25	1,959.375	106.25	39,187.5	2,125	94.86
	67	7	15	80	0	80	10	0	250	0	3,750	0	100.00
	73	7	16	76	8	84	9.5	1	237.5	25	3,800	400	90.48
	2	4	18	1,010	14	1,024	126.25	1.75	3,156.25	43.75	56,812.5	787.5	98.63
	3	4	18	947	10	957	118.375	1.25	2,959.375	31.25	53,268.75	562.5	98.96
	5	4	18	684	12	696	85.5	1.5	2,137.5	37.5	38,475	675	98.28
	6	4	22	785	6	791	98.125	0.75	2,453.125	18.75	53,968.75	412.5	99.24
	8	4	na	na	na	na	na	na	na	na	na	na	na
	9	4	na	na	na	na	na	na	na	na	na	na	na
	10	4	16	509	110	619	63.625	13.75	1,590.625	343.75	25,450	5,500	82.23
	11	4	na	na	na	na	na	na	na	na	na	na	na
	12	4	18	513	13	526	64.125	1.625	1,603.125	40.625	28,856.25	731.25	97.53
	13	4	20	514	8	522	64.25	1	1,606.25	25	32,125	500	98.47
	29	4	18	482	4	486	60.25	0.5	1,506.25	12.5	27,112.5	225	99.18
	31	4	na	na	na	na	na	na	na	na	na	na	na
	33	4	20	505	20	525	63.125	2.5	1,578.125	62.5	31,562.5	1,250	96.19
	35	4	na	na	na	na	na	na	na	na	na	na	na
	44	4	na	na	na	na	na	na	na	na	na	na	na
	52	4	18	739	22	761	92.375	2.75	2,309.375	68.75	41,568.75	1,237.5	97.11
Bodenalm	2	5	14	387	38	425	48.375	4.75	1,209.375	118.75	16,931.25	1,662.5	91.06
	4	4	na	na	na	na	na	na	na	na	na	na	na
	5	5	18	158	1	159	19.75	0.125	493.75	3.125	8,887.5	56.25	99.37
	6	4	20	141	33	174	17.625	4.125	440.625	103.125	8,812.5	2,062.5	81.03

8	4	18	404	2	406	50.5	0.25	1,262.5	6.25	22,725	112.5	99.51
9	4	16	57	12	69	7.125	1.5	178.125	37.5	2,850	600	82.61
10	5	14	577	105	682	72.125	13.125	1,803.125	328.125	25,243.75	4,593.75	84.60
15	5	16	224	1	225	28	0.125	700	3.125	11,200	50	99.56
18	4	18	109	3	112	13.625	0.375	340.625	9.375	6,131.25	168.75	97.32
20	4	16	502	22	524	62.75	2.75	1,568.75	68.75	25,100	1,100	95.80
21	4	16	583	3	586	72.875	0.375	1,821.875	9.375	29,150	150	99.49
22	4	20	121	34	155	15.125	4.25	378.125	106.25	7,562.5	2,125	78.06
25	5	18	130	5	135	16.25	0.625	406.25	15.625	7,312.5	281.25	96.30
26	5	20	210	0	210	26.25	0	656.25	0	13,125	0	100.00
27	5	18	790	14	804	98.75	1.75	2,468.75	43.75	44,437.5	787.5	98.26
28	4	19	96	11	107	12	1.375	300	34.375	5,700	653.125	89.72
29	4	18	356	7	363	44.5	0.875	1,112.5	21.875	20,025	393.75	98.07
30	4	14	437	4	441	54.625	0.5	1,365.625	12.5	19,118.75	175	99.09
35	5	18	177	1	178	22.125	0.125	553.125	3.125	9,956.25	56.25	99.44
36	4	na	na	na	na	na	na	na	na	na	na	na
37	4	18	232	3	235	29	0.375	725	9.375	13,050	168.75	98.72
47	4	16	253	10	263	31.625	1.25	790.625	31.25	12,650	500	96.20
49	4	14	141	3	144	17.625	0.375	440.625	9.375	6,168.75	131.25	97.92
50	4	na	na	na	na	na	na	na	na	na	na	na
69	5	18	391	57	448	48.875	7.125	1,221.875	178.125	21,993.75	3,206.25	87.28
77	4	20	182	6	188	22.75	0.75	568.75	18.75	11,375	375	96.81
96	5	18	455	18	473	56.875	2.25	1,421.875	56.25	25,593.75	1,012.5	96.19
97	5	20	214	3	217	26.75	0.375	668.75	9.375	13,375	187.5	98.62
99	5	18	892	20	912	111.5	2.5	2,787.5	62.5	50,175	1,125	97.81
100	5	16	533	28	561	66.625	3.5	1,665.625	87.5	26,650	1,400	95.01

Supplementary table 4. Descriptive statistics of the flow cytometric measurement of seeds of *Potentilla puberula* Krašan. For each "pollen recipient" and "pollen donor" used in the crosses the "Population", and "Individual" number is provided. The internal biological standard was for all measurements *Pisum sativum* cv. Kleine Rheinländerin. "Count", "Mean" and "CV" are the number of particles registered, the mean fluorescence, and the variation coefficient, respectively, calculated for the internal "Standard" and the "Embryo". A) 35 seeds from homoploid tetraploid fertilizations, B) 67 seeds from heteroploid fertilizations and C) 871 seeds from mixed-ploidy fertilizations. Population numbers refer to the studied regions as follows: Zabernig 10, Obersteiner 45, Ossenigo/Scaiola 64/68, Raas/Fortezza 121/119 and Bodenalm 144.

A) Homoploid tetraploid fertilization seeds

Pollen recipient			Pollen donor			Standard			Embryo			Embryo:Standard ratio
Population	Individual	Ploidy [x]	Population	Individual	Ploidy [x]	Count	Mean	CV	Count	Mean	CV	
10	125	4	10	142	4	573	705.98	3.07	413	99.96	5.21	0.141590
45	103	4	45	43	4	389	715.67	2.79	701	102.17	5.29	0.142761
45	103	4	45	43	4	343	704.00	2.95	946	102.62	6.31	0.145767
45	103	4	45	44	4	785	712.7	1.9	373	102.02	5.08	0.143145
45	103	4	45	44	4	846	711.87	2.26	770	101.4	3.82	0.142442
45	104	4	45	140	4	211	707.76	3.80	964	100.54	4.42	0.142054
45	104	4	45	140	4	539	727.67	2.74	929	102.96	5.60	0.141493
45	116	4	45	45	4	806	712.47	2.62	616	100.06	4.81	0.140441
45	116	4	45	45	4	437	724.71	2.80	753	103.53	5.23	0.142857
45	116	4	45	45	4	637	709.69	2.76	454	100.28	5.96	0.141301
45	116	4	45	45	4	397	709.52	2.80	778	101.77	6.91	0.143435
45	146	4	45	127	4	747	710.55	1.8	1512	101.27	4.48	0.142523
45	146	4	45	127	4	517	727.33	1.93	715	101.79	3.34	0.139950
64	18	4	64	14	4	587	692.65	2.46	710	97.44	4.52	0.140677
64	18	4	64	14	4	293	713.84	2.93	926	99.55	4.62	0.139457

64	18	4	64	12	4	532	706.47	1.98	1132	99.98	4.06	0.141520
64	18	4	64	12	4	402	709.84	2.01	1170	100.05	3.54	0.140947
64	18	4	64	12	4	302	707.03	1.97	986	98.97	3.19	0.139979
64	27	4	64	20	4	1052	715.16	2.45	1032	103,68	4,05	0.144974
64	48	4	64	55	4	653	715.2	2.36	718	101.98	4.36	0.142589
64	48	4	64	55	4	214	719.33	3.67	1425	104.26	5.18	0.144940
121	20	4	121	10	4	409	707.22	2.56	869	104.16	6.24	0.147281
121	20	4	121	10	4	463	714.43	2.88	1075	101.18	4.54	0.141623
121	20	4	121	10	4	266	714.13	2.60	612	100.47	3.90	0.140689
121	22	4	121	52	4	600	710.64	2.19	1716	99.74	3.88	0.140352
121	22	4	121	52	4	635	705.71	2.45	927	100.07	3.73	0.141800
121	40	4	121	3	4	514	721.40	2.82	678	100.13	4.41	0.138800
121	40	4	121	6	4	685	715.03	1.92	702	100.1	4.11	0.139994
121	40	4	121	6	4	551	713.26	2.42	417	101.44	4.75	0.142220
121	40	4	121	3	4	328	724.60	3.17	847	102.67	5.48	0.141692
144	29	4	144	6	4	604	713.67	6.21	805	102.23	6.67	0.143245
144	29	4	144	20	4	780	728.66	3.17	459	98.14	5.16	0.134686
144	29	4	144	20	4	810	727.18	3.09	1358	101.61	5.22	0.139732
144	37	4	144	30	4	755	708.84	1.67	463	99.51	3.68	0.140384
144	37	4	144	30	4	727	729.86	1.8	917	101.01	3.98	0.13839

B) Heteroploid fertilizations seeds

Pollen recipient			Pollen donor			Standard			Embryo			Embryo:Standard ratio
Population	Individual	Ploidy [x]	Population	Individual	Ploidy [x]	Count	Mean	CV	Count	Mean	CV	
10	140	4	10	56	5	185	735.79	2.53	334	103.81	4.25	0.141086
10	140	4	10	56	5	723	699.04	1.58	951	100.69	4.88	0.144040

45	103	4	45	143	5	687	730.13	1.94	592	104.03	4.26	0.142481
45	103	4	45	145	5	287	716.87	3.58	627	107.18	4.94	0.149511
45	103	4	45	145	5	444	721.06	3.52	356	117.97	7.03	0.163606
45	105	4	45	41	5	1147	709.25	3.94	1026	109.14	4.53	0.153881
45	106	4	45	121	5	649	718.41	2.89	925	117.07	4.71	0.162957
45	106	4	45	121	5	606	725.01	2.75	898	136.81	3.56	0.188701
45	109	4	45	41	5	674	716.1	2.9	1103	119.14	3.56	0.166373
45	109	4	45	41	5	672	722.84	3.05	476	128.16	4.36	0.177301
45	116	4	45	36	5	538	698.03	1.77	861	96.85	3.72	0.138747
45	116	4	45	58	5	1627	716.72	2.84	592	104.88	3.57	0.146333
45	116	4	45	58	5	400	700.61	2.42	550	114.51	3.82	0.163443
45	143	4	45	121	5	571	691.24	2.25	1332	99.69	5.7	0.144219
45	143	4	45	121	5	531	719.75	2.86	593	114.6	5.69	0.159222
45	146	4	45	111	5	248	721.46	1.96	1176	101.5	3.89	0.140687
45	146	4	45	111	5	1145	708.02	2.42	678	103.16	3.35	0.14570
64	14	4	68	37	5	319	717.87	2.03	865	106.07	4.61	0.147756
64	14	4	68	37	5	1196	714.01	1.62	821	106.94	3.64	0.149774
64	14	4	68	37	5	649	722.31	1.89	734	120.9	4.08	0.167379
64	14	4	68	117	5	742	721.54	3.17	407	132.54	3.31	0.183690
64	14	4	68	117	5	662	716.69	1.79	618	138.81	4.39	0.193682
64	14	4	68	117	5	671	726.33	2.53	654	141.07	3.45	0.194223
121	36	4	119	12	5	520	744.59	2.16	694	130.36	3.33	0.175076
121	37	4	119	13	5	763	716.84	1.62	576	122.13	3.15	0.170373
121	40	4	119	25	5	327	724.27	1.87	858	103.83	3.48	0.143358
121	40	4	119	25	5	286	708.34	1.43	788	120.24	3.41	0.169749
121	40	4	119	25	5	263	699.52	2.79	897	123.49	4.99	0.176535
121	40	4	119	32	5	508	716.59	1.62	912	126.57	3.74	0.176628
121	43	4	119	31	5	851	707.9	2.27	963	119.44	3.46	0.168724
121	43	4	119	31	5	588	726.71	1.84	379	128.19	3.16	0.176397
144	31	4	144	10	5	689	722.24	1.9	487	105.01	4.15	0.145395

144	31	4	144	10	5	493	726.61	2.1	578	124.15	3.43	0.170862
144	37	4	144	10	5	328	730.1	1.83	310	125.97	4.82	0.172538
144	47	4	144	27	5	219	706.91	2.11	505	113.58	4.37	0.160671
144	47	4	144	27	5	472	719.45	2.06	1225	122.16	3.51	0.169796
144	50	4	144	100	5	350	709.01	2.19	922	115.56	4.25	0.162988
64	14	4	68	5	7	453	727.6	1.65	720	134.11	3.67	0.184318
64	14	4	68	5	7	487	726.23	1.75	1170	135.73	3.51	0.186896
64	14	4	68	13	7	179	701.85	1.88	703	136.34	2.99	0.194258
64	14	4	68	13	7	576	739.91	2	962	137	3.63	0.185157
64	14	4	68	13	7	438	700.65	1.8	1769	130.87	3.85	0.186783
64	14	4	68	13	7	787	711.09	1.79	1088	126.59	3.27	0.178022
64	14	4	68	71	7	448	718.58	1.79	616	143.37	4.15	0.199518
64	14	4	68	71	7	1108	712.43	1.97	288	141.83	3.62	0.199079
64	27	4	68	100	7	327	776.33	2.25	626	156.69	3.31	0.201834
64	27	4	68	100	7	452	732.43	2.21	594	139.85	3.47	0.190939
64	27	4	68	100	7	566	722.78	1.58	838	140.65	2.98	0.194596
121	20	4	119	18	7	655	713.59	2.05	726	126.66	3.86	0.177497
121	20	4	119	18	7	647	742.62	3.53	621	146.27	4.66	0.196965
121	20	4	119	18	7	555	730.22	2.15	1245	133.86	3.37	0.183314
121	20	4	119	18	7	659	703.64	1.76	1260	138.57	3.79	0.196933
121	20	4	119	18	7	817	717.12	2.35	645	147.84	3.59	0.206158
121	20	4	119	18	7	583	713.55	2.74	878	126.82	3.59	0.177731
121	20	4	119	18	7	617	716.07	1.99	818	129.63	3.2	0.181029
121	20	4	119	18	7	566	714.18	2.08	1069	145.34	3.54	0.203506
121	27	4	119	16	7	541	702.29	1.89	952	133.88	3.61	0.190633
121	27	4	119	16	7	1134	708.88	1.98	1249	142.76	3.84	0.201388
121	36	4	119	23	7	301	713.51	1.94	1602	125.79	4.73	0.176297
121	36	4	119	23	7	356	713.27	2.43	777	141.15	3.97	0.197891
121	36	4	119	29	7	920	712.75	1.97	1189	125.78	3.5	0.176471
121	36	4	119	29	7	1453	710.42	1.82	1579	142.47	3.46	0.200543

121	36	4	119	29	7	488	715.47	2.07	713	141.64	3.48	0.197967
121	37	4	119	53	7	342	700.76	2.2	891	128.26	4.14	0.183029
121	37	4	119	53	7	375	713.46	1.75	690	145.37	3.25	0.203753
121	37	4	119	53	7	273	711.08	2	798	134.35	3.48	0.188938
121	40	4	119	63	7	521	704.76	1.8	864	131.07	4.16	0.185978

C) Mixed-ploidy fertilizations seeds

Pollen recipient			Pollen donor 1			Pollen donor 2			Standard			Embryo			
Population	Individual	Ploidy [x]	Population	Individual	Ploidy [x]	Population	Individual	Ploidy [x]	Count	Mean	CV	Count	Mean	CV	Embryo:Standard ratio
10	140	4	10	111	4	10	106	5	758	684.58	3.34	638	96.22	5.63	0.140553332
10	140	4	10	111	4	10	106	5	744	712.85	3.22	1320	98.35	6.14	0.137967314
10	140	4	10	111	4	10	106	5	707	722.38	3.01	1442	100.66	5.54	0.139344943
10	142	4	10	147	4	10	97	5	1166	725.35	3.48	986	101.20	5.38	0.139518853
10	142	4	10	147	4	10	97	5	728	722.16	3.23	1120	102.58	5.80	0.142046084
10	142	4	10	147	4	10	97	5	1759	703.71	2.84	1674	97.89	6.74	0.139105597
10	142	4	10	147	4	10	97	5	1071	675.64	4.26	1392	100.44	6.30	0.148659049
10	142	4	10	147	4	10	97	5	1067	703.81	3.48	1679	98.57	6.43	0.140052003
10	142	4	10	147	4	10	97	5	734	726.38	3.35	1509	102.13	5.57	0.140601338
10	142	4	10	147	4	10	97	5	782	723.23	3.16	927	98.16	5.76	0.135724458
10	142	4	10	147	4	10	97	5	1085	708.16	3.20	534	136.60	5.44	0.192894261
10	142	4	10	147	4	10	97	5	1145	706.67	2.96	854	119.51	4.91	0.169117127
10	142	4	10	147	4	10	97	5	1042	710.32	3.01	1241	99.03	5.85	0.139416038
10	142	4	10	147	4	10	97	5	940	702.78	3.00	1209	99.30	6.53	0.141295996
10	142	4	10	147	4	10	97	5	1064	703.96	3.11	1394	96.89	5.55	0.137635661
45	103	4	45	43	4	45	36	5	1062	712.54	3.79	2099	101.01	6.03	0.141760463
45	103	4	45	44	4	45	145	5	1414	683.93	3.72	1818	96.38	5.96	0.140920854
45	103	4	45	44	4	45	145	5	685	709.74	3.27	1212	101.19	5.35	0.142573337
45	103	4	45	44	4	45	145	5	1145	703.32	3.01	1541	99.71	5.26	0.14177046
45	103	4	45	44	4	45	145	5	1308	682.98	3.19	1588	93.87	4.92	0.137441799
45	103	4	45	44	4	45	145	5	191	713.22	2.58	967	103.73	4.86	0.145438995
45	103	4	45	44	4	45	145	5	1679	671.50	3.11	1974	93.06	5.63	0.138585257
45	103	4	45	44	4	45	145	5	1139	729.05	2.20	1262	105.70	5.31	0.144983197

45	103	4	45	44	4	45	145	5	674	687.09	3.83	1863	102.46	7.59	0.149121658
45	103	4	45	36	5	45	43	4	1999	697.19	3.24	1370	98.46	5.91	0.141224057
45	103	4	45	36	5	45	43	4	1390	724.09	2.79	857	112.09	6.36	0.154801199
45	103	4	45	36	5	45	43	4	1974	712.14	3.43	839	101.67	5.62	0.142766872
45	103	4	45	36	5	45	43	4	1258	717.38	2.81	822	104.33	5.34	0.145431989
45	103	4	45	36	5	45	43	4	1162	719.21	4.11	2108	104.54	5.45	0.14535393
45	103	4	45	145	5	45	44	4	1136	700.30	2.96	2584	98.72	6.11	0.140968157
45	103	4	45	145	5	45	44	4	988	753.29	3.00	909	109.09	5.23	0.144818065
45	103	4	45	145	5	45	44	4	2193	720.54	3.22	1145	101.37	6.38	0.140686152
45	103	4	45	145	5	45	44	4	182	718.46	2.88	1940	101.21	6.24	0.140870751
45	103	4	45	44	4	45	145	5	887	743.04	3.51	1591	103.61	5.06	0.139440676
45	103	4	45	44	4	45	145	5	1795	713.29	2.45	1295	101.29	5.24	0.142003954
45	103	4	45	44	4	45	145	5	268	723.22	3.33	1045	145.27	4.63	0.200865573
45	103	4	45	44	4	45	145	5	1347	714.86	2.21	1516	101.18	4.97	0.141538203
45	103	4	45	145	5	45	44	4	289	724.15	3.08	1359	102.27	4.85	0.141227646
45	103	4	45	145	5	45	44	4	2004	710.57	2.66	975	100.75	4.19	0.141787579
45	103	4	45	145	5	45	44	4	450	714.68	2.71	2642	99.36	5.69	0.139027257
45	103	4	45	145	5	45	44	4	615	708.02	3.13	875	98.25	3.86	0.138767266
45	104	4	45	44	4	45	58	5	186	692.68	2.67	1416	96.90	4.60	0.139891436
45	104	4	45	44	4	45	58	5	598	743.59	2.33	1618	107.61	5.28	0.144716847
45	104	4	45	44	4	45	58	5	160	712.50	3.06	989	127.27	3.73	0.178624561
45	104	4	45	127	4	45	54	5	499	707.18	2.95	1658	103.02	4.74	0.145677197
45	104	4	45	127	4	45	54	5	917	716.15	3.18	1661	102.62	4.49	0.143294003
45	104	4	45	127	4	45	54	5	768	751.18	2.26	1642	105.74	4.79	0.140765196
45	104	4	45	140	4	45	145	5	239	709.76	3.48	1400	130.24	4.49	0.183498647
45	104	4	45	140	4	45	145	5	477	735.69	2.28	1523	105.00	4.04	0.142723158
45	104	4	45	140	4	45	145	5	283	718.05	2.46	1061	123.71	4.46	0.172286053
45	104	4	45	140	4	45	145	5	561	712.50	2.62	1531	120.15	4.38	0.168631579
45	104	4	45	58	5	45	44	4	702	722.69	2.96	1641	104.62	4.99	0.144764699
45	104	4	45	58	5	45	44	4	880	741.93	2.88	1649	107.09	4.94	0.144339763
45	104	4	45	54	5	45	127	4	693	729.41	3.05	1986	104.73	5.35	0.143581799
45	104	4	45	54	5	45	127	4	252	748.01	2.53	1135	106.92	4.24	0.142939266
45	104	4	45	54	5	45	127	4	210	721.05	2.55	1085	103.61	4.90	0.143693225
45	104	4	45	54	5	45	127	4	138	724.80	3.13	2461	104.77	5.09	0.144550221
45	104	4	45	54	5	45	127	4	632	748.72	2.50	1099	105.93	4.60	0.141481462
45	104	4	45	145	5	45	140	4	623	737.47	2.71	2772	105.19	5.24	0.142636311
45	104	4	45	145	5	45	140	4	1236	718.35	3.31	1758	101.85	4.95	0.141783253

45	104	4	45	145	5	45	140	4	1587	727.07	2.61	1862	102.62	4.61	0.141141843
45	105	4	45	106	4	45	41	5	1343	689.49	3.81	2288	96.04	6.15	0.13929136
45	105	4	45	106	4	45	41	5	926	706.67	2.44	1005	101.63	4.04	0.143815359
45	105	4	45	106	4	45	41	5	992	680.29	3.34	2174	95.62	6.16	0.140557703
45	105	4	45	106	4	45	41	5	669	729.16	2.63	865	102.81	3.47	0.140997861
45	105	4	45	47	4	45	38	5	364	700.24	3.17	1395	93.91	5.15	0.134111162
45	105	4	45	47	4	45	38	5	454	713.68	3.08	1652	99.29	5.25	0.139123977
45	105	4	45	41	5	45	106	4	207	720.27	2.53	2316	99.94	5.09	0.138753523
45	105	4	45	41	5	45	106	4	971	732.29	2.63	1271	102.70	5.43	0.140244985
45	105	4	45	41	5	45	106	4	344	713.07	2.64	1397	100.30	4.23	0.140659402
45	105	4	45	41	5	45	106	4	1551	770.66	2.61	821	108.67	4.83	0.141009005
45	105	4	45	38	5	45	47	4	299	715.68	4.50	2221	97.12	5.61	0.135703108
45	105	4	45	38	5	45	47	4	984	712.40	3.07	793	101.05	4.21	0.141844469
45	105	4	45	38	5	45	47	4	575	693.29	3.86	2618	92.90	6.87	0.13399876
45	105	4	45	38	5	45	47	4	1105	716.15	2.86	1646	101.41	4.94	0.141604412
45	106	4	45	46	4	45	33	5	767	707.91	4.11	2277	100.39	5.33	0.141811812
45	106	4	45	46	4	45	33	5	362	748.27	3.55	948	105.93	4.50	0.141566547
45	106	4	45	121	5	45	35	4	843	754.70	3.24	2038	103.29	5.05	0.136862329
45	106	4	45	121	5	45	35	4	990	755.12	2.77	1227	105.32	4.48	0.139474521
45	106	4	45	121	5	45	35	4	429	763.55	3.97	1633	115.14	5.63	0.150795626
45	106	4	45	121	5	45	35	4	1745	805.80	3.22	1284	118.45	4.96	0.146996773
45	106	4	45	139	5	45	48	4	423	734.99	3.44	1870	104.56	5.85	0.142260439
45	106	4	45	139	5	45	48	4	1367	746.94	2.73	1452	105.04	4.32	0.140627092
45	106	4	45	139	5	45	48	4	278	723.72	3.88	1446	104.15	4.84	0.143909247
45	106	4	45	139	5	45	48	4	1317	762.55	2.35	1327	105.36	4.33	0.138167989
45	109	4	45	139	5	45	48	4	1012	799.12	4.40	1206	117.35	5.03	0.146849034
45	109	4	45	43	4	45	45	5	626	718.42	3.01	1112	100.37	4.18	0.139709362
45	109	4	45	43	4	45	45	5	765	727.33	3.14	1997	100.27	4.47	0.137860393
45	109	4	45	43	4	45	45	5	1133	780.69	2.84	727	112.82	4.04	0.144513187
45	109	4	45	48	4	45	139	5	544	715.44	5.21	1737	103.88	4.97	0.145197361
45	109	4	45	48	4	45	139	5	600	671.76	2.94	1036	97.22	5.03	0.144724306
45	109	4	45	48	4	45	139	5	515	717.80	4.56	1795	103.67	4.84	0.144427417
45	109	4	45	48	4	45	139	5	612	761.44	2.95	340	104.38	3.82	0.13708237
45	109	4	45	103	4	45	138	5	684	687.27	5.61	1340	113.02	4.05	0.164447743
45	109	4	45	103	4	45	138	5	836	764.85	3.03	1198	107.00	4.37	0.139896712
45	109	4	45	103	4	45	138	5	697	711.28	3.70	1135	100.40	4.35	0.141153976
45	109	4	45	103	4	45	138	5	1224	758.24	3.17	1219	157.57	3.34	0.207810192

45	109	4	45	41	5	45	43	4	469	728.34	2.85	1668	100.30	4.56	0.13771041
45	109	4	45	41	5	45	43	4	606	759.61	2.67	1226	104.74	3.90	0.137886547
45	109	4	45	41	5	45	43	4	805	704.52	6.70	1251	103.95	4.16	0.147547266
45	109	4	45	139	5	45	48	4	1715	726.15	3.33	451	103.13	4.90	0.142022998
45	109	4	45	139	5	45	48	4	2421	747.74	3.00	1056	103.98	4.51	0.139059031
45	109	4	45	139	5	45	48	4	1775	721.71	3.47	1011	99.97	5.09	0.138518241
45	109	4	45	138	5	45	103	4	859	676.14	6.49	542	112.29	3.93	0.166075073
45	109	4	45	138	5	45	103	4	539	731.11	2.93	2302	101.39	5.23	0.138679542
45	109	4	45	138	5	45	103	4	796	634.79	6.37	1836	110.61	4.09	0.174246601
45	109	4	45	138	5	45	103	4	335	750.50	3.89	554	106.80	5.35	0.142305130
45	110	4	45	127	4	45	145	5	371	730.03	3.69	2779	104.93	5.31	0.143733819
45	110	4	45	127	4	45	145	5	1864	729.28	2.93	1135	101.34	4.42	0.138958973
45	110	4	45	106	4	45	36	5	239	738.38	3.98	2198	107.87	5.53	0.146090089
45	110	4	45	47	4	45	58	5	791	707.85	2.82	2508	99.58	4.88	0.140679522
45	110	4	45	47	4	45	58	5	978	731.39	2.66	1817	105.46	5.46	0.144191198
45	110	4	45	47	4	45	58	5	854	710.79	2.40	1513	101.50	4.53	0.142798858
45	110	4	45	47	4	45	58	5	1657	710.56	4.73	1409	100.16	5.95	0.140959243
45	110	4	45	47	4	45	58	5	1223	723.10	2.81	1119	103.21	4.99	0.142732679
45	110	4	45	145	5	45	127	4	1523	698.62	2.98	1161	99.25	5.49	0.142065787
45	110	4	45	36	5	45	106	4	1754	716.92	2.58	1734	99.71	4.09	0.139081069
45	110	4	45	36	5	45	106	4	1391	723.61	3.30	423	99.92	4.23	0.138085433
45	110	4	45	36	5	45	106	4	1478	678.14	3.38	1055	95.51	5.84	0.140841124
45	110	4	45	58	5	45	47	4	1123	735.64	2.79	1364	102.11	4.61	0.138804306
45	110	4	45	58	5	45	47	4	1478	703.36	2.87	554	99.34	5.03	0.141236351
45	110	4	45	58	5	45	47	4	1551	722.68	2.73	1883	101.16	4.65	0.139978967
45	110	4	45	58	5	45	47	4	2182	694.92	2.87	1099	96.63	4.86	0.139051977
45	113	4	45	106	4	45	111	5	1533	731.68	2.54	1973	100.54	4.09	0.137409797
45	113	4	45	46	4	45	125	5	889	714.70	3.11	1601	98.36	4.89	0.137624178
45	113	4	45	46	4	45	125	5	1171	763.78	2.85	1869	105.38	5.43	0.137971667
45	113	4	45	46	4	45	125	5	1200	736.14	3.15	2443	102.38	4.84	0.139076806
45	113	4	45	46	4	45	125	5	737	684.68	2.46	1088	99.69	5.01	0.145600865
45	113	4	45	125	5	45	46	4	2284	726.30	3.63	1036	100.44	4.84	0.138289963
45	113	4	45	125	5	45	46	4	1473	720.47	3.77	1895	100.54	4.29	0.139547795
45	116	4	45	45	4	45	148	5	1333	708.51	3.18	892	100.40	5.30	0.141705833
45	116	4	45	45	4	45	148	5	1599	748.64	3.33	535	104.06	6.40	0.138998718
45	116	4	45	45	4	45	148	5	1580	743.72	3.00	1706	106.66	5.13	0.143414188
45	116	4	45	45	4	45	148	5	1018	734.87	3.10	518	100.01	4.36	0.136092098

45	116	4	45	103	4	45	139	5	1463	732.72	2.87	1691	103.38	5.39	0.14109073
45	116	4	45	103	4	45	139	5	1156	720.27	3.49	1145	101.25	4.96	0.140572285
45	116	4	45	103	4	45	139	5	1301	715.03	3.29	1137	99.86	5.00	0.139658476
45	116	4	45	103	4	45	139	5	1246	746.08	4.25	1247	105.20	5.45	0.141003646
45	116	4	45	103	4	45	139	5	1674	738.38	3.33	901	102.91	5.04	0.139372681
45	116	4	45	148	5	45	45	4	1186	718.87	3.46	1475	98.83	5.96	0.137479656
45	116	4	45	148	5	45	45	4	1161	731.44	2.65	1260	105.17	6.01	0.143784863
45	116	4	45	148	5	45	45	4	1606	701.91	2.84	2074	96.61	5.02	0.137638729
45	116	4	45	148	5	45	45	4	1693	748.98	3.20	558	105.97	5.29	0.141485754
45	126	4	45	103	4	45	36	5	1747	730.90	3.96	1120	100.95	5.44	0.138117390
45	126	4	45	103	4	45	36	5	949	718.34	2.76	731	99.58	5.04	0.138625164
45	126	4	45	103	4	45	36	5	1449	723.95	2.99	1421	101.41	5.49	0.140078735
45	126	4	45	103	4	45	36	5	1758	732.59	2.86	1026	105.39	5.67	0.143859458
45	126	4	45	103	4	45	36	5	1361	696.04	2.58	1050	99.28	6.19	0.142635481
45	126	4	45	103	4	45	36	5	1382	739.60	2.60	1498	103.82	4.94	0.140373175
45	126	4	45	103	4	45	36	5	2483	713.14	3.04	399	99.57	5.34	0.139621954
45	126	4	45	36	5	45	103	4	1008	733.32	3.69	228	101.44	5.22	0.138329788
45	126	4	45	36	5	45	103	4	517	744.77	2.90	2487	103.83	5.23	0.139412168
45	126	4	45	36	5	45	103	4	1900	733.64	3.38	1163	105.24	4.92	0.143449103
45	126	4	45	36	5	45	103	4	987	774.55	3.37	946	109.88	5.15	0.141863017
45	126	4	45	36	5	45	103	4	1629	710.74	2.77	257	98.80	4.18	0.139010046
45	126	4	45	36	5	45	103	4	2166	721.57	2.63	1230	103.86	6.14	0.143936139
45	126	4	45	36	5	45	103	4	1632	709.47	2.77	696	98.94	4.60	0.139456214
45	126	4	45	36	5	45	103	4	1216	715.90	2.82	1358	98.36	4.85	0.137393491
45	143	4	45	45	4	45	133	5	542	730.09	3.30	1096	100.63	5.03	0.137832322
45	143	4	45	45	4	45	133	5	1029	763.28	3.00	1600	108.63	4.66	0.142319987
45	143	4	45	45	4	45	133	5	1062	751.13	2.48	1586	107.64	5.41	0.143304089
45	143	4	45	45	4	45	133	5	1237	730.88	3.00	1862	103.41	4.77	0.141486975
45	143	4	45	43	4	45	121	5	1260	744.85	3.01	1583	104.05	4.68	0.139692556
45	143	4	45	43	4	45	121	5	1676	704.45	3.16	763	91.57	6.45	0.129987934
45	143	4	45	43	4	45	121	5	1221	724.45	2.60	2110	100.82	5.73	0.139167644
45	143	4	45	43	4	45	121	5	1203	762.45	3.80	1428	107.07	5.63	0.140428881
45	143	4	45	45	4	45	57	5	1259	716.34	2.82	889	101.66	5.02	0.141915850
45	143	4	45	45	4	45	57	5	1118	720.82	2.76	1949	105.26	5.94	0.146028135
45	143	4	45	45	4	45	57	5	1062	737.61	2.88	1568	106.49	5.25	0.144371687
45	143	4	45	45	4	45	57	5	1378	733.96	3.30	1508	105.82	5.67	0.144176794
45	143	4	45	133	5	45	45	4	1333	746.88	3.02	1160	103.52	4.37	0.138603256

45	143	4	45	133	5	45	45	4	1558	732.67	4.00	2171	103.91	5.34	0.141823741
45	143	4	45	133	5	45	45	4	746	753.34	3.20	1762	124.87	5.04	0.165755170
45	143	4	45	133	5	45	45	4	976	734.27	3.84	1562	103.37	4.49	0.140779277
45	143	4	45	121	5	45	43	4	2228	733.12	3.66	775	103.30	5.07	0.140904627
45	143	4	45	121	5	45	43	4	1432	741.46	3.48	1681	104.09	4.45	0.140385186
45	143	4	45	121	5	45	43	4	1316	742.08	3.19	1270	103.82	4.56	0.139904053
45	143	4	45	121	5	45	43	4	1633	742.87	3.15	1689	104.62	4.69	0.140832178
45	143	4	45	121	5	45	43	4	1509	708.82	2.88	1256	98.64	4.69	0.139160859
45	146	4	45	127	4	45	41	5	1480	732.51	2.68	1541	103.78	4.44	0.141677247
45	146	4	45	127	4	45	41	5	879	754.62	2.91	2312	106.41	4.91	0.141011370
45	146	4	45	127	4	45	41	5	1802	733.66	2.68	1399	121.65	4.48	0.165812502
45	146	4	45	127	4	45	41	5	1416	742.61	3.46	2150	104.11	4.36	0.140194719
45	146	4	45	104	4	45	111	5	2649	725.52	3.07	634	99.49	5.48	0.137129231
45	146	4	45	104	4	45	111	5	1357	733.81	3.71	953	99.94	5.05	0.136193293
45	146	4	45	104	4	45	111	5	1282	733.79	3.32	1734	103.75	5.10	0.141389226
45	146	4	45	31	4	45	36	5	1180	737.32	3.10	1340	101.39	4.38	0.137511528
45	146	4	45	31	4	45	36	5	1106	733.64	3.28	2249	102.97	5.19	0.140354942
45	146	4	45	31	4	45	36	5	1478	739.56	2.29	1281	103.81	4.94	0.140367245
45	146	4	45	31	4	45	36	5	861	729.09	3.49	1905	102.98	5.03	0.141244565
45	146	4	45	111	5	45	104	4	1074	713.94	3.47	862	98.24	5.07	0.137602600
45	146	4	45	111	5	45	104	4	1115	726.05	2.90	1584	101.17	5.26	0.139343020
45	146	4	45	111	5	45	104	4	2176	748.19	3.78	411	102.05	4.41	0.136395835
45	146	4	45	111	5	45	104	4	908	764.60	3.59	1677	111.04	6.59	0.145226262
45	146	4	45	36	5	45	31	4	1113	692.78	2.99	1110	120.40	4.00	0.173792546
45	146	4	45	36	5	45	31	4	1274	713.18	3.37	1857	120.87	4.59	0.169480356
45	146	4	45	36	5	45	31	4	691	713.59	2.59	1109	115.84	5.13	0.162334113
45	146	4	45	36	5	45	31	4	1395	751.54	3.51	1550	125.43	4.65	0.166897304
64	14	4	64	20	4	68	117	5	491	694.48	3.23	761	126.40	5.01	0.182006681
64	14	4	64	20	4	68	117	5	571	688.97	3.24	893	95.15	5.25	0.138104707
64	14	4	64	20	4	68	117	5	585	730.05	3.21	810	133.56	5.72	0.182946374
64	14	4	64	20	4	68	117	5	615	720.30	3.13	822	98.90	4.42	0.137303901
64	14	4	64	20	4	68	117	5	527	714.12	3.27	1654	97.54	5.41	0.136587688
64	14	4	64	20	4	68	117	5	866	697.67	3.00	2157	92.93	4.87	0.133200510
64	14	4	64	43	4	68	37	5	756	709.06	5.52	879	100.10	6.42	0.141172820
64	14	4	64	43	4	68	37	5	562	723.08	6.90	1703	103.78	7.34	0.143524921
64	14	4	64	18	4	68	30	5	907	699.95	7.25	1300	97.98	8.07	0.139981427
64	14	4	64	18	4	68	30	5	976	716.10	3.24	1205	95.83	5.81	0.133822092

64	14	4	64	18	4	68	30	5	370	713.65	3.74	678	101.41	5.51	0.142100469
64	14	4	64	18	4	68	30	5	356	716.78	3.40	1343	98.69	6.08	0.137685203
64	14	4	68	37	5	64	43	4	848	709.10	3.39	1282	98.35	5.70	0.138696940
64	14	4	68	37	5	64	43	4	581	692.69	3.69	704	95.95	5.06	0.138517952
64	14	4	68	37	5	64	43	4	573	709.13	3.41	1067	99.46	4.98	0.140256370
64	14	4	68	37	5	64	43	4	662	687.59	3.38	979	95.94	5.36	0.139530825
64	14	4	68	37	5	64	43	4	287	714.42	3.35	857	97.99	5.21	0.137160214
64	14	4	68	37	5	64	43	4	362	712.49	3.43	1410	97.43	5.25	0.136745779
64	14	4	68	37	5	64	43	4	460	692.28	4.18	845	98.05	5.18	0.141633443
64	14	4	68	37	5	64	43	4	785	725.54	4.22	1272	103.48	6.13	0.142624804
64	14	4	68	30	5	64	18	4	658	704.78	4.33	682	98.95	4.91	0.140398422
64	14	4	68	30	5	64	18	4	435	713.50	4.72	564	96.66	6.30	0.135473020
64	14	4	68	30	5	64	18	4	707	715.63	4.28	1000	100.54	5.68	0.140491595
64	14	4	68	30	5	64	18	4	661	692.13	3.49	1055	95.43	4.85	0.137878722
64	14	4	68	30	5	64	18	4	615	727.02	3.93	1102	102.53	5.79	0.141027757
64	14	4	68	30	5	64	18	4	511	696.07	4.92	1011	99.65	5.12	0.143160889
64	14	4	68	30	5	64	18	4	559	695.10	4.78	932	97.43	5.85	0.140166882
64	14	4	68	30	5	64	18	4	591	695.99	4.23	1363	97.17	6.09	0.139614075
64	14	4	64	49	4	68	34	5	491	688.05	5.81	1006	98.37	7.44	0.142969261
64	17	4	64	49	4	68	34	5	529	707.84	3.89	1889	100.89	6.78	0.142532211
64	17	4	64	49	4	68	34	5	730	725.70	4.65	1813	105.10	6.11	0.144825686
64	17	4	64	49	4	68	34	5	539	724.17	3.45	746	100.81	6.47	0.139207645
64	17	4	64	49	4	68	34	5	636	704.27	4.56	1083	100.08	5.43	0.142104591
64	17	4	64	12	4	68	**29**	5	523	706.50	5.27	1418	99.20	6.47	0.140410474
64	17	4	64	12	4	68	29	5	810	693.69	3.50	1781	98.09	6.09	0.141403220
64	17	4	64	12	4	68	29	5	315	695.13	4.55	1029	97.11	5.55	0.139700488
64	17	4	64	55	4	68	7	5	505	723.95	3.57	606	103.18	6.90	0.142523655
64	17	4	68	34	5	64	49	4	551	709.77	3.40	1743	99.19	6.15	0.139749496
64	17	4	68	34	5	64	49	4	433	696.32	5.09	1737	95.16	6.65	0.136661305
64	17	4	68	34	5	64	49	4	475	694.87	4.30	1413	96.35	7.37	0.138659030
64	17	4	64	21	4	68	68	5	285	722.98	4.66	1025	101.67	5.83	0.140626297
64	20	4	68	32	5	64	21	4	286	727.80	4.72	1237	104.26	5.89	0.143253641
64	20	4	68	32	5	64	21	4	400	726.65	4.34	1253	101.84	6.32	0.140150003
64	20	4	68	32	5	64	21	4	568	692.82	3.62	1760	101.90	6.98	0.147080050
64	20	4	68	32	5	64	21	4	507	708.52	3.84	666	101.77	5.39	0.143637441
64	27	4	64	12	4	68	33	5	652	708.75	2.84	1271	97.47	5.48	0.137523810
64	27	4	64	12	4	68	33	5	526	700.88	3.51	1063	111.51	4.65	0.159099989

64	27	4	64	12	4	68	33	5	305	703.06	3.34	748	96.78	6.04	0.137655392
64	27	4	64	12	4	68	33	5	575	695.26	3.42	1226	94.66	6.31	0.136150505
64	27	4	64	12	4	68	33	5	557	718.63	3.52	743	123.53	4.97	0.171896525
64	27	4	64	12	4	68	33	5	433	705.74	3.39	1014	98.18	5.88	0.139116388
64	27	4	64	12	4	68	33	5	378	703.84	3.13	1164	96.54	6.57	0.137161855
64	27	4	64	12	4	68	33	5	283	715.39	3.05	495	98.37	3.84	0.137505417
64	36	4	64	12	4	68	35	5	316	695.03	2.31	1600	95.23	5.64	0.137015668
64	36	4	64	12	4	68	35	5	262	703.68	3.31	1538	97.04	6.75	0.137903593
64	36	4	64	12	4	68	35	5	201	719.49	3.57	915	98.47	5.31	0.136860832
64	44	4	64	22	4	68	27	5	1000	715.62	3.38	1728	99.62	6.60	0.139207960
64	44	4	68	27	5	64	22	4	875	708.01	3.05	791	101.89	5.34	0.143910397
64	44	4	68	27	5	64	22	4	944	711.95	2.99	2451	98.12	5.88	0.137818667
64	48	4	68	29	5	64	43	4	162	696.64	3.96	658	98.48	5.31	0.141364263
64	48	4	68	29	5	64	43	4	799	696.13	3.13	846	96.76	5.43	0.138997026
64	48	4	68	29	5	64	43	4	470	708.40	3.23	1358	98.62	5.74	0.139215133
64	48	4	68	29	5	64	43	4	524	714.52	3.13	1044	98.28	6.41	0.137546885
64	48	4	68	29	5	64	43	4	604	698.46	4.53	2022	95.35	6.23	0.136514618
64	48	4	68	29	5	64	12	4	654	714.14	3.10	781	95.93	4.92	0.134329403
64	14	4	64	20	4	68	71	7	515	687.73	3.06	340	109.10	6.60	0.158637838
64	14	4	64	20	4	68	71	7	201	698.02	3.56	155	111.42	6.76	0.159622933
64	14	4	68	71	7	64	20	4	320	697.42	2.90	410	96.20	5.31	0.137936968
64	14	4	68	71	7	64	20	4	558	704.30	2.58	410	132.86	4.30	0.188641204
64	14	4	68	71	7	64	20	4	461	687.95	3.15	623	93.67	5.23	0.136158151
64	14	4	68	71	7	64	20	4	617	695.21	3.27	976	96.13	6.89	0.138274766
64	14	4	68	71	7	64	20	4	839	709.37	2.92	667	97.52	5.95	0.137474097
64	14	4	68	71	7	64	20	4	448	699.09	3.12	1098	96.63	5.54	0.138222546
64	14	4	68	71	7	64	20	4	1816	693.90	3.15	367	130.40	7.40	0.187923332
64	14	4	68	71	7	64	20	4	2155	713.35	2.53	647	136.41	4.55	0.191224504
64	14	4	68	71	7	64	20	4	551	692.19	3.09	1466	95.69	5.42	0.138242390
64	14	4	68	71	7	64	20	4	732	702.96	3.42	705	97.76	6.30	0.139069079
64	14	4	68	71	7	64	20	4	1114	700.30	2.74	188	163.36	5.64	0.233271455
64	17	4	68	6	7	64	55	4	262	694.63	3.16	781	148.01	5.64	0.213077466
64	17	4	68	6	7	64	55	4	351	706.63	2.84	1299	96.74	5.69	0.136903330
64	17	4	68	6	7	64	55	4	192	702.11	3.17	1548	100.09	6.03	0.142556010
64	17	4	68	6	7	64	55	4	702	699.92	2.89	1872	99.08	6.10	0.141559035
64	17	4	68	6	7	64	55	4	363	696.86	3.20	1055	96.42	5.84	0.138363516
64	17	4	68	6	7	64	55	4	526	705.40	3.05	1548	95.75	6.50	0.135738588

64	20	4	64	18	4	68	16	7	765	689.09	3.06	903	93.78	5.61	0.136092528
64	20	4	64	18	4	68	16	7	454	697.54	3.48	228	94.60	5.82	0.135619463
64	20	4	64	18	4	68	16	7	339	697.56	3.19	2205	96.70	5.99	0.138626068
64	20	4	68	16	7	64	18	4	557	693.32	3.60	1209	94.32	7.00	0.136041078
64	20	4	68	16	7	64	18	4	444	696.38	2.76	2264	94.53	5.63	0.135744852
64	27	4	64	32	4	68	16	7	452	698.65	3.32	897	96.02	5.17	0.137436485
64	27	4	64	32	4	68	16	7	496	693.75	3.74	1109	97.85	6.16	0.141045045
64	27	4	64	32	4	68	16	7	1667	706.71	5.02	681	94.37	5.18	0.133534264
64	27	4	64	32	4	68	16	7	801	686.43	5.09	952	92.98	6.28	0.135454453
64	27	4	64	32	4	68	16	7	479	704.45	3.28	478	97.38	5.20	0.138235503
64	27	4	68	100	7	68	16	7	517	687.17	3.89	1160	100.45	5.07	0.146179257
64	27	4	68	100	7	68	16	7	231	691.07	3.73	1522	97.57	6.30	0.141186855
64	27	4	64	32	4	68	16	7	866	697.60	3.50	1063	94.75	5.92	0.135822821
64	27	4	64	32	4	68	16	7	179	704.26	3.98	300	136.00	4.89	0.193110499
64	27	4	64	32	4	68	16	7	1106	702.63	3.37	926	97.13	5.34	0.138237764
64	27	4	64	32	4	68	16	7	1038	683.84	3.78	844	97.55	5.13	0.142650328
64	27	4	64	32	4	68	16	7	749	681.56	3.41	1456	96.64	5.43	0.141792359
64	27	4	64	32	4	68	16	7	589	700.50	3.30	538	95.48	7.04	0.136302641
64	27	4	64	32	4	68	16	7	1024	706.39	3.74	2504	96.26	7.10	0.136270332
64	27	4	64	32	4	68	16	7	476	725.27	4.07	1228	98.04	6.14	0.135177244
64	27	4	64	32	4	68	16	7	852	715.40	4.05	861	98.77	5.63	0.138062622
64	27	4	68	120	7	64	12	4	1336	702.43	3.87	2058	98.56	6.32	0.140312914
64	27	4	68	120	7	64	12	4	1338	702.98	3.81	790	95.58	5.24	0.135964039
64	27	4	68	120	7	64	12	4	958	682.00	3.76	2502	96.32	5.84	0.141231672
64	27	4	68	120	7	64	12	4	529	702.81	3.37	833	95.48	5.23	0.135854641
64	27	4	68	120	7	64	12	4	548	687.00	3.79	928	131.87	5.71	0.191950509
64	27	4	68	120	7	64	12	4	810	702.54	3.29	1003	96.79	6.34	0.137771515
64	27	4	68	120	7	64	12	4	267	696.15	4.55	1130	95.91	6.77	0.137772032
64	27	4	68	120	7	64	12	4	1575	695.71	3.35	1863	96.96	6.06	0.139368415
64	44	4	64	34	4	68	55	7	408	697.70	4.19	1007	95.74	5.51	0.137222302
64	44	4	64	34	4	68	55	7	862	691.32	4.16	1354	96.27	7.29	0.139255338
64	44	4	64	34	4	68	55	7	798	711.05	3.83	1023	98.31	5.88	0.138260319
64	44	4	64	34	4	68	55	7	602	691.54	3.65	1011	94.28	5.42	0.136333401
64	44	4	64	34	4	68	55	7	794	703.91	3.83	836	94.17	5.32	0.133781307
64	44	4	64	34	4	68	55	7	260	688.14	4.77	359	91.69	6.04	0.133243235
64	44	4	64	34	4	68	55	7	598	685.39	3.77	1011	95.64	5.94	0.139540991
64	44	4	68	55	7	64	34	4	1142	698.53	3.22	2367	99.43	6.56	0.142341775

64	44	4	68	55	7	64	34	4	932	697.70	2.92	1076	96.85	6.44	0.138813244
64	48	4	64	12	4	68	46	7	575	678.30	7.75	783	92.92	6.26	0.136989533
64	48	4	64	27	4	68	74	7	615	695.60	4.06	702	127.87	5.50	0.183826912
64	48	4	64	27	4	68	74	7	650	697.59	2.87	771	121.11	5.11	0.173612007
64	48	4	64	27	4	68	74	7	912	689.08	3.48	1563	93.55	5.67	0.135760724
64	48	4	64	27	4	68	74	7	668	693.72	3.18	914	123.83	5.23	0.178501413
64	48	4	64	27	4	68	74	7	522	700.70	3.24	1236	94.84	5.29	0.135350364
64	48	4	64	27	4	68	74	7	646	685.73	3.14	500	180.11	5.81	0.262654398
64	48	4	68	46	7	64	12	4	440	701.54	3.35	814	144.12	5.12	0.205433760
64	48	4	68	46	7	64	12	4	605	713.13	3.37	463	97.59	5.59	0.136847419
64	48	4	68	46	7	64	12	4	692	707.50	3.50	1363	98.68	4.79	0.139477032
64	48	4	68	74	7	64	27	4	437	695.22	3.53	1529	97.04	5.85	0.139581715
64	48	4	68	74	7	64	27	4	717	695.37	3.66	1045	99.60	4.93	0.143233099
121	16	4	119	48	5	121	31	4	356	709.50	3.67	881	108.68	5.45	0.153178295
121	18	4	121	33	4	119	13	5	293	734.14	3.23	713	103.22	5.10	0.140599886
121	18	4	121	33	4	119	13	5	377	712.93	3.28	1189	101.45	5.76	0.142300086
121	18	4	121	33	4	119	13	5	639	703.36	2.77	2174	99.34	5.68	0.141236351
121	18	4	119	19	5	121	29	4	767	714.20	3.15	1227	101.62	5.68	0.142285074
121	18	4	119	19	5	121	29	4	106	708.59	4.01	855	98.42	5.62	0.138895553
121	18	4	119	19	5	121	29	4	407	749.75	3.56	981	103.72	4.95	0.138339446
121	18	4	119	19	5	121	29	4	1004	722.79	2.63	633	99.19	4.85	0.137232114
121	18	4	119	19	5	121	29	4	433	721.06	2.84	892	102.81	4.51	0.142581755
121	18	4	119	19	5	121	29	4	362	697.52	5.81	1505	103.20	6.87	0.147952747
121	18	4	119	19	5	121	29	4	546	779.88	3.14	1852	112.66	6.13	0.144458122
121	18	4	119	19	5	121	29	4	260	705.60	3.18	831	99.64	5.41	0.141213152
121	18	4	119	19	5	121	29	4	263	706.63	3.87	704	99.47	5.87	0.140766738
121	18	4	119	12	5	121	31	4	290	695.38	4.52	1769	95.13	7.05	0.136802899
121	18	4	119	12	5	121	31	4	496	706.13	3.19	1195	100.10	6.82	0.141758600
121	18	4	119	12	5	121	31	4	331	694.77	3.89	570	105.72	5.05	0.152165465
121	18	4	119	12	5	121	31	4	534	742.66	3.64	865	105.00	5.19	0.141383675
121	18	4	119	12	5	121	31	4	393	703.74	3.33	833	98.00	4.91	0.139255975
121	18	4	119	12	5	121	31	4	432	740.24	3.15	837	102.97	5.49	0.139103534
121	18	4	119	12	5	121	31	4	777	732.17	3.60	837	106.64	5.57	0.145649234
121	18	4	119	12	5	121	31	4	396	704.88	3.36	1893	99.29	6.27	0.140860856
121	18	4	119	12	5	121	31	4	332	693.79	3.32	904	98.34	5.40	0.141743179
121	18	4	119	13	5	121	33	4	321	716.05	3.11	1567	103.78	5.07	0.144934013
121	18	4	119	13	5	121	33	4	517	721.56	3.31	1685	102.71	5.83	0.142344365

121	18	4	119	13	5	121	33	4	313	721.03	3.61	1144	103.42	5.27	0.143433699
121	20	4	121	10	4	119	24	5	466	712.33	3.82	706	103.34	4.97	0.145073210
121	20	4	121	10	4	119	24	5	468	713.19	3.45	635	103.27	5.15	0.144800123
121	20	4	121	10	4	119	24	5	179	716.40	3.44	1026	104.12	6.03	0.145337800
121	20	4	121	10	4	119	24	5	409	711.85	3.74	820	100.84	4.82	0.141659057
121	20	4	121	10	4	119	24	5	461	707.23	3.49	1048	102.73	6.27	0.145256847
121	20	4	121	10	4	119	24	5	479	707.31	3.05	868	119.82	5.15	0.169402384
121	20	4	121	10	4	119	24	5	683	705.63	3.00	870	99.57	4.96	0.141107946
121	20	4	121	10	4	119	24	5	271	680.34	4.96	333	124.45	8.14	0.182923244
121	20	4	121	31	4	119	60	5	284	731.55	3.00	1002	129.13	4.80	0.176515618
121	20	4	121	31	4	119	60	5	246	709.76	3.17	1756	98.98	5.55	0.139455591
121	20	4	121	31	4	119	60	5	455	734.96	3.47	1272	116.82	5.64	0.158947426
121	20	4	121	31	4	119	60	5	467	727.35	3.53	899	105.81	5.86	0.145473293
121	20	4	121	31	4	119	60	5	345	706.82	3.72	1039	100.85	5.68	0.142681305
121	20	4	119	3	5	121	3	4	546	699.37	3.71	1302	100.73	5.65	0.144029627
121	20	4	119	60	5	121	31	4	650	711.41	5.76	745	123.25	6.74	0.173247494
121	20	4	119	60	5	121	31	4	379	762.95	3.18	436	106.86	5.29	0.140061603
121	20	4	119	60	5	121	31	4	306	718.79	4.11	512	101.09	5.59	0.140639130
121	22	4	121	52	4	119	25	5	464	730.59	3.07	2825	101.97	5.65	0.139572127
121	22	4	121	52	4	119	25	5	359	711.36	3.43	690	102.09	6.49	0.143513833
121	22	4	121	6	4	119	28	5	334	741.77	3.58	1759	110.62	5.95	0.149129784
121	22	4	119	28	5	121	6	4	691	733.55	3.03	1209	108.07	4.90	0.147324654
121	26	4	121	11	4	119	44	5	543	744.40	3.24	1932	106.66	4.94	0.143283181
121	26	4	121	11	4	119	44	5	191	731.74	2.95	859	101.12	6.31	0.138191161
121	26	4	121	11	4	119	44	5	357	720.17	3.25	2326	101.06	6.45	0.140327978
121	26	4	121	10	4	119	28	5	353	734.67	3.39	487	102.63	4.69	0.139695373
121	26	4	121	10	4	119	28	5	288	731.36	3.55	1254	103.05	5.61	0.140901881
121	26	4	121	10	4	119	28	5	500	723.77	3.65	846	125.92	5.30	0.173977921
121	26	4	121	10	4	119	28	5	1141	719.71	3.65	2127	99.07	6.10	0.137652666
121	26	4	121	10	4	119	28	5	389	732.65	3.46	949	103.89	4.84	0.141800314
121	26	4	121	10	4	119	28	5	541	707.98	3.82	883	136.20	5.83	0.192378316
121	26	4	121	2	4	119	47	5	297	715.13	3.19	638	119.30	4.01	0.166822815
121	26	4	121	2	4	119	47	5	1326	714.82	2.68	1118	101.21	3.83	0.141588092
121	26	4	121	2	4	119	47	5	520	721.63	3.00	647	97.91	4.45	0.135678949
121	26	4	121	2	4	119	47	5	457	716.98	2.83	863	98.54	4.76	0.137437585
121	26	4	121	2	4	119	47	5	399	717.17	3.12	1250	97.87	4.57	0.136466946
121	26	4	119	44	5	121	11	4	516	709.52	2.78	960	118.08	3.86	0.166422370

121	26	4	119	44	5	121	11	4	301	699.40	2.78	1072	96.83	4.22	0.138447240
121	26	4	119	44	5	121	11	4	966	705.97	2.89	895	121.54	3.61	0.172160290
121	26	4	119	44	5	121	11	4	410	705.84	2.82	753	95.17	3.32	0.134832257
121	26	4	119	44	5	121	11	4	650	724.59	3.00	575	100.59	4.87	0.138823335
121	26	4	119	47	5	121	2	4	949	733.20	3.12	685	102.38	4.06	0.139634479
121	26	4	119	47	5	121	2	4	566	711.78	2.99	467	101.25	3.62	0.142249010
121	27	4	121	9	4	119	1	5	479	722.21	2.89	609	98.50	3.58	0.136386923
121	27	4	121	9	4	119	1	5	727	726.12	3.17	846	103.60	4.31	0.142676142
121	27	4	121	9	4	119	1	5	536	725.01	3.39	814	101.68	4.32	0.140246341
121	27	4	121	9	4	119	1	5	658	687.10	3.49	605	93.49	4.37	0.136064619
121	27	4	121	9	4	119	1	5	956	726.00	3.33	1025	102.56	4.09	0.141267218
121	27	4	121	2	4	119	48	5	803	722.15	3.18	682	101.10	4.21	0.139998615
121	27	4	121	2	4	119	48	5	379	724.46	3.83	589	98.57	4.38	0.136059962
121	27	4	121	2	4	119	48	5	1199	705.57	3.47	1774	98.15	4.72	0.139107388
121	27	4	121	2	4	119	48	5	933	695.55	3.58	1849	97.86	4.69	0.140694414
121	27	4	121	6	4	119	8	5	1048	708.37	3.58	2961	97.40	4.49	0.137498765
121	27	4	121	6	4	119	8	5	1151	708.15	4.23	2877	100.29	4.57	0.141622538
121	27	4	121	6	4	119	8	5	3483	708.52	4.51	2020	99.77	4.60	0.140814656
121	27	4	121	6	4	119	8	5	1558	727.96	3.97	2714	103.09	4.76	0.141614924
121	27	4	119	1	5	121	9	4	1010	713.41	3.95	1697	101.52	4.93	0.142302463
121	27	4	119	1	5	121	9	4	543	707.11	4.35	1068	99.27	4.79	0.140388341
121	27	4	119	1	5	121	9	4	1257	721.01	3.99	2043	99.37	4.74	0.137820557
121	27	4	119	1	5	121	9	4	785	706.67	3.66	1673	98.75	4.34	0.139739907
121	27	4	119	8	5	121	6	4	329	726.95	3.27	1260	98.11	4.19	0.134961139
121	27	4	119	8	5	121	6	4	510	708.20	4.02	1559	94.70	3.93	0.133719288
121	27	4	119	8	5	121	6	4	359	682.47	3.36	1126	92.67	4.69	0.135786188
121	30	4	121	12	4	119	19	5	995	723.46	3.64	2721	98.69	4.30	0.136413900
121	30	4	121	12	4	119	19	5	1009	712.65	3.61	2763	99.90	4.57	0.140181015
121	30	4	121	13	4	119	31	5	485	714.91	3.30	2113	98.49	4.19	0.137765593
121	30	4	121	13	4	119	31	5	334	694.39	3.80	1858	93.21	5.01	0.134232924
121	30	4	119	31	5	121	52	4	1006	727.54	3.29	3228	102.39	4.62	0.140734530
121	30	4	119	31	5	121	52	4	922	713.16	2.98	2454	102.11	3.55	0.143179651
121	30	4	119	31	5	121	52	4	1008	700.13	3.41	3656	98.09	4.46	0.140102552
121	30	4	119	31	5	121	52	4	1071	712.90	3.87	3942	94.75	5.16	0.132907841
121	30	4	119	31	5	121	52	4	1014	724.32	3.47	4610	97.06	5.77	0.134001546
121	30	4	119	31	5	121	52	4	1504	700.41	3.82	2981	97.61	4.37	0.139361231
121	30	4	119	32	5	121	13	4	1507	701.50	3.65	4204	95.76	5.15	0.136507484

121	30	4	119	32	5	121	13	4	1827	698.62	3.61	3233	95.66	4.36	0.136927085
121	30	4	119	32	5	121	13	4	1798	694.79	3.81	2874	97.02	4.47	0.139639315
121	30	4	119	32	5	121	13	4	1579	710.42	2.76	3656	97.82	4.47	0.137693196
121	30	4	119	32	5	121	13	4	1876	703.94	3.50	3854	97.55	5.04	0.138577151
121	30	4	119	32	5	121	13	4	2064	708.15	3.69	3618	98.67	5.04	0.139334887
121	36	4	121	12	4	119	12	5	818	707.38	2.95	3528	98.73	4.71	0.139571376
121	36	4	121	5	4	119	1	5	1288	698.23	3.05	6072	94.26	5.34	0.134998496
121	36	4	121	5	4	119	1	5	824	701.96	2.85	3827	93.85	5.15	0.133697077
121	36	4	121	5	4	119	1	5	2192	699.47	3.43	3998	94.71	5.15	0.135402519
121	36	4	121	5	4	119	1	5	1370	694.95	3.41	2471	92.33	4.62	0.132858479
121	36	4	121	5	4	119	1	5	1311	701.71	3.37	3576	93.79	4.74	0.133659204
121	36	4	121	5	4	119	1	5	1680	705.69	3.47	3798	96.66	4.89	0.136972325
121	36	4	119	12	5	121	12	4	832	695.41	2.87	2200	95.95	3.95	0.137976158
121	36	4	119	12	5	121	12	4	1753	704.04	3.54	3577	96.32	4.68	0.136810408
121	36	4	119	12	5	121	12	4	981	701.89	3.05	3116	94.13	4.41	0.134109333
121	36	4	119	12	5	121	12	4	955	697.65	3.47	3068	95.08	4.35	0.136286103
121	36	4	119	1	5	121	5	4	1313	706.84	3.11	4130	96.37	4.92	0.136339200
121	36	4	119	1	5	121	5	4	649	706.67	3.17	3204	94.59	4.54	0.133853142
121	36	4	119	47	5	121	29	4	1116	698.50	3.45	1917	96.23	3.70	0.137766643
121	37	4	121	5	4	119	8	5	806	695.29	3.54	1987	97.66	4.00	0.140459377
121	37	4	121	13	4	119	13	5	802	694.19	3.22	2635	120.89	4.25	0.174145407
121	37	4	121	13	4	119	13	5	1534	701.96	3.79	3858	97.97	5.14	0.139566357
121	37	4	121	13	4	119	13	5	835	704.91	3.62	3607	97.96	5.01	0.138968095
121	37	4	121	13	4	119	13	5	527	712.09	2.81	1974	97.10	4.56	0.136359168
121	37	4	121	11	4	119	24	5	803	708.82	3.30	3722	97.02	5.19	0.136875370
121	37	4	121	11	4	119	24	5	1236	713.52	3.48	4323	96.25	4.95	0.134894607
121	37	4	121	11	4	119	24	5	1833	708.76	3.58	1901	98.44	4.23	0.138890457
121	37	4	121	11	4	119	24	5	887	692.35	4.07	1441	94.28	4.86	0.136173900
121	37	4	119	8	5	121	5	4	752	720.68	3.47	3051	98.47	4.90	0.136634845
121	37	4	119	8	5	121	5	4	399	692.67	3.43	3385	96.27	4.80	0.138983932
121	37	4	119	8	5	121	5	4	779	704.91	3.46	2973	97.96	4.70	0.138968095
121	37	4	119	8	5	121	5	4	415	705.94	3.21	3134	97.58	4.60	0.138227045
121	37	4	119	13	5	121	13	4	995	708.63	3.37	3551	97.57	4.57	0.137688215
121	37	4	119	13	5	121	13	4	925	714.14	3.13	4513	98.41	4.58	0.137802112
121	37	4	119	13	5	121	13	4	696	711.30	3.36	3245	98.27	4.35	0.138155490
121	37	4	119	13	5	121	13	4	448	682.23	4.11	2930	86.74	5.00	0.127141873
121	37	4	119	24	5	121	11	4	1543	704.27	3.71	6367	93.47	6.18	0.132718986

121	37	4	119	24	5	121	11	4	1275	693.02	3.71	4238	93.57	4.76	0.135017748
121	40	4	121	6	4	119	25	5	1548	692.10	3.25	4821	95.75	5.55	0.138347060
121	40	4	121	6	4	119	25	5	896	722.89	3.15	2691	100.26	5.18	0.138693301
121	40	4	121	3	4	119	66	5	1546	728.50	3.56	4555	97.78	5.52	0.134221002
121	40	4	121	3	4	119	66	5	1383	726.20	3.54	2853	100.55	4.27	0.138460479
121	40	4	121	3	4	119	66	5	1315	713.65	2.87	2543	99.11	4.55	0.138877601
121	40	4	121	3	4	119	66	5	742	702.02	3.09	3231	97.29	4.50	0.138585795
121	40	4	121	3	4	119	66	5	1084	710.48	3.44	1440	120.06	4.42	0.168984349
121	40	4	121	6	4	119	32	5	1850	703.36	3.99	3415	98.17	4.87	0.139572907
121	40	4	121	6	4	119	32	5	650	714.99	3.16	3400	100.49	4.91	0.140547420
121	40	4	121	6	4	119	32	5	661	723.70	3.08	3161	102.28	4.11	0.141329280
121	40	4	121	6	4	119	32	5	870	722.00	3.11	3168	100.40	4.19	0.139058172
121	40	4	121	6	4	119	32	5	610	709.56	3.36	3073	98.03	4.44	0.138156040
121	40	4	119	66	5	121	3	4	1104	697.16	3.28	3566	98.69	4.29	0.141560044
121	40	4	119	66	5	121	3	4	619	706.49	3.38	2463	99.64	4.79	0.141035259
121	40	4	119	66	5	121	3	4	1203	707.55	3.75	3633	99.39	4.80	0.140470638
121	40	4	119	66	5	121	3	4	814	697.03	3.29	2562	100.76	4.01	0.144556188
121	40	4	121	32	4	119	6	5	545	708.39	2.89	2933	98.64	4.61	0.139245331
121	40	4	121	32	4	119	6	5	675	713.02	2.99	2579	100.76	4.84	0.141314409
121	40	4	121	32	4	119	6	5	1802	700.49	3.53	2346	98.10	4.51	0.140044826
121	40	4	121	32	4	119	6	5	1050	712.88	3.03	2155	100.20	3.92	0.140556615
121	43	4	121	44	4	119	66	5	1300	700.82	3.06	2615	99.19	4.17	0.141534203
121	43	4	121	44	4	119	66	5	2174	700.84	2.69	3530	97.17	4.97	0.138647908
121	43	4	121	44	4	119	66	5	1370	712.37	3.35	3659	99.87	4.72	0.140194000
121	43	4	121	44	4	119	66	5	1462	709.72	3.15	3157	100.24	4.09	0.141238798
121	43	4	121	44	4	119	66	5	854	715.57	2.98	3280	99.63	4.84	0.139231661
121	43	4	121	31	4	119	31	5	1694	714.02	3.13	2607	100.01	4.45	0.140066105
121	43	4	121	31	4	119	31	5	669	700.43	2.97	3372	97.42	4.77	0.139085990
121	43	4	121	31	4	119	31	5	912	689.83	3.05	3133	97.97	4.65	0.142020498
121	43	4	121	31	4	119	31	5	851	695.73	3.42	3127	97.82	4.55	0.140600520
121	43	4	121	31	4	119	31	5	245	686.82	3.55	521	121.86	4.43	0.177426400
121	43	4	121	33	4	119	3	5	838	687.09	3.35	3918	95.94	4.80	0.139632363
121	43	4	121	33	4	119	3	5	1142	684.41	3.30	3983	100.27	5.03	0.146505749
121	43	4	121	33	4	119	3	5	1024	697.04	3.27	3711	96.80	5.07	0.138872948
121	43	4	121	33	4	119	3	5	534	705.09	3.05	2185	96.58	4.64	0.136975422
121	43	4	119	3	5	121	33	4	675	704.96	3.14	2511	97.59	4.79	0.138433386
121	43	4	119	3	5	121	33	4	1739	699.36	3.39	2945	98.99	4.04	0.141543697

121	43	4	119	3	5	121	33	4	607	711.89	2.72	1812	99.44	4.46	0.139684502
121	43	4	119	3	5	121	33	4	1004	723.22	3.14	3315	101.06	4.47	0.139736180
121	43	4	119	3	5	121	33	4	433	716.18	3.09	1926	101.22	4.76	0.141333184
121	18	4	121	52	4	119	53	7	955	715.66	3.70	1104	130.74	5.81	0.182684515
121	18	4	121	52	4	119	53	7	571	717.15	3.25	867	102.03	5.43	0.142271491
121	18	4	121	52	4	119	52	7	912	697.57	3.43	1178	99.13	5.60	0.142107602
121	18	4	119	53	7	121	52	4	1411	685.15	3.59	2093	99.53	6.55	0.145267460
121	18	4	119	53	7	121	52	4	1390	700.01	3.51	1786	96.90	6.39	0.138426594
121	18	4	119	53	7	121	52	4	801	695.39	3.26	1283	97.43	6.15	0.140108428
121	18	4	119	53	7	121	52	4	538	689.91	3.27	2568	93.28	7.05	0.135206041
121	18	4	119	53	7	121	52	4	992	705.87	3.62	1063	99.80	6.05	0.141385808
121	18	4	119	53	7	121	52	4	709	702.77	4.12	1094	97.22	6.82	0.138338290
121	18	4	119	53	7	121	52	4	691	699.79	3.71	649	94.76	6.37	0.135412052
121	18	4	119	53	7	121	52	4	807	697.71	4.04	475	95.91	5.64	0.137463989
121	18	4	119	53	7	121	52	4	552	697.41	3.98	1052	100.78	6.02	0.144506101
121	20	4	121	31	4	119	50	7	824	687.44	3.39	802	96.66	5.25	0.140608635
121	20	4	121	31	4	119	50	7	1004	693.05	3.49	2071	97.93	5.80	0.141302936
121	20	4	121	31	4	119	50	7	511	686.94	3.13	1646	94.31	5.90	0.137290011
121	20	4	121	31	4	119	50	7	607	712.53	3.53	817	97.25	5.42	0.136485481
121	20	4	121	31	4	119	50	7	776	698.85	3.09	1667	99.96	5.48	0.143034986
121	20	4	121	3	4	119	10	7	522	720.21	3.49	1246	98.45	6.27	0.136696241
121	20	4	121	3	4	119	10	7	1042	675.47	3.48	2255	92.48	5.65	0.136912076
121	20	4	121	3	4	119	10	7	378	706.90	3.52	1076	96.51	5.93	0.136525675
121	20	4	121	3	4	119	10	7	453	700.13	3.68	350	100.15	7.68	0.143044863
121	20	4	121	3	4	119	10	7	606	673.02	6.31	1054	92.24	6.39	0.137053877
121	20	4	119	50	7	121	31	4	645	690.46	3.70	2166	95.66	6.18	0.138545318
121	20	4	119	50	7	121	31	4	1008	698.76	3.26	1757	99.43	5.32	0.142294922
121	20	4	119	50	7	121	31	4	358	704.62	3.29	1026	97.52	4.98	0.138400840
121	20	4	119	50	7	121	31	4	1086	697.14	3.43	1657	100.11	6.22	0.143600998
121	20	4	119	10	7	121	3	4	515	698.53	3.15	692	95.56	4.66	0.136801569
121	20	4	119	10	7	121	3	4	281	696.24	4.84	737	95.70	4.94	0.137452603
121	20	4	119	10	7	121	3	4	470	694.18	3.26	1630	95.31	5.45	0.137298683
121	20	4	119	10	7	121	3	4	286	700.96	3.37	1510	96.53	5.05	0.137711139
121	22	4	121	3	4	119	14	7	291	677.73	3.52	831	90.01	5.05	0.132811001
121	22	4	121	3	4	119	14	7	519	710.59	3.28	834	95.55	4.98	0.134465726
121	22	4	121	3	4	119	14	7	700	701.25	3.28	888	94.00	5.93	0.134046346
121	22	4	121	3	4	119	14	7	459	686.03	3.66	1534	92.67	5.35	0.135081556

121	22	4	121	6	4	119	27	7	651	689.85	3.46	1654	94.56	6.97	0.137073277
121	22	4	121	6	4	119	27	7	624	688.87	3.26	1330	95.58	5.86	0.138748966
121	22	4	121	6	4	119	27	7	236	683.61	3.43	1270	96.50	4.45	0.141162359
121	22	4	121	6	4	119	27	7	741	700.10	4.11	936	92.53	6.70	0.132166833
121	22	4	121	6	4	119	27	7	250	718.74	3.82	442	95.20	5.14	0.132454017
121	22	4	121	52	4	119	10	7	636	704.28	3.30	945	98.41	5.07	0.139731357
121	22	4	121	52	4	119	10	7	479	706.58	3.60	1461	96.98	6.05	0.137252682
121	22	4	121	52	4	119	10	7	457	703.00	3.56	683	120.25	5.26	0.171052632
121	22	4	119	14	7	121	3	4	278	696.09	3.30	794	95.53	5.47	0.137238001
121	22	4	119	14	7	121	3	4	254	697.35	2.74	353	86.94	6.51	0.124671972
121	22	4	119	14	7	121	3	4	629	705.23	2.89	2029	95.19	5.64	0.134977241
121	22	4	119	27	7	121	6	4	931	709.07	3.34	1114	95.80	5.65	0.135106548
121	22	4	119	27	7	121	6	4	646	711.35	3.45	1135	96.00	7.32	0.134954664
121	22	4	119	27	7	121	6	4	376	692.70	3.45	1140	92.76	5.63	0.133910784
121	22	4	119	27	7	121	6	4	161	702.24	3.52	480	91.86	6.25	0.130809979
121	26	4	121	2	4	119	9	7	1144	731.91	3.80	1524	100.92	5.14	0.137885806
121	26	4	121	2	4	119	9	7	284	721.04	3.89	1216	98.48	5.32	0.136580495
121	26	4	121	2	4	119	9	7	481	718.63	3.08	617	96.01	6.33	0.133601436
121	26	4	121	2	4	119	9	7	564	698.13	3.02	1058	95.13	5.96	0.136264020
121	26	4	121	2	4	119	9	7	1203	684.77	3.02	1819	93.20	5.33	0.136104093
121	26	4	121	2	4	119	9	7	831	701.04	2.95	581	93.17	4.14	0.132902545
121	26	4	121	2	4	119	9	7	507	706.09	3.12	372	94.78	6.18	0.134232180
121	26	4	121	2	4	119	9	7	1051	704.73	2.97	600	98.24	4.30	0.139400905
121	26	4	119	11	7	121	10	4	254	699.99	3.24	998	98.01	5.96	0.140016286
121	26	4	119	11	7	121	10	4	397	712.16	2.84	1004	98.60	4.50	0.138452033
121	26	4	119	9	7	121	2	4	415	687.94	2.81	1296	92.76	5.26	0.134837340
121	26	4	119	9	7	121	2	4	677	701.25	2.72	674	92.62	4.54	0.132078431
121	26	4	119	9	7	121	2	4	742	700.46	3.02	504	94.60	5.82	0.135054107
121	26	4	119	9	7	121	2	4	1018	687.77	2.96	1173	91.24	5.44	0.132660628
121	26	4	119	9	7	121	2	4	895	698.87	2.74	922	124.89	6.05	0.178702763
121	26	4	119	9	7	121	2	4	314	712.34	3.94	279	92.86	6.51	0.130359098
121	26	4	119	9	7	121	2	4	895	688.61	2.82	1203	91.16	5.43	0.132382626
121	26	4	119	9	7	121	2	4	902	688.14	2.86	1199	91.31	5.42	0.132691022
121	26	4	119	9	7	121	2	4	465	698.22	3.44	460	94.50	5.50	0.135344161
121	27	4	121	6	4	119	49	7	369	689.44	2.65	287	91.92	4.29	0.133325598
121	27	4	121	6	4	119	49	7	547	697.42	3.04	621	96.79	5.38	0.138782943
121	27	4	121	6	4	119	49	7	683	689.34	3.28	645	94.99	5.25	0.137798474

121	27	4	121	6	4	119	49	7	291	689.08	2.87	383	92.69	4.34	0.134512684
121	27	4	121	6	4	119	49	7	805	698.06	3.00	507	93.75	4.72	0.134300776
121	27	4	119	46	7	121	2	4	316	695.80	3.74	1162	96.73	5.60	0.139019833
121	27	4	119	46	7	121	2	4	603	703.79	2.99	774	131.55	4.58	0.186916552
121	27	4	119	46	7	121	2	4	1287	700.55	2.69	1456	95.18	4.85	0.135864678
121	27	4	119	46	7	121	2	4	861	694.81	2.71	414	92.09	4.71	0.132539831
121	27	4	119	46	7	121	2	4	886	711.49	3.27	237	94.91	6.45	0.133396112
121	27	4	119	46	7	121	2	4	1223	692.08	3.15	950	95.47	5.53	0.137946480
121	27	4	119	46	7	121	2	4	999	701.51	2.80	852	99.19	5.74	0.141394991
121	27	4	119	46	7	121	2	4	397	701.61	2.96	584	92.93	4.49	0.132452502
121	27	4	119	46	7	121	2	4	862	684.99	2.91	819	92.90	4.80	0.135622418
121	27	4	119	46	7	121	2	4	1133	688.34	2.70	1029	90.92	5.77	0.132085888
121	27	4	119	46	7	121	2	4	404	699.11	2.79	669	93.54	4.81	0.133798687
121	27	4	121	49	4	119	6	7	412	709.21	2.46	473	99.05	5.89	0.139662441
121	27	4	121	49	4	119	6	7	467	687.31	2.64	1002	93.15	4.94	0.135528364
121	27	4	121	49	4	119	6	7	659	691.62	2.85	984	92.77	4.99	0.134134351
121	27	4	121	49	4	119	6	7	713	694.32	2.91	1525	94.85	5.66	0.136608480
121	27	4	121	49	4	119	6	7	714	699.51	3.05	1503	94.23	4.90	0.134708582
121	30	4	121	12	4	119	22	7	592	712.93	4.15	1303	97.24	5.49	0.136394877
121	30	4	121	12	4	119	22	7	343	695.41	3.35	832	90.52	5.64	0.130167815
121	30	4	121	12	4	119	22	7	472	696.38	3.40	963	109.57	6.27	0.157342256
121	30	4	121	12	4	119	22	7	433	729.04	3.25	857	104.09	5.76	0.142776802
121	30	4	121	12	4	119	22	7	686	693.65	3.62	1736	101.09	8.22	0.145736322
121	30	4	121	12	4	119	22	7	404	709.13	3.09	1314	95.92	5.53	0.135264338
121	30	4	121	12	4	119	22	7	162	693.47	3.31	561	96.24	5.81	0.138780337
121	30	4	121	12	4	119	22	7	1054	702.15	3.29	995	95.70	4.96	0.136295663
121	30	4	121	12	4	119	22	7	296	689.82	3.12	1054	93.12	5.90	0.134991737
121	30	4	121	12	4	119	22	7	125	695.19	4.19	1829	98.80	5.19	0.142119421
121	30	4	121	52	4	119	73	7	515	690.12	3.01	545	96.95	4.57	0.140482815
121	30	4	121	52	4	119	73	7	798	747.24	2.97	624	104.29	4.18	0.139566940
121	30	4	121	52	4	119	73	7	726	701.51	4.30	987	100.77	5.93	0.143647275
121	30	4	121	52	4	119	73	7	1030	690.62	4.05	666	97.63	5.60	0.141365729
121	30	4	121	13	4	119	67	7	939	716.85	3.56	641	101.60	5.02	0.141731185
121	30	4	121	13	4	119	67	7	991	729.19	2.93	1461	101.54	4.23	0.139250401
121	30	4	121	13	4	119	67	7	644	694.81	3.35	548	96.74	5.43	0.139232308
121	30	4	121	13	4	119	67	7	908	707.45	5.22	2557	98.72	6.64	0.139543431
121	30	4	121	13	4	119	67	7	524	720.24	3.88	460	99.70	4.56	0.138426080

121	30	4	119	21	7	121	12	4	608	728.26	3.55	1246	101.94	5.76	0.139977481
121	30	4	119	21	7	121	12	4	335	718.43	3.27	779	101.84	5.47	0.141753546
121	30	4	119	21	7	121	12	4	673	700.36	3.08	451	99.15	5.57	0.141570050
121	30	4	119	73	7	121	52	4	526	707.94	2.65	499	98.95	6.01	0.139771732
121	30	4	119	73	7	121	52	4	740	703.19	3.26	413	101.16	3.92	0.143858701
121	30	4	119	73	7	121	52	4	897	708.63	3.22	492	103.10	4.84	0.145492006
121	30	4	119	67	7	121	13	4	357	718.90	2.71	1165	101.06	4.94	0.140575880
121	30	4	119	67	7	121	13	4	359	726.93	3.59	1287	100.56	4.79	0.138335190
121	36	4	121	29	4	119	29	7	306	714.90	3.37	1074	99.47	5.68	0.139138341
121	36	4	121	5	4	119	26	7	437	728.33	3.33	829	103.94	5.39	0.142710035
121	36	4	121	5	4	119	26	7	510	719.96	2.85	766	100.74	4.17	0.139924440
121	36	4	121	5	4	119	26	7	476	721.30	3.25	1214	100.36	5.59	0.139137668
121	36	4	121	5	4	119	26	7	218	687.68	2.74	1231	96.88	4.13	0.140879479
121	36	4	121	5	4	119	26	7	490	712.07	3.16	1282	100.34	5.75	0.140913112
121	36	4	121	2	4	119	64	7	564	713.76	2.83	1120	101.61	4.55	0.142358776
121	36	4	121	2	4	119	64	7	599	704.58	3.36	1280	99.92	5.93	0.141814982
121	36	4	121	2	4	119	64	7	805	713.12	2.78	1450	100.83	4.66	0.141392753
121	36	4	121	2	4	119	64	7	335	734.90	2.96	1175	103.76	5.53	0.141189277
121	36	4	119	29	7	121	29	4	281	713.11	2.89	1047	99.48	6.82	0.139501620
121	36	4	119	29	7	121	29	4	325	723.54	2.73	1488	100.53	4.19	0.138941869
121	36	4	119	29	7	121	29	4	440	730.79	3.13	1120	103.30	5.23	0.141353877
121	36	4	119	29	7	121	29	4	381	720.71	3.07	1196	98.16	4.44	0.136199026
121	36	4	119	26	7	121	5	4	501	726.49	2.67	1741	101.48	4.91	0.139685336
121	36	4	119	26	7	121	5	4	1287	727.61	2.80	1246	98.96	4.24	0.136006927
121	36	4	119	26	7	121	5	4	242	720.04	3.97	432	106.33	6.77	0.147672352
121	36	4	119	26	7	121	5	4	534	702.35	2.96	1667	96.10	5.00	0.136826369
121	36	4	119	64	7	121	12	4	668	699.84	2.57	1081	94.92	4.42	0.135631001
121	36	4	119	64	7	121	12	4	926	724.61	2.82	1305	100.22	4.64	0.138308883
121	36	4	119	64	7	121	12	4	735	702.04	3.08	825	96.70	4.04	0.137741439
121	37	4	121	5	4	119	53	7	741	715.25	3.16	1990	99.13	4.57	0.138594897
121	37	4	121	5	4	119	53	7	325	719.98	2.43	1062	102.98	5.83	0.143031751
121	37	4	121	5	4	119	53	7	339	706.23	3.10	904	97.86	4.71	0.138566756
121	37	4	121	5	4	119	53	7	470	720.91	3.25	1233	99.68	5.30	0.138269687
121	37	4	121	13	4	119	51	7	413	714.98	2.47	2381	99.25	4.53	0.138815072
121	37	4	121	13	4	119	51	7	445	715.21	3.22	385	95.02	7.78	0.132856084
121	37	4	121	13	4	119	51	7	129	694.60	3.27	349	100.01	5.37	0.143982148
121	37	4	121	52	4	119	5	7	581	694.07	3.32	681	99.34	5.88	0.143126774

121	37	4	121	52	4	119	5	7	318	701.56	3.14	534	100.34	4.82	0.143024118
121	37	4	121	52	4	119	5	7	1249	694.66	3.07	605	96.54	3.93	0.138974462
121	37	4	119	53	7	119	5	7	440	701.97	2.76	1519	96.49	4.45	0.137456017
121	37	4	119	53	7	119	5	7	723	722.71	2.63	699	130.60	6.63	0.180708721
121	37	4	119	53	7	119	5	7	305	723.87	2.99	1036	98.26	3.97	0.135742606
121	37	4	119	51	7	121	13	4	388	709.46	2.51	365	96.77	5.95	0.136399515
121	37	4	119	51	7	121	13	4	336	691.62	2.68	503	94.02	3.94	0.135941702
121	37	4	119	51	7	121	13	4	336	700.34	2.45	1080	95.28	4.20	0.136048205
121	37	4	119	51	7	121	13	4	486	714.18	3.39	1690	100.96	5.14	0.141364922
121	43	4	121	12	4	119	16	7	249	715.84	2.33	658	102.25	5.03	0.142839182
121	43	4	121	12	4	119	16	7	490	718.27	2.28	1187	102.37	4.74	0.142523007
121	43	4	121	12	4	119	16	7	594	711.84	3.27	1069	97.55	4.39	0.137039222
121	43	4	119	16	7	121	12	4	414	689.84	2.78	677	96.77	5.31	0.140278905
121	43	4	119	16	7	121	12	4	808	696.18	2.90	1397	98.67	4.58	0.141730587
121	43	4	119	16	7	121	12	4	1223	703.84	2.67	554	107.76	5.70	0.153102978
121	43	4	119	16	7	121	12	4	1125	710.69	2.77	573	100.07	5.47	0.140806822
144	29	4	144	8	4	144	2	5	774	703.94	2.85	2502	97.23	5.60	0.138122567
144	29	4	144	8	4	144	2	5	709	715.94	3.49	1397	102.12	4.82	0.142637651
144	29	4	144	8	4	144	2	5	1737	712.44	3.14	1269	100.70	5.06	0.141345236
144	29	4	144	8	4	144	2	5	1249	737.96	3.25	1449	124.80	4.35	0.169114857
144	29	4	144	8	4	144	2	5	868	684.29	3.07	1104	92.56	6.04	0.135264289
144	29	4	144	8	4	144	2	5	1087	714.19	3.14	1788	98.40	6.54	0.137778462
144	29	4	144	8	4	144	2	5	2186	687.71	2.88	424	89.45	4.98	0.130069361
144	29	4	144	8	4	144	2	5	1341	741.00	2.95	1657	112.88	5.63	0.152334683
144	29	4	144	8	4	144	2	5	1622	716.99	2.55	726	97.78	4.91	0.136375682
144	29	4	144	8	4	144	2	5	1046	715.02	3.07	914	97.66	4.46	0.136583592
144	29	4	144	8	4	144	2	5	1932	704.50	3.45	602	94.09	4.31	0.133555713
144	31	4	144	8	4	144	26	5	855	766.93	2.85	2012	106.14	4.24	0.138395942
144	31	4	144	8	4	144	26	5	1529	720.42	2.51	1469	98.05	4.23	0.136101163
144	31	4	144	8	4	144	26	5	1180	723.81	3.24	1672	98.33	4.83	0.135850569
144	31	4	144	6	4	144	20	5	2056	714.99	3.16	1366	100.59	4.31	0.140687282
144	31	4	144	4	4	144	5	5	1622	754.25	2.91	1940	111.45	4.87	0.147762678
144	31	4	144	4	4	144	5	5	1048	714.60	2.61	946	122.52	4.59	0.171452561
144	31	4	144	4	4	144	5	5	1262	743.22	2.80	1504	131.81	4.15	0.177349910
144	31	4	144	4	4	144	5	5	1153	710.74	3.05	1514	97.25	5.21	0.136829220
144	31	4	144	4	4	144	5	5	1120	714.45	2.78	883	95.38	5.62	0.133501295
144	31	4	144	26	5	144	8	4	1915	762.85	2.68	1788	106.62	4.72	0.139765354

144	31	4	144	10	5	144	6	4	1523	737.71	2.88	671	101.60	4.31	0.137723496
144	31	4	144	10	5	144	6	4	1409	726.51	2.84	700	97.49	4.75	0.134189481
144	31	4	144	10	5	144	6	4	1399	735.19	2.60	1156	100.79	4.52	0.137093812
144	31	4	144	10	5	144	6	4	1410	717.25	2.49	1070	97.99	5.93	0.136619031
144	31	4	144	10	5	144	6	4	1406	709.52	3.69	1515	119.82	5.03	0.168874732
144	31	4	144	5	5	144	4	4	1537	705.53	2.85	1700	97.65	4.72	0.138406588
144	31	4	144	5	5	144	4	4	2462	700.52	2.59	1160	95.29	4.91	0.136027522
144	31	4	144	5	5	144	4	4	1866	728.76	3.69	1485	100.69	4.90	0.138166200
144	31	4	144	5	5	144	4	4	1556	704.24	2.63	1089	95.54	5.41	0.135663978
144	31	4	144	5	5	144	4	4	942	718.88	3.75	418	120.14	4.90	0.167121077
144	33	4	144	25	5	144	20	4	1967	708.40	2.83	869	116.85	4.81	0.164949181
144	33	4	144	25	5	144	20	4	2097	707.41	2.60	594	96.41	4.35	0.136285888
144	33	4	144	25	5	144	20	4	1761	726.22	3.14	1618	107.85	5.22	0.148508716
144	33	4	144	25	5	144	20	4	2182	719.61	3.35	761	98.00	5.57	0.136184878
144	33	4	144	25	5	144	20	4	1098	705.18	2.58	1244	96.06	7.16	0.136220539
144	33	4	144	25	5	144	20	4	1252	718.77	2.84	1428	100.58	4.54	0.139933498
144	33	4	144	96	5	144	21	4	1250	694.08	3.01	1655	99.37	5.17	0.143167935
144	33	4	144	96	5	144	21	4	2055	724.97	3.00	901	101.58	5.57	0.140116143
144	33	4	144	96	5	144	21	4	364	725.21	3.78	843	99.71	5.16	0.137491209
144	33	4	144	96	5	144	21	4	731	733.76	3.24	776	103.48	4.43	0.141027039
144	33	4	144	27	5	144	22	4	1553	724.30	3.50	693	108.53	4.68	0.149841226
144	33	4	144	27	5	144	22	4	777	731.91	2.95	1081	101.83	5.56	0.139129128
144	33	4	144	27	5	144	22	4	1525	724.33	3.06	1649	102.65	4.93	0.141717173
144	33	4	144	27	5	144	22	4	796	705.09	2.71	1281	98.02	5.29	0.139017714
144	33	4	144	27	5	144	22	4	1250	715.34	3.05	1201	100.57	4.89	0.140590488
144	33	4	144	27	5	144	22	4	720	717.66	2.86	1453	100.77	4.90	0.140414681
144	33	4	144	27	5	144	22	4	916	695.43	2.59	1419	124.98	5.03	0.179716147
144	37	4	144	77	4	144	2	5	1311	709.32	3.40	2242	99.62	5.08	0.140444369
144	37	4	144	77	4	144	2	5	1758	728.62	3.38	1556	101.57	4.89	0.139400511
144	37	4	144	30	4	144	99	5	1183	729.66	2.71	1873	102.65	4.65	0.140681961
144	37	4	144	30	4	144	99	5	865	722.24	3.40	910	104.79	5.28	0.145090275
144	37	4	144	30	4	144	99	5	1510	710.34	2.89	1737	125.11	4.72	0.176126925
144	37	4	144	50	4	144	10	5	1330	734.10	3.06	468	101.08	3.92	0.137692412
144	37	4	144	50	4	144	10	5	784	709.85	2.65	1474	117.20	4.35	0.165105304
144	37	4	144	50	4	144	10	5	664	716.66	3.12	1736	99.06	5.35	0.138224542
144	37	4	144	50	4	144	10	5	448	720.84	3.04	688	99.21	3.87	0.137631097
144	37	4	144	2	5	144	77	4	1236	689.20	4.28	2319	96.57	5.60	0.140118979

144	37	4	144	2	5	144	77	4	716	712.56	2.95	1307	100.87	4.79	0.141560009
144	37	4	144	2	5	144	77	4	1108	683.06	3.45	1367	95.85	6.61	0.140324422
144	37	4	144	2	5	144	77	4	1192	687.85	3.55	1497	96.58	4.74	0.140408519
144	37	4	144	99	5	144	30	4	617	712.90	3.31	755	104.66	5.18	0.146808809
144	37	4	144	99	5	144	30	4	728	674.75	3.31	1233	92.72	4.37	0.137413857
144	37	4	144	99	5	144	30	4	664	732.66	2.84	1275	102.19	5.27	0.139478066
144	37	4	144	10	5	144	50	4	513	686.32	3.37	2220	95.29	4.73	0.138841940
144	37	4	144	10	5	144	50	4	466	705.16	3.33	807	97.00	4.50	0.137557434
144	37	4	144	10	5	144	50	4	1027	697.76	3.13	1978	98.68	4.72	0.141423985
144	37	4	144	10	5	144	50	4	1180	701.92	3.21	1123	98.69	4.74	0.140600068
144	47	4	144	9	4	144	27	5	1267	690.04	2.90	1379	95.37	4.95	0.138209379
144	47	4	144	9	4	144	27	5	1025	725.21	2.81	1442	101.36	4.98	0.139766412
144	47	4	144	9	4	144	27	5	1121	695.71	2.94	1270	96.60	4.88	0.138850958
144	47	4	144	9	4	144	27	5	883	709.15	3.11	1267	98.43	5.66	0.138799972
144	47	4	144	9	4	144	27	5	1236	718.98	3.09	1199	101.35	5.94	0.140963587
144	47	4	144	9	4	144	27	5	344	713.62	2.45	1105	100.16	3.71	0.140354811
144	47	4	144	18	4	144	15	5	1747	689.14	3.03	1815	97.30	4.65	0.141190469
144	47	4	144	18	4	144	15	5	806	706.61	2.73	1544	100.34	5.11	0.142001953
144	47	4	144	18	4	144	15	5	1482	700.69	3.14	656	96.72	5.28	0.138035365
144	47	4	144	30	4	144	69	5	225	699.28	3.05	711	96.85	4.48	0.138499600
144	47	4	144	30	4	144	69	5	994	703.01	2.80	1540	99.62	6.03	0.141704954
144	47	4	144	30	4	144	69	5	487	719.02	2.91	861	100.77	5.22	0.140149092
144	47	4	144	30	4	144	69	5	1573	715.60	2.89	1076	97.59	4.98	0.136375070
144	47	4	144	27	5	144	9	4	1503	701.83	3.31	1187	98.14	5.14	0.139834433
144	47	4	144	27	5	144	9	4	454	724.37	2.78	182	98.26	4.01	0.135648909
144	47	4	144	27	5	144	9	4	952	722.64	2.73	1976	101.85	5.06	0.140941548
144	47	4	144	27	5	144	9	4	775	733.63	3.91	1017	102.94	4.53	0.140315963
144	47	4	144	15	5	144	18	4	1420	698.01	3.49	1469	97.83	5.30	0.140155585
144	47	4	144	15	5	144	18	4	1525	723.05	2.77	1755	100.99	4.47	0.139672222
144	47	4	144	15	5	144	18	4	1615	702.97	2.98	1424	100.35	5.14	0.142751469
144	47	4	144	15	5	144	18	4	1404	728.24	2.67	1166	102.74	4.27	0.141079864
144	47	4	144	15	5	144	18	4	460	741.68	3.26	708	105.90	4.01	0.142783950
144	47	4	144	15	5	144	18	4	1822	720.44	3.44	1094	102.96	4.84	0.142912664
144	47	4	144	15	5	144	18	4	1266	729.37	3.41	1066	101.97	5.22	0.139805586
144	49	4	144	50	4	144	96	5	813	718.34	2.60	1360	99.49	5.41	0.138499875
144	49	4	144	50	4	144	96	5	424	695.94	2.92	814	97.26	5.46	0.139753427
144	49	4	144	50	4	144	96	5	417	706.29	3.28	996	100.41	5.21	0.142165399

144	49	4	144	50	4	144	96	5	479	706.17	2.92	956	100.61	5.01	0.142472776
144	49	4	144	37	4	144	100	5	802	714.11	2.57	611	99.71	5.33	0.139628349
144	49	4	144	47	4	144	15	5	1756	701.53	2.99	1168	98.54	5.01	0.140464413
144	49	4	144	47	4	144	15	5	1710	701.80	2.76	1223	98.33	5.45	0.140111143
144	49	4	144	96	5	144	50	4	1548	722.38	2.74	1620	100.99	4.97	0.139801766
144	49	4	144	96	5	144	50	4	828	716.37	2.53	1432	99.91	6.34	0.139467035
144	49	4	144	96	5	144	50	4	236	718.94	2.43	557	99.75	5.42	0.138745932
144	49	4	144	100	5	144	37	4	1893	681.55	2.98	1222	93.26	5.36	0.136835155
144	49	4	144	100	5	144	37	4	1701	699.15	2.76	1859	99.55	6.33	0.142387184
144	49	4	144	100	5	144	37	4	2139	716.24	2.45	1095	99.13	4.44	0.138403328
144	49	4	144	15	5	144	47	4	1046	707.31	2.80	495	98.45	4.34	0.139189323
144	49	4	144	15	5	144	47	4	1852	712.32	2.58	1061	98.51	4.24	0.138294587
144	49	4	144	15	5	144	47	4	1114	712.55	2.45	1193	100.24	4.56	0.140677847
144	50	4	144	77	4	144	100	5	902	711.89	2.51	1206	100.19	3.81	0.140738035
144	50	4	144	77	4	144	100	5	852	725.64	2.51	731	100.21	4.61	0.138098782
144	50	4	144	77	4	144	100	5	623	735.59	2.75	1042	101.15	4.63	0.137508667
144	50	4	144	77	4	144	100	5	417	720.77	2.13	980	100.90	4.23	0.139989178
144	50	4	144	77	4	144	100	5	696	724.14	2.36	1596	102.86	4.79	0.142044356
144	50	4	144	37	4	144	35	5	500	693.52	2.61	1832	112.12	4.79	0.161668012
144	50	4	144	37	4	144	35	5	1320	708.30	2.41	2090	99.46	5.11	0.140420726
144	50	4	144	37	4	144	35	5	882	737.66	2.60	940	100.96	5.08	0.136865222
144	50	4	144	37	4	144	35	5	702	717.57	2.99	1491	100.61	4.88	0.140209318
144	50	4	144	49	4	144	25	5	1006	742.51	3.04	1248	102.54	5.70	0.138099150
144	50	4	144	49	4	144	25	5	669	714.78	2.50	1712	104.49	4.56	0.146184840
144	50	4	144	49	4	144	25	5	1296	753.81	2.63	1401	105.37	4.85	0.139783235
144	50	4	144	100	5	144	77	4	645	712.54	2.51	2306	100.76	5.11	0.141409605
144	50	4	144	100	5	144	77	4	564	737.97	3.05	1164	103.32	5.32	0.140005691
144	50	4	144	35	5	144	37	4	695	737.89	3.05	1971	120.18	5.49	0.162869804
144	50	4	144	35	5	144	37	4	493	740.60	3.37	626	101.71	5.32	0.137334594
144	50	4	144	25	5	144	49	4	604	714.07	2.85	1990	99.69	5.69	0.139608162
144	50	4	144	25	5	144	49	4	659	716.03	2.77	1592	98.83	5.31	0.138024943
144	50	4	144	25	5	144	49	4	504	727.28	2.74	1812	102.43	5.19	0.140839842
144	50	4	144	25	5	144	49	4	1040	711.34	3.16	1034	97.50	5.09	0.137065257
144	51	4	144	4	4	144	26	5	1360	731.38	3.33	777	101.63	4.87	0.138956493
144	51	4	144	6	4	144	15	5	591	729.18	3.84	915	99.94	4.95	0.137058065
144	51	4	144	6	4	144	15	5	878	748.54	3.74	1085	100.63	5.50	0.134435034
144	51	4	144	6	4	144	15	5	598	744.77	2.71	1714	103.08	4.84	0.138405145

144	51	4	144	6	4	144	15	5	738	780.42	3.26	649	106.00	4.74	0.135824300
144	51	4	144	26	5	144	4	4	464	754.09	2.89	1755	104.22	4.94	0.138206315
144	51	4	144	26	5	144	4	4	800	774.07	3.58	1220	106.82	4.70	0.137997855
144	51	4	144	26	5	144	4	4	872	721.46	3.08	578	98.07	5.38	0.135932692
144	51	4	144	97	5	144	18	4	370	755.90	2.72	818	103.49	5.28	0.136909644
144	51	4	144	97	5	144	18	4	573	696.09	2.67	1574	96.77	6.50	0.139019380
144	51	4	144	97	5	144	18	4	398	711.21	2.94	653	96.32	5.71	0.135431167
144	56	4	144	28	4	144	97	5	178	723.16	2.86	2760	102.63	5.24	0.141918801
144	56	4	144	28	4	144	97	5	562	721.62	4.14	602	96.04	6.39	0.133089438
144	56	4	144	28	4	144	97	5	162	753.46	2.85	1125	103.91	5.19	0.137910440
144	56	4	144	28	4	144	97	5	537	709.81	3.37	1180	97.98	4.75	0.138036939
144	56	4	144	29	4	144	2	5	594	699.81	3.27	1248	96.66	5.00	0.138123205
144	56	4	144	47	4	144	99	5	1193	730.86	2.73	1340	100.33	5.44	0.137276633
144	56	4	144	97	5	144	28	4	788	721.20	2.92	1456	100.07	5.82	0.138754853
144	56	4	144	97	5	144	28	4	1199	712.47	2.54	1336	98.23	5.46	0.137872472
144	56	4	144	97	5	144	28	4	645	727.31	2.38	829	98.62	4.12	0.135595551
144	56	4	144	2	5	144	29	4	1944	719.52	2.70	1024	100.03	4.79	0.139023238
144	56	4	144	2	5	144	29	4	1834	718.72	2.62	1102	99.96	4.62	0.139080588
144	56	4	144	2	5	144	29	4	1273	745.88	2.78	1461	103.90	5.24	0.139298547
144	56	4	144	2	5	144	29	4	1375	736.30	2.93	562	100.52	4.69	0.136520440
144	56	4	144	2	5	144	29	4	1396	698.63	3.27	1130	96.09	5.93	0.137540615
144	56	4	144	99	5	144	47	4	994	741.68	2.59	1348	101.91	4.46	0.137404271
144	56	4	144	99	5	144	47	4	1294	694.67	2.75	981	93.66	5.05	0.134826608
144	56	4	144	99	5	144	47	4	2512	748.17	2.67	1054	102.87	4.83	0.137495489
144	56	4	144	99	5	144	47	4	1216	720.37	2.61	1143	98.35	5.75	0.136527062
144	57	4	144	77	4	144	27	5	1445	736.35	2.91	1806	101.93	5.33	0.138426020
144	57	4	144	77	4	144	27	5	914	711.67	2.79	1133	97.24	5.38	0.136636362
144	57	4	144	77	4	144	27	5	1969	727.32	2.50	1347	101.07	3.88	0.138962217
144	57	4	144	77	4	144	27	5	756	717.27	2.67	690	96.03	4.52	0.133882638
144	57	4	144	9	4	144	5	5	789	739.60	3.29	1293	102.63	5.68	0.138764197
144	57	4	144	9	4	144	5	5	627	703.28	2.60	1700	95.22	5.69	0.135394153
144	57	4	144	9	4	144	5	5	801	725.28	2.69	900	102.46	4.43	0.141269579
144	57	4	144	9	4	144	5	5	1104	722.19	2.85	1050	97.76	5.48	0.135366039
144	57	4	144	27	5	144	77	4	1148	728.31	3.15	1325	100.85	4.96	0.138471255
144	57	4	144	5	5	144	9	4	583	699.80	2.46	1460	98.13	5.07	0.140225779
144	57	4	144	5	5	144	9	4	694	724.05	2.65	669	99.02	4.83	0.136758511
144	57	4	144	5	5	144	9	4	807	727.06	2.72	1669	101.26	5.00	0.139273237

144	58	4	144	21	4	144	100	5	919	721.95	2.91	1843	101.08	5.30	0.140009696
144	58	4	144	21	4	144	100	5	943	710.45	2.34	1057	96.81	4.97	0.136265747
144	58	4	144	21	4	144	100	5	1146	707.15	2.94	1644	96.24	4.98	0.136095595
144	58	4	144	21	4	144	100	5	878	700.46	2.87	851	96.77	5.75	0.138152071
144	58	4	144	29	4	144	96	5	915	737.09	2.95	842	122.87	4.34	0.166696062
144	58	4	144	29	4	144	96	5	997	698.67	2.72	1498	94.97	5.07	0.135929695
144	58	4	144	29	4	144	96	5	681	686.25	3.07	879	92.75	5.85	0.135154827
144	58	4	144	100	5	144	21	4	952	693.66	2.91	1683	92.63	5.21	0.133538045
144	58	4	144	100	5	144	21	4	1164	699.08	2.96	1357	97.51	4.78	0.139483321
144	58	4	144	100	5	144	21	4	1252	717.56	3.05	1032	100.34	4.49	0.139834996
144	58	4	144	100	5	144	21	4	563	707.48	3.94	830	94.96	5.73	0.134222876
144	58	4	144	100	5	144	21	4	582	705.22	3.26	1138	93.90	5.64	0.133149939
144	58	4	144	100	5	144	21	4	379	697.34	2.95	1416	94.19	6.13	0.13507041
144	60	4	144	9	4	144	35	5	1207	712.34	5.75	1275	102.75	5.62	0.144242918
144	60	4	144	9	4	144	35	5	540	710.22	3.35	1459	95.99	6.40	0.135155304
144	60	4	144	9	4	144	35	5	1205	724.33	3.39	1795	120.96	5.66	0.166995706
144	60	4	144	9	4	144	35	5	768	696.06	3.46	1278	93.55	6.28	0.134399333
144	60	4	144	22	4	144	25	5	2334	723.07	2.81	849	98.20	5.49	0.135809811
144	60	4	144	22	4	144	25	5	425	698.28	2.68	782	94.29	5.98	0.135031792
144	60	4	144	22	4	144	25	5	1561	708.35	2.56	1597	97.79	4.97	0.138053222
144	60	4	144	22	4	144	25	5	753	703.84	2.69	1660	97.13	6.37	0.138000114
144	60	4	144	47	4	144	97	5	669	728.10	3.17	535	101.21	5.40	0.139005631
144	60	4	144	47	4	144	97	5	845	710.17	3.46	1034	97.44	5.79	0.137206584
144	60	4	144	47	4	144	97	5	696	717.74	2.40	343	100.75	5.17	0.140371165
144	60	4	144	47	4	144	97	5	608	711.17	2.91	785	97.04	5.78	0.1364512
144	60	4	144	35	5	144	9	4	955	708.16	2.40	1708	98.09	5.33	0.138513895
144	60	4	144	35	5	144	9	4	634	707.87	2.76	1094	97.08	5.40	0.137143826
144	60	4	144	25	5	144	22	4	1361	716.63	3.01	769	98.73	5.04	0.137769839
144	60	4	144	25	5	144	22	4	358	716.32	3.48	999	96.87	6.18	0.135232857
144	60	4	144	25	5	144	22	4	1363	700.28	2.65	1619	95.24	4.63	0.136002742
144	60	4	144	97	5	144	47	4	2006	714.31	2.87	1295	98.48	5.05	0.137867313
144	60	4	144	97	5	144	47	4	313	699.04	3.12	256	93.17	5.17	0.133282788
144	60	4	144	97	5	144	47	4	977	721.30	3.38	807	100.19	5.36	0.138901983
144	60	4	144	97	5	144	47	4	250	707.15	4.14	798	98.54	5.57	0.139348087