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EVOLUTIONARY IMPLICATIONS OF SELF-COMPATIBILITY AND REPRODUCTIVE FITNESS IN THE APOMICTIC *RANUNCULUS AURICOMUS* POLYPLOID COMPLEX (RANUNCULACEAE)

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Abstract

Uniparental reproduction has often been regarded as advantageous for colonization. In pseudogamous apomicts, reproduction via single individuals requires self-pollination and consequently self-compatibility (SC) for production of viable seeds. SC and reproductive fitness have been studied in diploid and polyploid taxa of the *Ranunculus auricomus* complex via pollinator exclusion tests, assessment of seed set, and germination rates. Reproductive fitness of sexuals exceeds that of apomicts and of F₁ hybrids but may fluctuate more strongly between years than is the case in apomicts. Diploid sexual taxa and also their F₁ hybrids are completely self-incompatible (SI). Auto-polyploid sexual cytotypes are also predominantly SI, which may have restricted their range expansion. The observed breakdown of SI in the rather widespread allohexaploid apomicts may be explained by initial partial SC inherited from semi-self-compatible ancestors and strong selection for SC genotypes. It is concluded that higher reproductive fitness of sexuals may help to maintain sexual populations when cross-pollination is available, whereas SC in apomicts may be advantageous in temporally and spatially unstable environments and also for colonization events. Results suggest that SC in connection with pseudogamous apomixis is an important factor for the observed distribution pattern of geographical parthenogenesis.

Keywords

apomixis; fitness; geographical parthenogenesis; polyploidy; self-compatibility

Introduction

Apomixis, a mode of reproduction via asexually formed seed (Asker and Jerling 1992), has often been thought to be advantageous for colonization because of the potential for uniparental reproduction (Stebbins 1950). Especially after long-distance dispersal, the ability to found a new population with a single individual has been regarded as a main factor for the wide distributions of apomictic biotypes (an expansion of Baker's law; Baker 1967). Baker's law more generally invokes the importance of reproductive assurance in selecting for self-fertilization. In a metapopulation model, selection for reproductive assurance is highest when colony occupancy rate is low (Pannell and Barrett 1998). Beyond the colonization phase, uniparental reproduction might alleviate reduced fecundity resulting from pollen limitation in small founder populations and compensate for lack of suitable pollinators in the

colonized region (Rambuda and Johnson 2004). Indeed, similar to selfing, apomixis has been frequently reported for invasive plants (Carino and Daehler 1999; Brock 2004; Rambuda and Johnson 2004; Fehrer et al. 2007). Contrary to selfing, apomixis functions without meiosis and fertilization of egg cells, thus resulting in offspring with genotypes identical to that of the mother plant. Consequently, apomixis infers no increase in homozygosity or inbreeding depression in the offspring. Maintenance of heterozygosity, which is usually higher in allopolyploid apomicts than in related sexual species (Gornall 1999; Hörandl and Paun 2007), is probably an important advantage to apomixis in bottleneck situations or founder events. The advantages of uniparental reproduction are probably one important causal factor for the general phenomenon that asexual organisms often have larger distribution areas than their sexual relatives (geographical parthenogenesis; Van Dijk 2003; Kearney 2005; Hörandl 2006; Hörandl et al. 2008).

Nevertheless, selfing is actually a frequent mode of reproduction in colonizers, and various mechanisms may compensate for any disadvantages when compared with apomixis (see table 1 in Hörandl 2006). In the long term, apomicts are expected to need a longer time to recover genotypic variation after a bottleneck than do selfers because even highly heterozygous genotypes remain fixed; selfers maintain recombination and segregation at heterozygous loci during meiosis, resulting in individual variation (Burt 2000). In sexual selfers, selection against homozygous deleterious alleles may lead to purging effects and a subsequent increase in fitness after inbreeding (Carr and Dudash 2003). Selfing is frequently connected to polyploidy, which can cause a breakdown of self-incompatibility (SI), especially in gametophytic SI systems (Mable 2004; Barringer 2007). Theoretically, polyploidy may reduce rates of homozygote formation (Richards 1997) and can buffer inbreeding depression because of masking deleterious mutations by multiple copies of the genome (Lande and Schemske 1985). In autotetraploids, deleterious mutations expressed in homozygotes are more efficiently purged by selection, and inbreeding depression is in general lower than in diploids for a given selfing rate (Ronfort 1999). Under the theoretical assumption of relaxed inbreeding depression, self-compatible (SC) sexual and apomictic polyploids would have rather equal advantages. Against SI sexual polyploids, apomicts would still have the advantage of uniparental reproduction. Compared with diploid, SI sexuals, apomixis infers an even greater advantage, that is, both uniparental reproduction and avoidance of inbreeding depression in small founder populations. Actual advantages of apomixis versus selfing have to be studied in the context of polyploidy.

In most apomictic plants, production of viable seed depends on the presence of pollen; that is, fertilization of the primary endosperm nuclei is required for endosperm development (pseudogamy). Sexual diploid relatives of apomicts are generally SI (Asker and Jerling 1992); exceptions have been reported in some early diverging sexual species of *Taraxacum* (Hughes and Richards 1988). This implies that pseudogamous apomixis might be connected to a breakdown of SI to allow for uniparental reproduction and reproductive assurance without pollinators. Mathematical modeling on resource allocation to the male and female functions in pseudogamous apomicts predicts a selection for male sterility in the case of SI and therefore the maintenance of SC genotypes (Noirot et al. 1997). SC of apomicts has been frequently observed in Rosaceae (Dickinson et al. 2007) but has been studied only for few apomictic complexes of other plant families (Noirot et al. 1997).

SI of sexual species may also break down if pollen of another related species is present on the stigma (Tas and Van Dijk 1999; Mraz 2003; Brock 2004). This so-called mentor effect (Richards 1997) represents a special case of induced selfing depending on cross-pollination and will occur only when sexuals and apomicts are in the same vicinity. Here I focus on the comparison of reproductive systems in spatial isolation of sexuals and apomicts.

Fitness parameters can be also influenced by apomixis. The reproductive success of sexuals usually differs quantitatively from that of related apomicts (Asker and Jerling 1992). Pseudogamous apomicts usually have a higher percentage of aborted fruits and malformed pollen than do related sexuals (Asker and Jerling 1992), which was studied in more detail in, for example, *Ranunculus auricomus* (Izmailow 1996; Hörandl et al. 1997; Lohwasser 2001) and *Boechea* (Voigt et al. 2007). Malformed pollen of apomicts is a result of disturbed meiosis, usually referred to as the hybrid origin of polyploids (Asker and Jerling 1992). In autonomous apomicts, seed set of apomicts exceeds that of sexuals (Michaels and Bazzaz 1986; Van Dijk 2007), suggesting that differences in the pollination system have an influence on fitness and on actual advantages of apomixis.

The actual levels of fitness under the conditions of SI or SC systems are best studied in the comparison of closely related sexual and apomictic taxa. The Eurasian *R. auricomus* complex provides an appropriate model system for study. This predominantly apomictic group comprises two very closely related sexual species, *Ranunculus cassubicifolius* and *Ranunculus carpaticola*, and the more distant diploid *Ranunculus notabilis* (Hörandl 2004; Hörandl et al. 2005; Paun et al. 2005; table A1 in the online edition of the *International Journal of Plant Sciences*). *Ranunculus cassubicifolius* is exclusively sexual and has diploid and autotetraploid cytotypes with a disjunct distribution in the Alps and Prealps (Nogler 1984; Hörandl et al. 1997; Hörandl and Greilhuber 2002; Paun et al. 2006a), which allows an assessment of effects of genome doubling without hybridization on the reproductive system. *Ranunculus carpaticola* comprises diploid sexual and hexaploid apomictic cytotypes and is widespread in the Carpathians (Hörandl and Greilhuber 2002; Paun et al. 2006a, 2006b). Within Central Slovakia, the apomictic cytotypes of *R. carpaticola* are more widely distributed than the diploid sexual ones (maps in Paun et al. 2006a, 2006b). The apomicts are of allopolyploid origin, with autotetraploid *R. cassubicifolius* and diploid *R. carpaticola* as likely parents (Hörandl and Greilhuber 2002; Paun et al. 2006a). Since the apomicts probably originated from a single event (Paun et al. 2006a, 2006b), the wide distribution of apomicts is rather referable to dispersal and not to multiple origins. The apomictic populations consist of a single clone or a few genotypes and may have been founded by single genotypes (Paun et al. 2006b). Thus, *R. carpaticola* is an interesting model system for studying the influence of reproductive biology on colonization abilities, with *R. cassubicifolius* as a close relative for comparison of sexual diploid and polyploid cytotypes. The third sexual species, *R. notabilis*, is exclusively diploid, distributed in southeastern Austria, and both morphologically and genetically distinct (Hörandl et al. 2000; Hörandl 2004; Paun et al. 2005).

All species of the complex have flowers arranged in cymes and the typical bowl-shaped flowers of buttercups, with glossy yellow petals and a nectary on the base of the petals. Insect pollination by bees, bumblebees, beetles, and flies has been observed for other species of the *R. auricomus* complex and other species of the genus (Steinbach and Gottsberger 1994). Apomicts often have partly aborted petals and lower frequencies of pollinator visits (Steinbach and Gottsberger 1994). Previous studies on pollen stainability have shown that all the sexual cytotypes have good pollen quality (~80%–100% of stainable pollen), whereas that of the apomicts is partly aborted; the percentage of stainable pollen ranges from 20% to 90%, but the majority of measures are below 50% (Izmailow 1996; Hörandl et al. 1997; Lohwasser 2001). Pollen germination rates range from 28.0% to 48.3% in the apomicts, compared with 95.9% in the diploid sexuals (Izmailow 1996). *Ranunculus* forms single-seeded achenes, with all achenes of a mature flower forming a collective fruit until they separate and drop down at maturity.

Apomictic taxa of the *R. auricomus* complex are aposporous and pseudogamous; that is, fertilization of endosperm nuclei by pollen nuclei is necessary for endosperm development

and formation of fertile achenes (Rutishauser 1954; Izmailow 1967). Apomixis in the *R. auricomus* complex is a heritable, genetically controlled trait (Nogler 1984). Apomicts often have partly aborted achenes; Lohwasser (2001) reports a range of 20%–50% good achenes, and Izmailow (1996) reports a mean value of 35.2%. Partly aborted pollen and reduced seed set suggest higher reproductive success of diploid sexuals compared with polyploid apomicts, but previous studies lacked a quantitative statistical analysis to support actual differences in fitness between sexuals and apomicts. The complex as a whole shows geographical parthenogenesis, with apomictic cytotypes distributed abundantly all over Europe and sexual taxa restricted to comparatively small distribution areas in Central and Eastern Europe; moreover, apomicts cover a broader spectrum of habitats than do sexual species (Hörandl and Paun 2007).

The observed differences in seed set of sexual and apomictic cytotypes raise the question of the extent to which fitness is depressed in pseudogamous apomicts. The ability of uniparental reproduction remains unexplored. The main aim of this study is to experimentally assess differences in reproductive success and germination rates and the potential for uniparental reproduction as a potential advantage to apomixis. Furthermore, effects of genome doubling (autopolyploidy) or hybridization on SI systems of sexuals are being tested. This approach should help to disentangle the effects of polyploidy and genome constitution from the effects of apomixis in the hypothetical framework. The following questions are asked: (1) Do sexuals have quantitatively a higher fitness than do apomicts after open pollination? (2) Are diploid and tetraploid sexual cytotypes SC, and does hybridization of diploid sexuals influence SI systems? (3) Can allopolyploid apomicts reproduce via self-pollination, and how can the breakdown of SI in apomicts be explained? (4) What conclusions can be drawn for colonization abilities of apomicts versus sexuals? Answers to these questions come from experimental work on cultivated, wild-collected plants and from plants produced in experimental crosses.

Material and Methods

Plants were collected from natural populations (see table A1; Hörandl et al. 2000; Hörandl and Greilhuber 2002; Paun et al. 2006a, 2006b) and cultivated in the experimental garden of the Botanical Garden of the University of Vienna in a half-shaded area under a large tree, resembling the natural forest habitats of the plants. The plants flowered regularly over all years between March and April. Flowers were regularly visited by insects (bees, bumblebees, beetles, and flies; E. Hörandl, personal observation), which suggests that sufficient pollinators were available in the experimental garden. Plants were selected to represent different ploidy levels, autopolyploid versus allopolyploid origin, and sexual versus apomictic reproduction, according to information from earlier studies (Hörandl et al. 1997, 2000; Hörandl and Greilhuber 2002; Paun et al. 2006a, 2006b). For the apomicts, plants of clonal lineages were used, as assessed by isozyme and amplified fragment length polymorphism (AFLP) studies (Hörandl and Greilhuber 2002; Paun et al. 2006a).

The necessity of pollen for seed set in apomicts (pseudogamy), as reported by Rutishauser (1954) and Izmailow (1967), was confirmed by emasculation and bagging of each two flowers on five hexaploid individuals, thus excluding any pollen, which led to complete seed abortion. For assessment of SI, flowers were bagged before anthesis with cellophane bags and kept bagged until maturity of achenes. Control plants from the same population were left untreated for open pollination. In the flowers, mature stamens are in close vicinity of the carpels during anthesis, thus allowing spontaneous self-pollination. On two diploid sexual individuals, self-pollination was performed by hand on each three bagged flowers but resulted in the failure of seed set, as in bagged-only flowers of the same individual, indicating the SI is not due to spatial distance of anthers and stigmas. For studying effects of

hybridization on SC, experimentally produced F₁ hybrids between *Ranunculus notabilis* and *Ranunculus carpaticola* were studied. To test the effects of polyploidy on SI and fitness in a sexual system, randomly collected plants from natural populations of autotetraploid *R. cassubicifolius* were used. A potential influence of genetic diversity on expression of SI was studied in offspring of experimental crosses of the genetically most distinct individuals from the same wild population (parental plants selected after genetic distance of AFLPs of the data set of Paun et al. 2006a).

Seed set was assessed as the total number and as the percentage of well-developed achenes of the total number of pistils per flower. Good versus aborted achenes are easily recognized by color (turning from dark green to brown in the former; turning to yellow in the latter), endosperm development (full, hard achene bodies in the former; empty achene bodies in the latter), size (2–3 mm length of achene body in the former; dwarfed, ~1 mm length of achene body in the latter), and ease of removal from the receptaculum (falling off in the former; sticking to the receptaculum in the latter). In addition to the total number of well-developed seed, the percentage of well-developed achenes per collective fruit was used as the main unit for comparison of fitness; the number of flowers per plant varies under environmental conditions such as light and water support (Lohwasser 2001) and depends on the general vigor of the plants (E. Hörandl, personal observation).

Experiments were conducted over 3 yr (2005–2007) to assess the constancy of observations on SI and to obtain a long-term estimate of fitness for reproductive systems and ploidy levels, which is especially important for perennial plants (individuals collected as adults still lived 10–15 yr in cultivation; E. Hörandl, personal observation). The effects of homoploid hybridization—that is, of crosses of the same ploidy level—and intrapopulational cross-fertilization on fitness and SI could be tested only in 2007, when the experimentally produced plants flowered for the first time. Germination was tested with seeds from the 2005 season. Achenes were stored in a refrigerator for a few days and then sown on a moist soil mixture of humus and needle soil, which produces high germination rates in the *R. auricomus* complex (Lohwasser 2001; E. Hörandl, personal observation from previous cultivation experiments). Plants were kept outside over the winter and covered with snow to avoid damage due to frost. Germination began in the fall of 2005 and continued, with a break in January and February, until March 2006. Germination rates were recorded monthly and revealed no loss of seedlings during the winter. The final number of seedlings was assessed in June 2006.

Statistical evaluations were performed with the program SPSS 11.0 for Windows. To test for a potential influence of seasonal fluctuations, differences in seed set were calculated for open-pollinated sexuals versus apomicts and for bagged versus open pollination for all years (table 1). Box plots showing medians and interquartile ranges were produced for summaries of results for all 3 yr. Levene's statistics were used to test for homogeneity of variances. Levels of significance were tested using one-way ANOVAs after arcsine transformation of percentages and Scheffe post hoc tests for all cases where the assumption of equal variances was met.

Results

Reproductive Fitness after Open Pollination

Flowers of the studied plants produced between 12 and 98 pistils (mean = 38.6). After open pollination, the total number of well-developed achenes was highest in diploid and tetraploid sexuals (pooled), lowest in homoploid hybrids, and intermediate in apomicts (fig. 1); differences between these three main groups were all highly significant ($P < 0.001$). The number of well-developed achenes was significantly higher in diploids than in tetraploids (P

= 0:019) and in hexaploids ($P < 0:001$). Differences between tetraploids and hexaploids ($P = 0:407$) were not significant (fig. 2a). The percentage of good achenes per collective fruit ranged in the sexuals from 9 to 96 (mean = 64.6) in the diploids and from 21 to 100 (mean = 62.8) in the tetraploids; these two groups were not significantly different ($P = 0:681$). In the hexaploid apomicts, percentages of good achenes ranged from 3 to 86 (mean = 36.9), which differed significantly ($P < 0:001$) from both diploid and tetraploid sexuals (fig. 2b). The homoploid hybrids had the lowest mean values of percentages of good achenes (table 1). Germination rates of good achenes did not differ significantly between the three ploidy levels and the two reproductive systems, respectively ($P = 0:999$ between 2x and 4x, $P = 0:898$ between 4x and 6x, and $P = 0:876$ between 2x and 6x; fig. 3).

The observation of higher seed set of sexuals (diploids and tetraploids pooled) compared with apomicts was constant over 3 yr ($P = 0:013$ for 2005, $P < 0:001$ for 2006, and $P < 0:001$ for 2007; fig. 4). Sexuals had a significant higher reproductive output in 2006 compared with 2005 and 2007 ($P = 0:001$ and $P < 0:001$, respectively), whereas 2005 versus 2007 was not different ($P = 0:530$). Hexaploid apomicts showed no significant differences in seed set between the 3 yr ($P = 0:600$ between 2005 and 2006, $P = 0:088$ between 2005 and 2007, and $P = 0:796$ between 2006 and 2007; fig. 4).

Self-Compatibility in Sexuals, Apomicts, and Hybrids

The pollinator exclusion experiments revealed that all diploid sexual individuals from all three species are completely SI with zero seed set in all 3 yr (for open controls and test statistics, see table 1). Pistils of the diploids failed to enlarge (~1 mm long), became yellow, remained attached to the receptacle, and failed to germinate (differences of germination rates compared with achenes from open controls are significant; $P < 0:001$). In autopolyploid *Ranunculus cassubicifolius*, all plants collected in the wild also showed complete SI over all 3 yr (table 1), whereas experimentally produced offspring from genetically distinct parents had low amounts of seed set (in six of the seven individuals and in 12 of 20 bagged flowers; percentages of good achenes ranged from 2.9 to 63.0, mean = 10.2). In these individuals, the total number of well-developed achenes reached a maximum of only 16 achenes in a single collective fruit; in another individual, the total number of achenes varied between 0 and 10 good achenes among nine collective fruits. Altogether in 2007, seed set in the autotetraploid sexuals was significantly reduced in comparison to open pollination (see test statistics in table 1), and the majority of flowers produced no achenes (summary in fig. 5a). In the autopolyploids, aborted achenes tended to be of normal size but had a yellow to greenish empty achene body and remained attached to the receptacle. Achenes of this appearance also completely failed to germinate (differences to achenes from open controls are significant; $P = 0:022$).

In contrast, the hexaploid apomicts produced well-developed achenes in all 3 yr; differences in seed set to unbagged control plants were not significant for all years pooled ($P = 0:083$; see fig. 5b) and for each year separately (see table 1), confirming a breakdown of SI in all allopolyploid lineages. Germination rates of achenes from bagged flowers were not significantly different from those of open-pollinated apomictic plants ($P = 0:153$), confirming full fertility after enforced self-pollination. Hybrids of two diploid species, in contrast, had zero seed set after bagging, indicating that hybridization alone has no immediate effect on SI systems. The open control had the lowest percentages of seed set compared with other groups, but the hybrids appeared not to be completely sterile; percentages of well-developed achenes ranged from 0.0 to 53.6 (mean = 21.8; fig. 5c; table 1).

Discussion

Fitness in Sexuals and Apomicts after Open Pollination

The results confirm a higher fitness of sexuals compared with apomicts with respect to seed set, as observed by previous authors (Izmailow 1996; Lohwasser 2001). In the study by Izmailow (1996), reproductive efficiency of diploid sexuals (mean of 53.7%) was higher than that of hexaploid apomicts (mean = 35.2%). In contrast to my results, Izmailow reports higher means of germination rates of diploids compared with apomicts (50.7% vs. 32.0%, respectively). Germination rates can be influenced by soil composition (Lohwasser 2001) and might be reduced by small insects feeding on the achenes (E. Hörandl, personal observation).

A high proportion of aborted seed is not a common feature of apomicts. In fact, a higher fecundity of apomicts compared with sexual relatives was reported for *Taraxacum* (Van Dijk 2007) and *Antennaria* (Michaels and Bazzaz 1986). In contrast to *Ranunculus*, these species reproduce via autonomous endosperm development and are thus not dependent on pollination. The results on pseudogamous *Ranunculus* raise the question as to whether pollen quality has an effect on successful seed set; the observed high proportions of aborted achenes may be caused not only by developmental disturbances (see Izmailow 1966) but also by low quantities of good pollen of apomicts. An effect of pollen quality on self seed set has also been suggested for *Ranunculus repens* (Lundqvist 1998). Quantitative pollen limitation affected achene mass and number of progeny in *Ranunculus acris* (Hegland and Totland 2007). Since one pollen tube is required for each ovule, pollen quality may cause the high variance of seed set in apomicts. Morphological mechanisms promoting active self-pollination are lacking in *Ranunculus*. In apomictic blackberries (*Rubus* subg. *Rubus*), successful seed set is quantitatively much higher in species with a type of stamens arching over the stigmas during senescence, thus suggesting active self-pollination (Nybom 1986). Availability of functional pollen may actually strongly limit seed set in pseudogamous apomicts.

Though I found no differences between sexuals and apomicts in terms of germination rates, sexuals are expected to have higher total offspring under the conditions of cross-pollination because of higher total seed set. Therefore, sexual populations should outperform apomicts if they are of sufficient population size and regularly visited by pollinators (all other conditions being equal). In *Ranunculus*, this may be an important factor that allows sexual species to persist in their natural habitats. In consideration of the observations in *Ranunculus*, it remains questionable whether fitness parameters alone can provide a general explanation for higher abundance and larger distribution areas of apomicts. Comprehensive studies on wild populations are required to address this question.

Seed set fluctuated more over the years in the sexuals than in the apomicts, suggesting a higher sensitivity to changing climatic conditions or fluctuating pollinator frequencies compared with apomicts. Similar observations have been made in long-term studies on dandelions in natural mixed populations (Van Dijk 2007). Complete independence from pollination because of autonomous endosperm development may be an important additional advantage of apomictic *Taraxaca*. Reproductive assurance in temporally variable environments might be another advantage of apomixis that needs further long-term studies on natural populations. In my study system, fluctuations of seed set in sexuals are too weak to infer an actual fitness advantage of apomixis.

Self-Incompatibility Systems in Diploid and Tetraploid Sexual Taxa

Results confirm SI in all three diploid sexual species of the *Ranunculus auricomus* complex, as was also observed in diploid sexual relatives of most other agamic complexes (Asker and

Jerling 1992). In *Ranunculus*, flowers are predominantly homogamous, and self-pollination is expected to occur both within and between flowers of the same individuals; thus, SI systems rely on pollen recognition. SI systems in *Ranunculus* are of the gametophytic type, as can be expected from binucleate pollen, wet stigmas, and pollen tube behavior (Rendle and Murray 1988). In *R. acris*, *Ranunculus polyanthemos*, and *R. repens*, complex four-locus gametophytic SI systems have been described (Lundqvist 1973, 1990, 1994, 1998; Osterbye 1977). Lundqvist (1994, 1998) suggested that in the allotetraploid species *R. repens*, a four-locus system is acting, which originated from two diploid parents with different two-locus systems.

My data set confirms that polyploidy in sexual taxa is not necessarily correlated with a breakdown of SI, as discussed by Mable (2004) and Barringer (2007). The observations in *R. auricomus* suggest that the mode of origin of polyploidy (autopolyploid vs. allopolyploidy) is important for this phenomenon. Autopolyploid individuals from a natural population of *Ranunculus cassubicifolius* appeared to be completely SI, and offspring from crosses of genetically distinct individuals were the only ones to show low to medium percentages of normal achenes. Newly arisen autotetraploids are expected to be partially homozygous for *S* alleles and to produce both homozygous and heterozygous diploid pollen. Dominance effects among alleles in heterozygous diploid pollen could result in SC in unilocus systems (de Nettancourt 2001). Newly arisen autotetraploids can be 50% semicompatible because of partial formation of pollen heterozygous for *S* alleles (reviewed in Richards 1997). The observed low frequencies of normal achenes in some individuals of tetraploid *R. cassubicifolius* do not meet these expectations. Autotetraploid *R. cassubicifolius* is probably not of recent origin, as inferred from biogeographical patterns: *R. cassubicifolius* as a whole occurs in disjunct and geographically isolated areas, located between the glacier tongues of the Würm glaciations, whereas autotetraploids occur only in the areas that did not experience glaciation near the eastern borders of the ice shield, in strong geographical isolation from the diploids (see map and discussion in Paun et al. 2006a). Thus, original frequencies of semicompatibility after autopolyploidization might have been reduced by a subsequent loss of diversity in *S* alleles because of geographical isolation. Inbreeding, as indicated by an excess of homozygotes in isozyme profiles in all populations (Hörandl and Greilhuber 2002), might also influence frequencies of SI phenotypes within a population. Further studies are required to test these hypotheses.

In contrast to polyploidy, hybridization alone does not influence SI systems. Even in hybrids of the more distantly related species *Ranunculus carpaticola* and *Ranunculus notabilis*, no breakdown of SI is observed; failure of seed set in these F_1 hybrids after bagging cannot be related to hybrid sterility alone because all open control hybrid plants produced at least low amounts of well-developed achenes.

Alternatively, low frequencies of selfing in otherwise SI polyploids could be explained by pseudo-self-compatibility (Levin 1996), a temporary phenomenon usually associated with environmental effects such as high temperature or timing of pollination (e.g., bud pollination or delayed pollination) and with low heritability (de Nettancourt 2001; Brennan et al. 2005). In the experimental setup, external conditions were equal for all of the plants studied; outside temperatures never reached ranges of 32°–60°C as required for a temperature-induced breakdown of SI (de Nettancourt 2001); timing effects (e.g., in overmature flowers), if present, should have been observed in all plants studied. Pseudo-self-compatibility is therefore a less likely explanation for the observed low amounts of seed set.

Breakdown of Self-Incompatibility in Apomicts

Full SC was observed in allohexaploid apomictic populations of *R. carpaticola*. The constancy of observations over 3 yr (table 1) also speaks against environmental effects on

SI. These hexaploid apomicts most likely originated from an ancient hybridization event of autotetraploid *R. cassubicifolius* and diploid *R. carpaticola* during the Pleistocene (Paun et al. 2006a). The breakdown of SI in the allohexaploid apomicts may be influenced by different S loci in the parents, which is less likely because of the close relationship of the parental taxa (Hörandl 2004).

Another hypothetical pathway to SC might be evolution via original partial SC in the hybrids and subsequent selection for SC genotypes. Partial SC could be inherited from the originally semicompatible autotetraploid parent or caused by the existence of nonfunctional S-RNases or mutational change (de Nettan-court 2001), as it was suggested for partially SC allopolyploid *Prunus* (Hauck et al. 2002). In a newly arisen apomictic pseu-dogamous cytotype, three distinct factors are expected to select for SC genotypes. (1) Hexaploids represent minority cytotypes in the parental population and will have problems receiving pollen from another individual of the same ploidy level. In many angiosperms, a 2 : 1 maternal : paternal ratio in the endosperm is optimal for seed development because of genomic imprinting (Spielman et al. 2003; Vinkenoog et al. 2003). Apomictic plants have developed various modifications of developmental pathways to maintain this ratio in the endosperm (Savidan 2007). In polyploid apomictic *R. auricomus*, but also in many Rosaceae, fertilization of endosperm nuclei with both sperm nuclei maintains the 2 : 1 maternal : paternal ratio in the endosperm (Spielman et al. 2003; Savidan 2007; Talent and Dickinson 2007). Although small deviations from the endosperm balance are sometimes tolerated (e.g., in Rosaceae subtribe Pyrinae; Talent and Dickinson 2007), fertilization of the endosperm with haploid pollen from a sexual parent is expected to result in underdevelopment of seeds (Vinkenoog et al. 2003). (2) The newly arisen apomict will be surrounded by high numbers of offspring of the same maternal genotype, which should have the same SI or SC phenotype. Cross-compatible genotypes allocating pollen to the fertilization of the endosperm, but not the egg cell of another individual, will disappear from the population (Mogie 1992; Noirot et al. 1997). (3) Apomictic *Ranunculi* have reduced petals and therefore fewer flower visits of pollinators than do sexual congeneric species with complete and therefore more attractive flowers (Steinbach and Gottsberger 1994). Since successful self-pollination is advantageous in all these situations and SC apomictic genotypes do not segregate in the offspring, SC will be strongly selected for. Indeed, even individuals from one population with exceptional high clonal diversity (no. 8492; see table A1; Paun et al. 2006b) show complete SC, suggesting that SC established early after the origin of the hexaploid apomicts. Studies on pollen tube behavior in SC species of *Ranunculus* in New Zealand suggest strong pollen tube competition or selection of pollen tubes by the stylar tissue, such that only one pollen tube reaches the micropyle (Rendle and Murray 1988). This mechanism should reinforce selection of SC pollen tubes.

The Implications of Fitness and SC for Geographical Patterns

The observation that autopolyploid sexuals do not differ from diploid cytotypes with respect to fitness and SI challenges the view of a general advantage of polyploidy for colonization, as hypothesized by Bierzychudek (1985). Review articles have shown that sexual polyploidy is not correlated with large distribution areas, indicating that polyploidy alone is not a causal factor for distributional success (Stebbins and Dawe 1987; Hörandl 2006). My model system also confirms that polyploidy is not necessarily connected with large distributions: the autopolyploid cytotypes colonize a rather restricted distribution area of ~50 km diameter in northeastern Austria and remain restricted to riverine forest habitats, similar to the more widespread diploid cytodeme. SI may have restricted range expansions of autopolyploids. In contrast, hexaploid apomictic cytotypes of *R. carpaticola* in Central Slovakia have larger distribution ranges than the diploids and are also known to colonize man-made habitats (Paun et al. 2006a).

For range expansions of apomicts, SC is advantageous because it allows founding a population from a single individual. Theoretically, this ability has a similar advantage as autonomous (pollen-independent) apomixis, but the relation of modes of apomixis to geographical patterns needs further studies (Hörandl et al. 2008). Because of maintenance of high levels of heterozygosity, as shown by isozyme and microsatellite studies (Hörandl and Greilhuber 2002; Paun and Hörandl 2006), inbreeding depression can be avoided in small colonizing populations. For example, inbreeding depression was observed in *Senecio squalidus*, a successful SI sexual colonizer (Brennan et al. 2005). The combination of pseudogamous apomixis and SC may increase actual frequencies of successful founding of populations by single individuals. This advantage is expected to be most effective after long-distance dispersal (Baker 1967), which may not be frequent in *Ranunculus* because of the lack of specialized dispersal mechanisms; most achenes simply fall below the mother plant. Nevertheless, dispersal via ants (Müller-Schneider 1986) and flooding water (E. Hörandl, personal observation) have to be considered. An important advantage of SC in *Ranunculus* may be the ability to colonize rapidly new and moderately disturbed habitats. A frequent occurrence in man-made meadows is actually observed in *R. auricomus* (Paun et al. 2006b, Hörandl and Paun 2007). For establishment of populations, self-pollination reduces pollen limitation caused by small population size and by fluctuating pollinator frequencies.

In conclusion, SC in apomicts may provide reproductive assurance in both spatially and also temporally variable environments, which may outweigh or even exceed the disadvantage of reduced seed set for colonization. In contrast, higher fitness of sexuals may explain the maintenance of small distribution areas of SI sexual relatives in situations, where population density and pollinator availability is sufficient to provide reproductive assurance.

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Fig. 1.

Box plots of number of well-developed achenes after open pollination in sexuals, apomicts (pooled over 3 yr), and artificially produced hybrids (1 yr only). The box shows the twenty-fifth and seventy-fifth percentile ranges and the median; circles are outliers (cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box). *N*= number of collective fruits analyzed.

Fig. 2.
a, Number of achenes in diploid sexuals, autotetraploid sexuals, and allopolyploid apomicts (pooled over 3 yr). *b*, Percentage of well-developed achenes per collective fruit in diploid sexuals, autotetraploid sexuals, and allopolyploid apomicts (pooled over 3 yr). Box plots as in fig. 1.

Fig. 3. Germination rates (percentage of well-developed achenes) of diploid sexuals, autotetraploid sexuals, and allopolyploid apomicts. Box plots as in fig. 1. *N*= number of collective fruits analyzed.

Fig. 4. Mean percentage (plus confidence interval) of good achenes in 3 yr. Open bars = diploid and tetraploid sexuals (pooled), solid bars = hexaploid apomicts. *N* = number of collective fruits analyzed.

Fig. 5.

Percentages of good achenes per collective fruit, pooled over 3 yr. *a*, Autotetraploid sexuals (values above 0 only from artificial crosses). *b*, Allohexaploid apomicts. *c*, Artificial hybrids. Data from 2007. Bagged = bagged flowers, pollinators excluded; open = open pollination. Box plots as in fig. 1. Asterisks indicate extreme values (cases with values more than 3 box lengths from the upper or lower edge of the box). *N* = number of collective fruits analyzed.

Table 1
Seed Set in the Different Cytotypes/Reproductive Systems and in Hybrids, and Test Statistics for Bagged versus Open Flowers

	No. flowers	Total no. achenes	Mean % of well-developed achenes/collective fruit	SD	Minimum	Maximum	F	P
2005:								
2x sexuals:								
Bagged	13	586	.00	.00	.00	61.820	<.001	
Open	12	398	60.17	22.53	32.00	96.00		
4x sexuals: ^a								
Bagged	4	219	.00	.00	.00	32.705	<.001	
Open	6	186	54.91	17.74	21.05	72.22		
6x apomicts:								
Bagged	21	1301	38.69	11.61	19.61	66.67	.610	.439 (ns)
Open	22	1197	41.91	19.36	3.41	85.71		
2006:								
2x sexuals:								
Bagged	7	345	.00	.00	.00	85.628	<.001	
Open	15	715	73.78	14.70	55.77	95.45		
4x sexuals: ^a								
Bagged	10	317	.00	.00	.00	134.730	<.001	
Open	7	200	83.75	13.72	56.25	100.00		
6x apomicts:								
Bagged	4	138	17.93	12.22	3.70	33.33	2.405	.149 (ns)
Open	9	322	34.95	19.20	11.32	75.00		
2007:								
2x sexuals:								
Bagged	26	516	.00	.00	.00	179.892	<.001	
Open	11	370	58.70	20.88	9.00	81.00		
4x sexuals: ^b								
Bagged	32	1204	6.65	13.57	.00	63.00	86.352	<.001
Open	13	429	55.06	17.89	32.00	90.00		
6x apomicts:								

	No. flowers	Total no. achenes	Mean % of well-developed achenes/collective fruit	SD	Minimum	Maximum	F	P
Bagged	21	956	24.17	14.90	.00	54.00	2.323	.135 (ns)
Open	24	1106	30.71	13.67	.00	62.00		
F ₁ hybrids:								
Bagged	14	372	.00	.00	.00	.00	24.580	<.001
Open	51	1321	21.79	15.96	.00	54.00		

Note. In all cases, df = 1, ns, not significant.

^a Only individuals from natural populations.

^b Individuals from natural populations and from experimental crosses.