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Titel der Diplomarbeit

POSTZYGOTIC ISOLATION IN THE POLYPLOID COMPLEX OF A HIGH MOUNTAIN PLANT (*SENECIO CARNIOLICUS,* ASTERACEAE)

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TABLE OF CONTENT

PREFACE

Senecio carniolicus Willd is a European high mountain plant taxon forming a well studied polyploid complex. Formerly thought to be uniformly hexaploid, prevalence and spatial distribution of three main cytotypes (ploidy levels) are currently known for this species. A high percentage of all Eastern Alpine populations contain more than one cytotype, yet only very few individuals with an intermediate number of chromosome sets (inter-cytotype hybrids) have been found in the field. From what we know so far, ecological differentiation between plants of the three ploidy levels is strong but incomplete and therefore does not sufficiently explain the encountered near-absence of inter-cytotype hybrids in the field. We therefore suggest that strong postzygotic isolation barriers are at work keeping the percentage of hybrids in mixed populations negligibly low.

Postzygotic isolation barriers often entail high embryo and/or seedling mortality, or at least decreased fitness and fertility (Sobel et al., 2009). They can be intrinsic, for example seed abortion caused by imbalance of the ratio of maternal and paternal genomes contributing to endosperm formation, as encountered in experimental crosses between diploid *Arabidopsis thaliana* and tetraploid *A. arenosa* (Bushell et al., 2003). Alternatively, they may be extrinsic, e.g. slower development of hybrid seeds which provides an evolutionary disadvantage by drastically reducing survival chances when the seedling encounters intra- and/or interspecific competition, or any other phenotypical disadvantages becoming evident only in the natural environment but not in the laboratory (Martin and Willis, 2010).

Because of the low proportion (about 1%) of odd-ploidy individuals of *Senecio carniolicus* in the field, we expect that seedlings produced in crossings between all possible inter-cytotype combinations of the three main cytotypes would perform worse (that is grow more slowly and/or show higher mortality) than either of their parents' cytotypes in intra-cytotype crossings.

A germination experiment using seeds from artificial crossings in the field was designed to test this hypothesis. Plants from all three main cytotypes were cross pollinated resulting in twelve treatments (three intra-cytotype crossings, six inter-cytotype crossings – three possible combinations each in both directions - direction when egg and pollen donor were exchanged - and three unpollinated treatments to test for self-incompatibility). These seeds were germinated under controlled conditions in a climate chambers and their development was recorded.

The simplest explanation is that the progeny of an inter-cytotype crossing in an autopolyploid complex, which is nothing else than (intraspecific) hybridization the resulting progeny will display characteristics intermediate to those of its parents.

Therefore, we pooled the values of the two parental intra-cytotype crossings in the statistical analysis. Our null hypothesis was that the values for hybrid progeny would lie above (for duration)

and/or below (for survival rates) this pooled value. This hypothesis was tested using Generalized Linear Mixed-Effects Models.

In general, the crossing success of all inter-cytotype crossings, but especially for the two tetraploid – hexaploid combinations, was unexpectedly high. Seeds from inter-cytotype crossings displayed almost no disadvantages when compared to their parental intra-cytotype crossing in the beginning and only slight disadvantages in life stages beyond germination.

The duration of certain developmental stages in hybrid seeds does not influence their general performance and success. Likely, duration has important consequences under field conditions, and the (dis)advantage of accelerated/decelerated germination provides will depend on the respective habitat. Survival rates of seedlings strongly depend on the ratio of maternal to paternal genome in the endosperm and its deviation from the ratio 2:1. The higher the absolute deviation the less fit is the hybrid, but excess of paternal genome shows up earlier and has more devastating effects on the embryo, at least in the period of juvenile development investigated here.

1. INTRODUCTION

1.1 Polyploidy in higher plants

1.1.1 Incidence of polyploidy among flowering plants

More than a century after the discovery of the phenomenon of polyploidy, the "heritable possession of two or more chromosome sets" (Comai, 2005), research on this topic has ever since increased (Mable, 2003). While early studies uniformly underestimated its incidence as well as its evolutionary importance, we now know that chromosome doubling is common. Whereas polyploidy is widespread in ferns and angiosperms (e.g. Otto and Whitton, 2000), it is rarer in the animal kingdom for different reasons, e.g. complications with sex determination accompanied by chromosome doubling (Stebbins, 1968; but see Mable's (2004) review of studies based on the frequency of polyploidy in dioecious plants). Current estimations for the percentage of polyploid angiosperms vary between 30 and 80% (Otto and Whitton, 2000; Soltis et al., 2009). In gymnosperms (Cui et al., 2006) and wooden angiosperms (reviewed in Meyers and Levin, 2006) polyploidy appears to play a minor role. One possible explanation is that xylem fibres could hinder the increase in cell size that normally goes along with genome duplication (e.g. Otto and Whitton, 2000; Mable, 2004).

60 to 75% of the angiosperms are currently believed to have a least encountered polyploidy once in their ancestry (Köhler et al., 2009). Genomic data even provide evidence that all angiosperms except for *Amborella* are palaeopolyploid (Cui et al., 2006; Soltis et al., 2009).

The broad ranges for these estimations originate from the diverging definitions of the minimum genome size and therefore the threshold chromosome number below which a species is considered to be polyploid. The most conservative assumption is that every species with eleven or more chromosomes in the haploid genome has undergone chromosome doubling (reviewed in Otto and Whitton, 2000). But a number-based discrimination of polyploids is always problematic, because fusion or loss of entire chromosomes can lead to the paradox situation that even species with fewer chromosome numbers are nevertheless palaeopolyploid (e.g. *Arabidopsis thaliana* - Soltis et al., 2009).

In angiosperms, allopolyploids, also called amphidiploids (e.g. Stebbins, 1947; Chen, 2010) and defined as interspecific hybrids with doubled genome, seem to be more frequent than autopolyploids, which are characterized by chromosome doubling within a single species. But, as in some known autopolyploid taxa, e.g. *Chamerion angustifolium* (Husband and Sabara, 2003), *Ranunculus adoneus* (Baack, 2005) or some ecotypes of *Arabidopsis thaliana* (Bushell et al., 2003),

plants of different cytotype can not, or at least not easily, be distinguished morphologically, there is strong evidence that autopolyploids have only been repeatedly overlooked (Otto and Whitton, 2000; Soltis, 2007). Also the never-ending debate about nonuniform species concepts (e.g. Buggs et al., 2009) influences the discrimination of allo- and autopolyploids because every scientist has his/her own interpretation of what a species is.

To reduce these problems, some authors (Doyle et al., 2008) confess to the discrimination of auto- and allopolyploids according to their mode of chromosomal segregation during meiosis, referring to allopolyploids as polyploids exhibiting disomic segregation (the homologous chromosomes of the two parental taxa have diverged too much to pair any more) and to autopolyploids as polyploids with tetrasomic segregation (formation of tetravalents instead of bivalents).

But due to homoeologues gene loss (genome downsizing, e.g. Leitch and Bennett, 2004; Soltis et al., 2009) and structural chromosome rearrangements after polyploidization newly arisen autopolyploids usually undergo a process termed "diploidization" (Tate et al., 2009) which entails a return to disomic segregation.

These reasons make polyploids in general and (ancient) autopolyploids in particular hard to detect and recurrently overlooked. Indeed, in recent times an increasing number of autopolyploid complexes have been discovered (reviewed in Otto and Whitton, 2000). The evolutionary significance of autopolyploidy versus allopolyploidy remains unclear in large parts, though an increasing number of studies on this topic is currently undertaken (e.g. Parisod et al., 2010).

1.1.2 The relevance of polyploidy for speciation

Whereas the high incidence of polyploidy in higher plants has been pointed out and is widely accepted, no agreement has yet been found on the significance of chromosome doubling on speciation processes.

While Stebbins, one of the pioneers in plant polyploidy research, repeatedly stated that polyploidization does not play a major role in the evolution of plant taxa and only serves as a means to stabilize otherwise unviable hybrids (Stebbins, 1966, 1968), nowadays most evolutionary biologists emphasize the importance of polyploidy for speciation (Soltis et al., 2009; Tate et al., 2009; Ainouche and Jenczewski, 2010). The increasing interest in evolutionary consequences of polyploidy is certainly also due to its enormous potential in breeding new agricultural crops and increasing their yield, in the past as well as in present days (e.g. *Triticum* (wheat), *Zea* (maize) or *Brassica* (cabbage), to name just three of many important examples). Increased plant size (and therefore yield) as well as increased ecological adaptability and general vigour have often been associated with genome doubling (Stebbins, 1966; Parisod et al., 2010). But it must be considered that the majority of these crops are allo- rather than autopolyploids and therefore also incorporated the advantages of hybridization. No

consensus has been found on whether the chromosomal changes following polyploidization, which are commonly designated an evolutionary advantage, are primarily caused by genome merger or by genome doubling. Recent studies on *Arabidopsis thaliana* point at a higher positive influence of hybridization (Doyle et al., 2008). Baack and Rieseberg (2007) found that genomic and chromosomal consequences were very similar for hybrids and synthetic allopolyploids. Buggs et al. (2009) found a strong positive correlation between hybridization among genetically highly divergent species followed by polyploidization and the establishment of new species, but do also not provide an answer to the question if this increased successful speciation is the consequence of joining divergent genomes or of chromosome doubling. Similar studies have been undertaken to find out whether a correlation exists between polyploid lineages in general (thus no differentiation between auto- and allopolyploids) and elevated speciation rates, but whereas until recently agreement has reigned on a positive correlation (Soltis et al., 2009), no real evidence for this was found when Wood et al. (2009) repeated a far-reaching analysis of species numbers in genera for which chromosome numbers and therefore polyploid incidence are known.

It is widely accepted that chromosome duplication can result in an instantaneous reproductive barrier (Husband, 2000; Doyle et al., 2008; Tate et al., 2009). Some common explanations for its formation are instant habitat segregation of the ancestor and its polyploid progeny (Fowler and Levin, 1984), differences in flowering time (e.g. *Arrhenatherum elatius* - Petit et al., 1997; *Dactylis glomerata*, Lumaret et al., 1987), or pollinator preferences (*Chamerion angustifolium* – Husband, 2000; Husband and Schemske, 2000), different siring ability of pollen according to its ploidy (*Chamerion angustifolium* - Husband et al., 2002; *Ranunculus adoneus* - Baack, 2005), production of unviable seeds when egg cells are pollinated with pollen of different cytotypes (hybrid inviability, e.g. Bushell et al., 2003; triploid block, Köhler et al., 2009) or meiotic imbalances causing formation of aneuploid gametes which leads to sterility of hybrid offspring (hybrid sterility, Ramsey and Schemske, 1998; Comai et al., 2005). Whereas the first four examples belong to prezygotic isolation, the last two are postzygotic. Mechanisms leading to the formation of postzygotic reproductive barriers are more thoroughly discussed in chapter 1.1.4.

The possibility of immediate segregation potentially renders polyploidization an important driver of evolution. Nevertheless, in the view of some opponents of polyploid speciation the overall fitness and fertility of frequently formed neopolyploids are generally low, and only a small percentage of newly arising polyploids has the chance to survive long enough to be able to evolve to a new species (reviewed in Otto and Whitton, 2000). A high rate of (beneficial as well as deleterious) mutations in new polyploid lineages can boost subsequent radiation and divergence from the diploid parent (Yamauchi et al., 2004; for the role of deleterious mutations for polyploid speciation see Otto and Whitton, 2000).

Even though considerations made by Sobel et al. (2009) point out that even polyploid speciation always contains some ecological segregation which mostly results in geographical divergence, at least on microscale, polyploidization is nowadays seen as the "single most common mechanism of sympatric speciation in plants" (Otto and Whitton, 2000).

Ten years ago it was believed that polyploidization was involved in about 2-4% of all angiosperm speciation events (Otto and Whitton, 2000); newer estimates are as high as 15% (Köhler et al., 2009; Wood et al., 2009).

1.1.3 Mechanisms and pathways of polyploid formation and establishment

Polyploidization is usually either the result of duplication of a formerly diploid genome or of extension of a formerly polyploid one by another diploid chromosome set. Disregarding the possibilities of rare spontaneous chromosome doubling in somatic tissue (Ramsey and Schemske, 1998), which leads to chimeras in ploidy level, or polyspermy (fertilization of the egg by two sperm nuclei; Köhler et al., 2009), this can be achieved via two pathways. Either two unreduced gametes fuse at once to result in a zygote containing twice as many chromosomes as the somatic cells of either parent, or the initial cytotype mates with a transitory state that resulted from the fusion of one unreduced and one reduced gamete (Ramsey and Schemske, 1998; Husband, 2004; Köhler et al., 2009). As the transitory state involved in polyploidization most commonly is a triploid, the term "triploid bridge" was coined by Bretagnolle and Thompson (1995) for the second pathway. Polyploidization via triploids is far more probable, as the percentage of unreduced gametes is usually rather low. One appreciated estimation made for *Chamerion angustifolium* had also been adopted for other species as well as for general computer simulations of population genetics (Husband, 2004) and was 3% unreduced gametes (Burton and Husband, 2001). We ought to compare this to the general estimates by Ramsey and Schemske (1998), who revealed prevalences of unreduced gametes of about 0.56% for non-hybrid and 27.52% for hybrid systems, to find a positive correlation between hybridization and polyploidization (see chapter 1.1.2). Autopolyploid systems like that of *Chamerion angustifolium* also tend to produce more unreduced gametes than average non-hybrid taxa.

Once a polyploid successfully formed in a population, several factors determine its future fate: First, successful polyploid establishment requests their recurrent formation (Burton and Husband, 2001; Parisod et al., 2010); therefore high prevalence of the intermediate ploidy state (usually the triploid), which produces the respective polyploid in backcrosses with diploids, dramatically facilitates this process. Triploids usually display low overall fitness (e.g. Köhler et al., 2009). However, under changing conditions (e.g. rate of assortative mating and distribution of euploid gametes produced by

triploids) hybrid offspring has been proven to be really important for the spread or even fixation of the neopolyploid cytotype, even when its fitness values are considerably low (Husband, 2004).

Second, fitness of the neopolyploids in relation to the diploid ancestor is vitally important (Felber, 1991) – the higher the fitness of the polyploid (both in terms of viability and of fertility), the higher the probability for establishment in the population. In cases where overall fitness of a neopolyploid largely exceeds that of the diploid (or lower ploidy) ancestor, the new cytotype can even displace the old one (Burton and Husband, 2001; Husband, 2004; in combination with parthenogenesis: Yamauchi et al., 2004).

Third, the mating system has a large impact on the success of neopolyploids (Husband et al., 2008; for the significance of the mating system of the transitory state see Yamauchi et al., 2004): Self-fertilization facilitates polyploid establishment as these reproductive strategies lower the percentage of egg cells lost to fertilization with pollen of a wrong ploidy level. This is of particular importance at the initial phase of coexistence of two or more ploidy levels when the newly formed polyploid is still very rare, and under the assumption of no spatial clustering and equal pollen dispersion ability (minority cytotype disadvantage, e.g. Burton and Husband, 2001). Chromosome doubling lessens the effects of inbreeding depression and therefore reduces the disadvantages of autogamy (Rausch and Morgan, 2005), nonetheless are selfing costs supposed to rise with time as heterozygosity decreases which is why mixed mating systems are predicted to be most successful in neopolyploid taxa (Husband et al., 2008). A breakdown of self-incompatibility systems in case of inter-species pollination (the so called mentor effects) has been reported for some plant species (e.g. *Hieracium* - Mráz and Paule, 2006; *Ranunculus auricomus* - Hörandl and Temsch, 2009). Mentor effects can also favour polyploid establishment as they raise the prevalence of selfing, as it is the case for *Ranunculus auricomus*, and are therefore proposed as one of many consequences of genome doubling (Yamauchi et al., 2004).

For species which show high potential to reproduce asexually, polyploid establishment is also facilitated as minority cytotype disadvantage is evaded (e.g. Duchoslav et al., 2010). The same is true for species reproducing parthenogenetically; Stebbins (1979) already found a disproportionate incidence of polyploidy among these species. Reduction of the minority cytotype disadvantage it is also the approved explanation for the observation that polyploidy is more frequent among perennial than among short-lived or annual species, as the former have prolonged opportunities to cross with the correct ploidy level (Ramsey and Schemske, 1998; Otto and Whitton, 2000).

Migration from nearby populations - though the distance between recipient and donor population may be small, as even for anemochorous diaspores dispersal distances are limited to a few meters - and the composition of the donor population must be appropriate (see Levin (1975) for a comprehensive discussion) can also eventually help to raise the prevalence of a neopolyploid in a population.

Under most circumstances (that means in the majority of abiotic and biotic environments), in a mixed population one cytotype will be fixed whereas the other will be eliminated (Levin, 1975) or at least be restricted to very low frequencies (Husband and Schemske, 2000; Husband, 2004). Normally this would be the initial cytotype, as the initially rare polyploids are supposed to suffer badly from the recurrent production of less fit and/or infertile intermediate ploidy levels (i.e. the already mentioned minority cytotype exclusion principle (Levin, 1975). Yet, if one or more of the supporting factors described above act in favour of or reproductively isolate the minority cytotype, the later can rise in frequency so that subsequently the two sympatric cytotypes can coexist in a stable equilibrium. Else, if the habitat of a sympatric population is very heterogeneous and/or ecological segregation well-established so that mating from the beginning on is highly assortative rather than random, minority cytotype disadvantage can also be overcome by the neopolyploid plants. For example, Husband and Sabara (2003) found out that prezygotic isolation barriers alone accounted for 97.6% of the total isolation, yet note that the relative importance of any reproductive barrier can change over the history of a polyploid (Sobel et al., 2009) and more studies of newly arisen polyploids are needed.

Genome doubling is a major driving force of chromosomal change within a species or a hybrid lineage (Doyle et al., 2008). Commonly observed consequences of polyploidization include genomic shock (at least for allopolyploids: Doyle et al., 2008), significant rise in abundance of transposable DNA elements (especially in allopolyploids: Ainouche et al., 2009; also sometimes associated with speciation in general: Orr and Presgraves, 2000), widespread changes in DNA expression patterns (Leitch and Bennett, 1997; Liu and Adams, 2010), alterations in gene regulation (e.g. via DNA methylation; Doyle et al., 2008) or extensive chromosomal rearrangement (Leitch and Bennett, 1997). These alterations usually cause sub- and neofunctionalization (e.g. Wendel, 2000; Ainouche and Jenczewski, 2010), which is according to Comai (2005) one key advantage of polyploidization. As discussed in chapter 1.1.2, comparisons of diploid hybrids and allopolyploids resulted in the discovery that these changes are largely caused by hybridization rather than polyploidization and so the effective importance of these changes for polyploid complexes, especially for eventually following speciation among them, remains elusive (Doyle et al., 2008).

1.1.4 The nature and establishment of reproductive barriers

Postzygotic reproductive isolation between two species can result from interactions between nuclear and organelle DNA (Martin and Willis, 2010; Wolf et al., 2010), epigenetic modifications, different frequency and patterns of heterochromatin and many others (summarized by Brown and O'Neill, 2010). But the most profoundly studied and widely accepted trigger of postzygotic isolation are deleterious effects of the combination of otherwise neutral or beneficial alleles from different

genomes due to hybridization or gene introgression (e.g. Kondrashov, 2003; Welch, 2004). Such (Bateson-)Dobzhansky-Muller incompatibilities have been found to become more frequent with increasing genetic divergence between hybrid parents causing reproductive isolation among species, which diverged allopatrically but gained secondary contact. Therefore, Dobzhansky-Muller incompatibilities are the approved trigger of allopatric speciation.

In the case of polyploid speciation, which as discussed above is also possible in sympatry, other mechanisms of reproductive isolation must exist that enable immediately isolation of two lineages. The best-supported theory in this respect is genomic imprinting of the endosperm (e.g. Haig and Westoby, 1991; Bushell et al., 2003; Köhler et al., 2009; Chen, 2010). Genomic imprinting designates the fact that the degree of expression for identical alleles depends on whether they are part of the maternal or the paternal genome. Normally, in crossings between partners of the same ploidy levels, imprinting regulates levels of transcription and therefore doses of protein synthesis in a way that two maternal and one paternal genome complement each other perfectly (principle of dosage compensation: e.g. Haig and Westoby, 1991; Chen, 2010). For the endosperm, the target ratio of two maternal and one paternal genome (Endosperm Balance Number, EBN) results from the double fertilization mechanism fulfilled in the majority of angiosperms where the second sperm nucleus fertilizes two polar nuclei and thus initiates endosperm formation. When two individuals of different ploidy level mate, genomic imprinting inevitably leads to excess or shortage of gene products from all genes subjected to imprinting or to serious alterations in gene regulation when non-proteinogenic genes are involved. As the reproductive interests of pollen and egg donor are opposing each other (parental conflict theory: Haig and Westoby, 1991; Scott et al., 1998), the resulting phenotypes of an excess of paternal (termed paternalization) or an excess of maternal genome (maternalization) are also opposed. Paternalization has been shown to result in facilitated acquisition of the nutrients provided by the endosperm, increased mitotic rates in the corresponding embryo and accelerated development in early life stages (Haig and Westoby 1991; Bushell et al., 2003). After this initial overgrowth, paternalized embryos are more often doomed to perish than those with balanced ratios of paternal and maternal genome (Bushell et al., 2003). On the contrary, maternalized seeds are hindered in their growth performance, show earlier formation of cell walls in embryogenesis and have lower weight (Bushell et al., 2003).

A positive correlation between absolute deviation of the ratio of the respective crossing from the target ratio and the graveness of the deleterious effects on the embryo has been found for inter-ploidy *Arabidopsis thaliana* x *Arabidopsis arenosa* crosses (Bushell et al., 2003). In general, maternalization seems to have milder effects than paternalization (Bushell et al., 2003; Köhler et al., 2009).

Apart from its significance for inter-ploidy crossings, endosperm balance is also the explanation for the unexpectedly high abundance of pseudogamy (the unreduced female gametophyte

can only form a seedling when the endosperm is successfully fertilized) among apomictic taxa (Haig and Westoby, 1991), and explains the failure of some homoploid interspecific crossings (concept of effective ploidy, reviewed in Haig and Westoby, 1991).

If reproductive isolation by endosperm imprinting is not complete and hybrid offspring can flourish and produce flowers, the second most important and historically longer known barrier for inter-ploidy crosses (already mentioned in chapter 1.1.2) can be effective: hybrid sterility, resulting in F1-breakdown. Hybrid sterility is typically caused by problems occurring during meiosis of the (mostly odd-ploid) cells of inter-ploidy hybrids due to the formation of multivalents and chaotic chromosome segregation (e.g. Comai, 2005; Woodhouse et al., 2009). Hybrid sterility acts a lot later in a plant's life cycle and is therefore expected to contribute less to total reproductive isolation in polyploids than early acting imprinting failures. (Rieseberg and Willis, 2007).

Though no studies exist on the influence of Dobzhansky-Muller for inter-ploidy hybridization, it is likely that they also play a role, especially for allopolyploids and palaeoautopolyploids, when for whatever reason reproductive isolation via endosperm imprinting is incomplete.

1.2 Research species: *Senecio carniolicus*

1.2.1 Overall plant characteristics

Belonging to the family Asteraceae, to the genus *Senecio* sect. Jacobea, *Senecio carniolicus* Willd (syn. *S. incanus ssp. carniolicus* Braun-Blanquet, Carniolan Ragwort) is a common and abundant European mountain plant. As an endemic plant of the Eastern Alps and the Carpathians, it is documented for Austria, Switzerland, Italy, Slovenia, Poland, Slovakia, Romania and Ukrainia. *Senecio carniolicus* is closely related to its vicarious species *Senecio incanus L.*, found in the Western Alps (France, Italy and Switzerland) and was until recently treated as a subspecies. However, the two taxa can easily be differentiated based on their morphology, e.g. leaves of *S. incanus* have a dense, grey, silky or even woolly indumentum that persists even in senescent plants and leaf blades are more deeply lobed (Pitschmann, 1965; Reisigl, 1978; Suda et al., 2007). Additional differences occur on the cytological as well as the molecular level (Suda et al., 2007). Nevertheless, at the south-western edge of the distribution area of *S. incanus* L., a few presumably intermediate populations do exist (Alpi Lepontine, Alpi Bergamasche; Suda et al., 2007). These were alternatively classified as *S. incanus ssp. insubricus* and as *S. carniolicus var. insubricus* (Suda et al., 2007).

In general, *Senecio carniolicus* grows on silicious bedrock, but locally it can also be found on calcareous substrate covered by acid organic humus layers (Pitschmann, 1965), e.g. in the Northern Calcareous Alps of Upper Austria (Heberling et al., 2006). Occasionally, it can be found on often slightly dolomitic limestone, e.g. in the Karawanken (south-eastern Alps).

Senecio carniolicus is a characteristic species of alpine grasslands, dwarf shrub communities, glacier moraines and stable screes and bare rock habitats, ranging from about 1800 m a.s.l. to 3300m a.s.l. (alpine to subnival). It is most commonly found in the plant association *Primulo-Caricetum curvulae* (Oberdorfer, 2001). The *Primulo-Caricetum curvulae* is the local variety of *Carex curvula* communities in the Eastern Alps (Reisigl and Keller, 1987), which owes its name to different endemic *Primula* species (*P. minima, P. glutinosa, P. integrifolia*). Besides, *Senecio carniolicus* can sometimes also be found in mat-grass lawns between 1800 and 2500m (*Aveno-Nardetum, Curvulo-Nardetum*), as well as in *Loiseleuria* shrubs (*Loiseleurietum*) and in silicious bedrock communities (*Androsacetum vandellii*) (Reisigl and Keller, 1987).

The species sometimes displays pioneer character (Eggenberg and Möhl, 2007), especially the diploid cytotype which generally occupies habitats with low vegetation cover (Sonnleitner et al., 2010).

The inflorescences are hyper-pseudanthia comprising few to many few-flowered capitula measuring about one to two centimetres in diameter (Reisigl, 1978) and comprising three to six female egg-yolk yellow ray florets and five to ten hermaphroditic disc florets. The achenes possess a grey or brown pappus, the diaspores are therefore likely anemochorous, as are those of the majority of the composite family. Flowering time lasts from July to September (Pitschmann, 1965). *Senecio carniolicus* is mainly pollinated by flies (Oberdorfer, 2001). As a perennial, hemicryptophytic plant, it reaches a height of five to fifteen centimetres (Fischer et al., 2008).

Senecio carniolicus displays a high pollen-to-ovule-ratio (Fössinger, 2010), indicating a primarily (facultatively) allogamous reproduction. In autogamous species, pollen-to-ovule-ratios are usually low, as no considerable loss of pollen needs to be accounted for (Götzenberger, 2008).

Proterandry, a frequent phenomenon among the family of the Asteraceae, is also observed in *Senecio carniolicus* and provides another indication for allogamy, yet not an absolute one, as autogamy is possible and has been recorded in some proterandrous composite species.

In fact, the crossing experiments in summer 2009 revealed the fact that also selfed ovaries can produce intact, able to germinate diaspores in not negligible shares.

1.2.2 Differentiation among the main cytotypes

For a long time *Senecio carniolicus* was considered to be an exclusively hexaploid species; early reports of diploid individuals were regarded as dubious (Suda et al., 2007). Today it is not only clear that the population in the Alps comprises three main cytotypes (diploid – 2x, tetraploid – 4x and hexaploid – 6x), but also that an essential fraction of the populations contain more than one of these cytotypes.

The three main cytotypes of *Senecio carniolicus* can easily be distinguished on various levels. As to morphology (see Figure 2), diploid individuals are significantly smaller and more tender than the large hexaploid individuals. Tetraploids occupy an intermediate position, producing higher biomasses than diploids (Ruth Flatscher, unpubl.; Michaela Sonnleitner, unpubl.; personal observation). In this aspect our model species goes along with the general observation that higher ploidies lead to increased growth vigour as they trigger development of larger cells and nuclei (Otto and Whitton 2000; Chen, 2010). Apart from size, diploids can also be distinguished from tetraploid individuals by their strongly developed indumentum, and there generally less deeply lobed leaf blades. Young hexaploid leaves can possess thick indumenta but usually become glabrous with age.

On the ecological level, the recurrent impression of a more or less strict spatial segregation of the three main cytotypes was checked in an extensive study (Sonnleitner et al., 2010). Relating a

number of environmental parameters, such as percentage of rock and vegetation cover within a defined area surrounding each investigated plant individual, mean distance to the next individual of the same species, relative altitude or inclination to the cytotypes, ecological differences them were shown.

Diploids tend to grow in areas with high rock cover and display stronger clustering than hexaploids, which have, thanks have probably due to their size and vigour, the potential to grow in *Carex curvula* meadows or even *Loiseleuria* shrubs (see Figure 1).

The environmental requirements of the tetraploid cytotype are harder to delineate: Their microhabitats overlap with those of diploid and hexaploid individuals, but, unlike 2x and 6x, tetraploids sometimes also grow on more base-rich soils and were more abundant on steep, north-exposed slopes (Sonnleitner et al., 2010).

Concerning altitude, the findings of Schönswetter et al. (2007) and Hülber et al. (2009) that diploid individuals were more abundant on higher elevations, whereas hexaploids were exclusively found on altitudes below 2500 m, were confirmed by Sonnleitner et al. (2010). Similar patterns have been found in general studies by Knight et al. (2005) who discovered that plants with higher DNA content were absent from the highest altitudes and latitudes. Also in a uniformly diploid Andean genus closely related to *Senecio* was DNA content negatively correlated with mean sea level (*Lasiocephalus* - Dušková et al., 2010).

DNA content of plant individuals of all different cytotypes was recurrently determined via Flow Cytometry (Suda et al., 2007; Sonnleitner et al., 2010). Early studies of the polyploid complex of *Senecio carniolicus* yielded distinct mean fluorescence intensities for all cytotypes: 0.790±0.027; 1.498±0.029 and 2.151±0.051 for 2x, 4x and 6x plants respectively. *Pisum sativum* (2C=9.09 pg) was used as internal standard (Suda et al., 2007). To guarantee for accurate results, not only were measurements repeated on various days, but also fresh and silica dried samples compared, showing only negligible variation. Finally, Feulgen-stained chromosomes were counted for 32 individuals, showing that the distinct groups found via FCM corresponded to ploidy levels. The basic chromosome numbers for this species is x=20 with 2n=2x=40, 2n=4x=80 and 2n=6x=120 for di-, tetra- and hexaploids, respectively (Suda et al., 2007).

Last but not least, genetic research revealed the phylogenetic relationships of *Senecio carniolicus*, its major lineages and some related taxa. Analysis of AFLP (Amplified fragment length polymorphisms) and ITS (Internally transcribed Spacer) regions provided evidence for the existence of two diploid lineages, which also show significant variation in DNA content (Suda et al., 2007). The continuous range of the western diploid plants reaches the Isel valley in Eastern Tyrol (a disjunct occurrence is in the South Eastern Alps), whereas the eastern diploid lineage covers the Central Alps

eastwards, i.e. east of the Isel valley to the easternmost Niedere Tauern. Higher ploidy levels, all well separated from each other, occupy intermediate positions in the pedigree but are more closely related to the eastern diploid lineage. Yet the fact that tetraploids have only been located in two geographically separated areas makes multiple origins of polyploidization plausible. Even more, tetraploids can also easily be discriminated in an eastern and a western line on the genetic level. However, our knowledge about the historical distribution of the cytotypes and their postglacial (re)colonization pathways is still rather sparse (Suda et al., 2007).

Based on all the differences discussed, combined with the fact that hybridization in the field is very rarely observed, it seems very likely that the three main cytotypes of *Senecio carniolicus*, as well as the eastern and western diploid line will soon be granted species or at least subspecies state. This has yet rarely been done for autopolyploids because of usual high morphological similarities and ignorance on the degree of gene flow within the complex (Husband and Sabara, 2003; Soltis, 2007).

It is only on phenological level that the demarcation of the three main cytotypes remains problematic. Flowering time for the species ranges from July to September with slight differences between the cytotypes. Tetraploid individuals are the earliest and flower over a longer period than the other two cytotypes (Michaela Sonnleitner, unpubl.). However, these differences are not complete and can not be the only acting isolation mechanism that successfully keeps the main cytotypes apart.

Figure 1: Typical individuals of the diploid (left) and the hexaploid (right) cytotype of *Senecio carniolicus* in their respective habitat. Pictures kindly provided by Michaela Sonnleitner.

Figure 2: Representative plants of the three main cytotypes of *Senecio carniolicus*. Kindly provided by Ruth Flatscher.

1.2.3 Spatial distribution of the main cytotypes

Suda et al. (2007) discovered the cytotype distribution of 77 *Senecio carniolicus* populations in the Eastern Alps. A subsequent study (Sonnleitner et al., 2010), although increasing the number of sampled populations to 100 and strongly increasing the number of sampled plants per population to about 30 (compared to about five in Suda et al., 2007), did in large parts confirm the former findings but increased the percentage of mixed populations. According to Sonnleitner et al. (2010), 49.6% of the sampled plant individuals were hexaploid, followed by diploids (33.8%) and tetraploids (15.6%). The remaining 1.05% of the samples was aberrant cytotypes (3x, 5x, 7x, 8x and 9x).

Hexaploids were most widely spread over the Alpine range (and are the exclusive cytotype in the Carpathians: Suda et al., 2007) and are only missing in the western parts of the Hohe Tauern massif and the south-western edge of the species' distribution (Suda et al., 2007; Sonnleitner et al., 2010). Diploids are slightly less abundant, but are the only cytotype to inhabit the most south-western and south-eastern margins of the distribution range (Alpi Lepontini and Alpi Bergamasci, Italy; Belščica, Slovenia). Tetraploids are predominantly found in the northern areas of the distribution range as well as, less frequently, in the southern parts. These two occurrences are geographically isolated and correspond to areas which are thought to be important Pleistocene refugia (Schönswetter et al., 2005).

44% of the populations (Sonnleitner et al., 2010) consist of more than one cytotype (hereinafter referred to as mixed populations). The combination between diploid and hexaploid individuals hereby is the most frequent one (28% of the sampled populations, followed by 2x/4x/6x, 4x/6x and 2x/4x with 8%, 5% and 3%, respectively, of the sampled populations).

Figure 3 shows the distribution of the main cytotypes of *Senecio carniolicus* in the Eastern Alps.

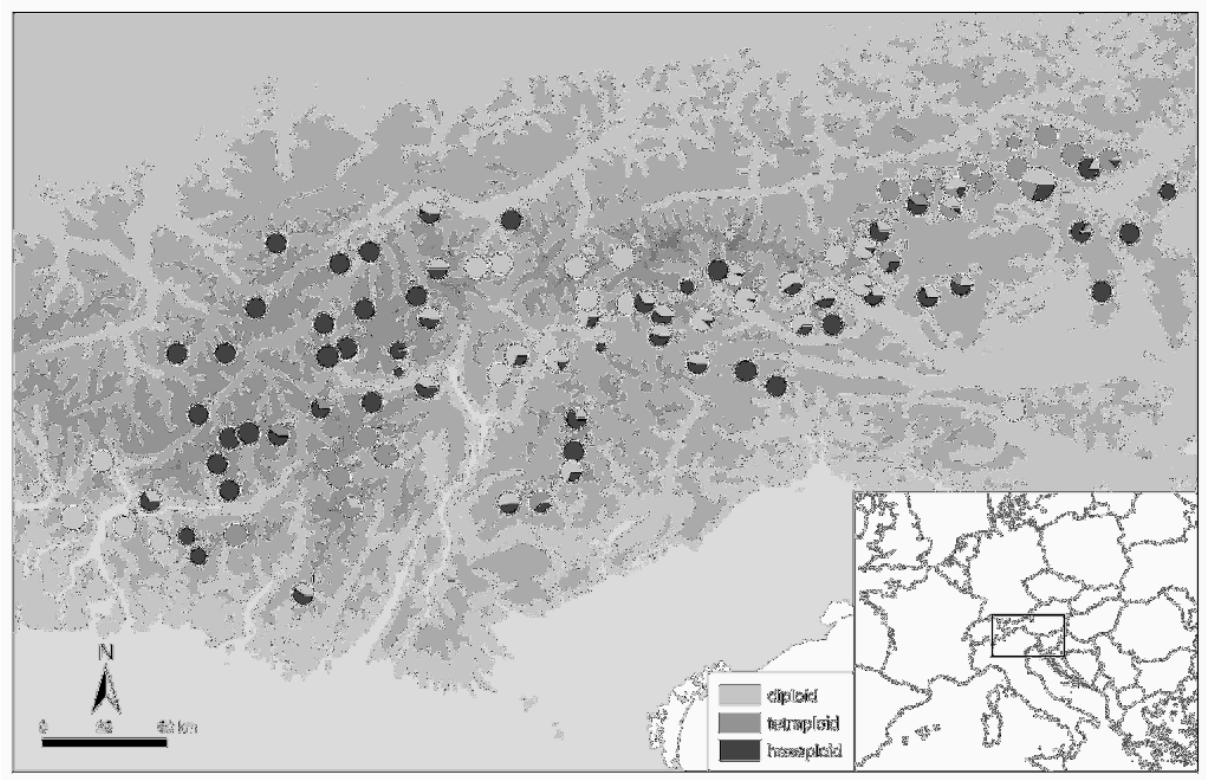


Figure 3: Spatial distribution of the three main cytotypes of *Senecio carniolicus* in the Eastern Alps (taken from Sonnleitner et al., 2010)

1.3 Research aims

As mentioned above, inter-cytotype-hybrids are rarely found in the field despite the high percentage of mixed populations and the close spatial proximity of individuals of *Senecio carniolicus*, leading to the suggestion that strong crossing barriers must be effective in this polyploid complex. Manual crossings yielded more or less high seed sets, which, as was proven through FCM, did not solely consist of selfed seeds. Therefore, we hypothesized that apart from prezygotic isolation also postzygotic reproductive barriers such as increased hybrid mortality or other fitness disadvantages act in this polyploid complex.

For the germination experiment, all possible crossing combinations between the three main cytotypes were realised in both directions, with intra-cytotype-crossings and unpollinated individuals to test for the degree of self-compatibility serving as control groups. Germinating these seeds in the laboratory and subsequently measuring performance parameters (mainly duration and survival from one developmental stage to the next, but also growth parameters such as length of radicle or seedling weight) we wanted to find out about the nature of the postulated postzygotic crossing barriers. Growth parameters were analysed visually only, data for duration and survival was used in statistical tests to look for any differential success of inter-cytotype hybrid progeny.

In detail we addressed the following questions (1) Does a lower performance of inter-cytotype hybrids compared to their parents indicate a selective disadvantage of the hybrid? and (2) Does the direction of an inter-cytotype crossing have any influence on growth performance?

2. MATERIAL AND METHODS

2.1 Germination experiment

a. Preparation

The seeds used in the germination studies were derived from crossing experiments on Mt. Schoberriegel, Kaserhöhe and Hoazhöhe (Turracher Höhe, Carinthia) during the growing period 2009. Originally previewed as a common garden experiment (as described in Schönswetter, 2007), crossings had to be repeated in the field because of an insufficient number of surviving plants. Plants of known cytotype were labeled and one average capitulum per plant was pollinated by pollen of a foreign cytotypes (inter-cytotype crossings). Intra-cytotype-crossings (pollination with pollen of the same cytotype) served as internal control. To test for the degree of self-incompatibility, some capitula of all three main cytotypes were not exposed to foreign pollen at all. Pollen was transferred to the stigmas by rubbing together the two capitula, and in order to exclude pollinators, the pollinated capitula were enwrapped with bags of fine net lace. The crossing experiments resulted in seeds from twelve different treatments, given in Table 1.

Mature fruits from pollinated capitula were harvested. The numbers of fructified and empty seeds, easy to discriminate via visual inspection, were determined. The seeds were then stored at approximately 8°C in the dark.

In February 2010, a pre-experiment was carried out to adapt the lab conditions for the germination experiment. Seeds of intra-cytotype crossings were germinated in different substrate types to get a realistic idea of germination and survival rates as well as necessary sample sizes. Manipulation of seeds and seedlings was optimized.

In March and April 2010, the set of fully developed seeds for each plant individual from the six intra-cytotype treatments was split in two parts, one of which was designated for germination, one of which for Flow Cytometry (FCM) to determine the cytotype of the embryo. This was necessary to get data on the prevalences of unreduced gametes and selfing among the seeds from the six inter-cytotype crossing treatments. In case of even seed numbers, the seeds were equally divided between FCM and germination, in case of an odd-numbered seed set, the exceeding seed was assigned to germination. The maximum number of seeds per individual used was restricted to 10 and 5 for germination and FCM, respectively. The remaining seeds were stored, as usual, at 8°C in the dark.

Cytotypes of seeds from intra-cytotype-crossings as well as from the selfed individuals were not determined using FCM, implicating that these should always have the maternal cytotype. Laboratory work to determine the cytotype of seeds was done at the FCM laboratory in Pruhonice under the supervision of Dr. Jan Suda (Department of Botany, Charles University, Prague).

The last preparatory measure was autoclaving of the needed material (petri dishes and filter paper).

Treatment	Plant individuals	Seeds for Germination	Seeds for FCM
2x2x	43	405	**-**
2x4x	35	195	133
2x6X	27	56	58
2xSI	21	92	-
4x2x	36	212	125
4x4x	35	334	-
4x6x	33	286	154
4xSI	27	130	-
6x2x	28	102	67
6x4x	38	365	186
6x6x	26	354	-
6xSI	19	54	-
Total	**378**	**2585**	**723**

Table 1: Overview of sample sizes for the germination experiment. The crossing formula has the following structure: maternal parent x paternal parent for actual crosses and cytotype x SI for pollinator-exclusion experiments. The first column gives the number of different plant individuals subjected to each treatment, the second column the number of seeds used for germination and the last one the number of seeds sent to FCM.

b. Soaking the diaspores

In May 2010, the visual pre-selection of seeds in developed and empty seeds was tested in order to account for the unlikely possibility that viable seeds from inter-cytotype crossings would generally look worse than average. Therefore, the empty seeds were placed in petri dishes (one per plant individual), on wet filter paper and left in the cold-storage chamber for two days at 4°C. At the same time, the whole set of developed seeds per individual was filled in a separate tea bag, watered, and, sorted by treatment, stored in the same facility.

After two days, the developed seeds were easily recognizable because of the size increase through soaking. For both subsets of seeds the numbers were corrected. The control of the visual pre-selection proved that even in dry state, the discrimination of the seeds was nearly always correct, with a negligibly low error rate (0.54%). Whereas the few empty seeds in between the full seeds were thrown away, the developed seeds within to empty ones were also used for germination.

c. Exposure of seeds

The germination experiment was done using numbered petri dishes. The dishes were disinfected with ethanol (96%), and then were covered with two layers of kitchen paper to retain

humidity and one layer of filter paper to prevent the roots from penetrating into the kitchen paper. Four equally sized sectors were marked on the filter paper with waterproof pen; sector A was marked with a little triangle. Inside each sector (named A to D), ten defined positions (1 to 10) in four lines following the given scheme, allowed to identify every single seed (see Figure 4).

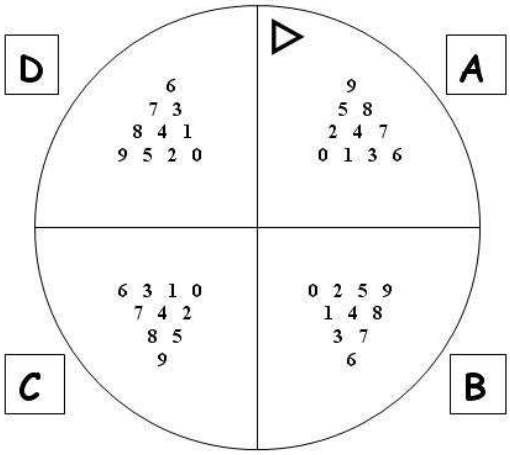


Figure 4: Scheme of seed exposure in the petri dishes

Whenever tea bags with viable seeds from one plant individual contained more than ten seeds, ten average ones were chosen arbitrarily. The seeds were put on the filter in continuing order, as described above. The few seeds wrongly categorized empty in the visual pre-selection of dry seeds were laid out in three separately numbered petri dishes.

The finished dishes were transferred to the Department of Pharmacology, where the facilities needed for the germination studies were available. Seeds were germinated in a climate chamber (Heraeus Vötsch, NPS1500 S-CTC, company information). The program for the climate chamber was the following:

Temperature during nighttime: 5°C

Lights on at 6:00 am, temperature rise during 6:00 am and 7:00 am

Temperature during daytime: 15°C

Lights off at 20:00 pm, temperature fall during 20:00 pm and 21:00 pm

Moisture: 90% relative humidity

Lighting was provided by two neon lights of type Osram powerstar HQ I-R 250W/NDL, illuminating the climate chambers with approximately 370 µmol photons/m^2/sec.

d. Daily observation and data recording

All 66 petri dishes were observed at a daily basis to estimate the viability of each single diaspore using the classes shown in Table 2. If necessary, water was applied to the dishes to ensure a constant level of moisture.

Status	Description
Nothing	no visible sign of germination
Mould	diaspore infected by mould (recognizable as a slight colour change to dark brown or black)
Radicle	root tip visible
Cotyledons	chlorophyll-containing plant parts (cotyledons and/or hypocotyl) visible
Transplantation	cotyledons sorted from testa and spread; as arranged in the run-up, this is the state at which the seedling is transplanted into substrate
Empty	empty position in petri dish, arisen because seedling has already been transplanted
Loss	empty position in petri dish due loss of diaspore because of manipulation errors or accidents
Death	death of the embryo or seedling; dead seeds were usually removed in order to avoid fungal infestations

Table 2: Status and description used for observation of seeds in petri dishes

As all petri dishes were sorted from the climate chamber daily and put back in an arbitrary order, rotation of the dishes on the shelf was guaranteed and no further measures had to be taken to account for the irregular illumination of the climate chamber (e.g. only 24% of the maximal light intensity was measured at the margins of the shelf).

In cases of excessive growth of mycelia in a petri dish, the seeds were disarranged or even put into fresh petri dishes to reduce the infection and to avoid its passing over to healthy seeds.

e. Transplantation of the seedlings

When both cotyledons were fully extended, the seedling was weighed (accuracy 1 mg), and the lengths of its root, its hypocotyl and the diameter of its two cotyledons were measured (accuracy 0.5 mm). Afterwards they were transplanted in multipot trays in arbitrary order, resulting in a mixture of cytotypes and seedling age within the trays. The used substrate was a mixture of coconut fibres, sand and fertilizer (approximate composition: 10l Cocopeat, soaked in $CaNO_3$/$MgSO_4$-solution, approximately 3l chalk-free sand and 20g Osmocoat® long-time fertilizer). PH was measured to ensure acidic conditions in the substrate.

Storage of the transplanted seedlings was previewed in two acclimatisation chambers, equipped with a PlantFog® moistening system, at approximately 22°C (±2°C) and an average relative humidity of 60% (±10%).

Seedlings were checked every second day. The state of the seedling (see Table 3) and the number of leaves were recorded. At the end of the experiment, the observation interval was extended to four days because of the less quick development of the seedlings.

Status	Description
Newly transplanted	The viability was not controlled on purpose, but any notable events (e.g. mechanical injuries) happening during transplantation were to be noted here
Good	Seedling looking healthy, all plant parts green
Neither good nor bad, or unclear	Seedling looking unhealthy due to abnormal development, colour changes, or mouldering
Bad	Seedling in bad condition, in most cases on the verge of dying

Table 3: Status and description for observation of seedlings in multipot trays

Whenever a transplanted seedling was obviously on the verge of dying, one or both cotyledons were stored in small Eppendorf tubes filled with silica gel for DNA ploidy level estimation.

f. End of the germination experiment

The experiment was finished using the following criterion: Germination rate (defined as the number of newly appearing radicles in relation to the non-germinated seeds) must not exceed 1.0% over a period of three days. In order to account for the different time spans necessary for germination, we applied this criterion to every treatment separately. After a maximum of fifty-five days in the germination experiment the remaining treatments were also finished. The duration of the germination experiment for all treatments is shown in Table 4.

Treatment(s)	Number of days in experiment
2x2x, 2x4x, 2x6x, 6x2x	49
2xSI, 6xSI	50
4xSI	51
4x2x, 4x4x, 4x6x, 6x4x, 6x6x	55

Table 4: Overview of the duration of the germination experiment

On July, 29th, the last control of the petri dishes was made. The seedlings in the multipot trays were kept in the climate chambers for one to three more weeks. According to their age, they were then transported to the experimental garden of the Institute of Botany of Vienna.

In the gardens, the seedlings were kept on tables covered with fleece to retain humidity and under a rack built to carry wooden blinds, in order to avoid intense sun light. The feet of the tables were posed in water-filled crates to keep away herbivores like slugs and ants. Watering was done usually once a day, sometimes every second day depending on weather conditions. Regularly, optical control was done not to miss seedlings which apparently would not survive the acclimatisation but could still be taken for cytotype analyses.

For each seedling, growth parameters (total number of leaves, length of the longest leaf, maximum diameter of the rosette and corresponding diameter in an angle of 90°) were recorded within two days after being transferred to the experimental garden. After about one month, one healthy leaf was cut off from all remaining plants, stored in silica gel and sent to FCM. Then the seedlings were transferred to the Alpine Gardens of the University of Innsbruck (Mt. Patscherkofel, Tyrol). Further observation of growth and measuring of the mentioned performance parameters of the crossings of the seedlings was repeated at the end of this summers' vegetation period and is supposed to take place once or twice every year in the future.

2.2 FCM

Flow Cytometry is a nowadays commonly used to determine DNA content of cells. Owing to its enormous potential to screen a large number of nuclei for their DNA amount, it is particularly important in research on the consequences of genome doubling as well as on spatial distribution of cytotypes in polyploid complexes and inferred from that, evolutionary history of polyploidization and distribution of polyploid taxa (Suda and Pyšek, 2010). Chromosomes are stained with a fluorochrome, in most cases as well as in ours, DAPI (4',6-diamidino-2-phenyl-indole), which specifically binds to the DNA base pairs. Fluorescence intensity of the stained nuclei, excited by a laser beam, is then technically multiplied, detected, measured and displayed as histograms.

As fluorescence intensity is directly proportional to the DNA amount in the nucleus, the resulting peaks can then be compared with an internal standard of known DNA content to assess ploidy for the probes (Doležel and Bartoš, 2005).

The machinery used for our purposes was a Partec Ploidy Analyser PA-II (equipped with UV mercury arc lamp and 488 mm argon ion laser).

2.3 Data processing

All data obtained through observation as well as the data from seed and seedling FCM was stored in a Microsoft Access 2002 (Microsoft Corp., Redmond, California) database.

Plausibility of data input was controlled automatically by selection queries wherever possible, and the remaining parameters were selectively scanned for errors. Impossible status sequences (e.g. radicle after death) were corrected after checking that they were not clustered in certain treatments, meaning that this could have a significant influence concerning seedling development.

Morphological abnormalities, such as the frequently observed formation of a third cotyledon, were also checked for possible correlations with treatment. As no such correlations had been found, they were not regarded in further statistical analysis.

On the contrary, the status “mould” seemed to have a not negligible influence on seedling development.

2.4 Statistical analyses

For the distribution of mouldering diaspores, a χ^2-test was performed to clarify its dependency or independency from the crossing parents.

To test for significant differences in developmental speed (duration) and survival of seedlings originating from inter-cytotype crossings and intra-cytotype crossings of both parental cytoypes we applied Generalized Linear Mixed-Effects Models using treatment as the only fixed-factor variable. For the binomially distributed survival data we used the canonical logit link-function. In case of duration we used a Poisson GLMM. We used mixed models instead of Generalized Linear Regression Models to account for the potential dependence of seeds derived from the same mother plant by integrating a random intercept for each plant. The use of herlmert-contrasts enables a comparison of the pooled parental intra-cytotype crossings with an inter-cytotype crossing. Significant marginal effects of the latter treatment can hence be interpreted as a deviation of the hybrid from the mean performance of its parents. Parameters were estimated based on the Laplacian approximation. Analyses were done using the package lme4 (Bates and Sarkar, 2008) of the statistical software platform R (R Core Development Team, 2008).

We applied the commonly used significance levels. Probabilities below 5% ($p<0.05$) designate a significant difference, those below 1% ($p<0.01$) a highly significant one and those below 0.1% ($p<0.001$) a very highly significant difference.

Three meaningful time spans were chosen for application of the Generalized Linear Mixed-Effects Models following personal observation of seedling behaviour during the experiment: We examined the periods from the beginning of the experiment to the first sign of the radicle (germination), from formation of the radicle to formation of the primary leaf and the overall duration (combination of the two cited time spans). The remaining combinations of start and end points were also statistically tested and graphically displayed in large parts to make sure that no major differences in development of hybrids and intra-cytotype crossings were overlooked (data not shown).

Cytotype distribution data obtained from FCM was displayed as barplots. Visual comparison was underpinned by Chi-Square tests which were performed to test for significant differences between the frequencies of hybrids created via fusion of two properly reduced gametes.

3. RESULTS

3.1. General results

Only 107 out of the 2585 exposed seeds (4.14%) were lost due to manipulation errors. 147 out of the 1551 germinated seeds (9.48%) displayed an abnormal development, most frequently by skipping one state. 165 of 1481 transplanted seedlings (11.41%) were mechanically injured in course of daily observation, a fact that could possible have affected their growth speed. However, because of the assumable random distribution of mechanical injury and the high incidence among the seedlings, these seedlings were not removed from the analyses. 153 out of 1551 seedlings (9.86%) showed morphological abnormalities, most commonly the formation of three or only one cotyledon instead of two. A Chi-Square test for independence revealed no significant deviation of the observed from the expected values (χ^2 =12.903, p=0.2997).

223 of the 628 germinated seeds from inter-cytotype-crossings (35.51%) perished before they could be sent to FCM, and 44 probes out of 588 (7.48%) failed to be cytometrizied.

19.74% of all seeds and 18.20% of all seedlings sent to FCM (from the inter-cytotype crossings only) were created via selfing and unreduced gametes were found in 4.82% of the screened seeds and 3.86% of the seedlings, though these are minimum values restricting interpretation (see legend to Table 29 for an explanation). Besides, selfing rates and prevalence of unreduced gametes was strongly clustered to certain treatments.

3.1.1. Germination and transplantation rates

Germination rates were almost equally high among all treatments (62.59±13.56). Only the germination rate for 4xSI diaspores was outside the borders of twice the standard deviation. However, given that treatments involving 4x gametes, especially when they were of maternal origin, display significantly longer germination duration, but our experiment was terminated after 55 days, the low germination rate must only be interpreted in relation to the other germination rates but may not be seen as absolute. Germination rates were lower than those of the two remaining intra-cytotype crossings for almost all inter-cytotype ones.

Transplantation rates were almost the same as germination rates (59.78±13.05), so once a diaspore produced a radicle it was very likely to end up as a transplantable seedling. Moderate deviations were only found in the treatments 2x4x and 6x2x, 6x6x and 6xSI (transplantation rate was -6.59%, -6.93%, -4.95% and -5.55% in relation to germination rate, respectively), which had seeds that

more frequently perished in radicle and cotyledon state than the other treatments. Table 5 gives an overview of germination and transplantation rates.

Treatment	Sample size	Number of germinated seeds	Germination rate [%]	Number of transplanted seeds	Transplantation rate [%]
2x2x	363	246	67.77	239	65.84
2x4x	182	110	60.44	98	53.85
2x6x	53	29	54.72	28	52.83
2xSI	92	70	76.09	69	75.00
4x2x	210	126	60.00	124	59.24
4x4x	300	130	43.33	123	41.00
4x6x	285	216	75.79	213	74.74
4xSI	129	42	32.56	42	32.56
6x2x	101	63	62.38	56	55.45
6x4x	365	220	60.27	210	57.53
6x6x	344	259	75.29	242	70.35
6xSI	54	40	74.07	37	68.52
Total	**2478**	**1551**	**62.59**	**1481**	**59.78**

Table 5: Comparision of germination and transplantation rates for the twelve treatments. Sample size is defined as total number of seeds used in the germination experiment minus those that were lost due to manipulation errors. Both rates were calculated using this sample size.

3.1.2. Death rates and mouldering diaspores

For all inter-cytotype crossings, death rates were higher than those of the respective intra-cytotype crossings. Apart from one exception (6xSI) they were also higher than those of the SI-treatments. Yet, as in the pursuit of the analysis dying diaspores (as well as those lost due to manipulation errors) were included for as long as data was available for them, no information loss or bias was risked in case that elevated death rates had any influence on the developmental speed of a treatment.

Mouldering of diaspores which was frequently observed during the germination experiment did not seem to influence their performance in terms of duration, but had a not negligible negative effect on survival rates (see chapter 3.2.3).

A Wilk-Shapiro test was performed to test for normal distribution of mould ($W=0.988$, $p=0.999$), and as the normality criterion was met, a Chi-Square test was performed to check for independence of observed and expected mould rates ($\chi^2=52.277$, $p<0.05$). On this basis, as well as on the basis of a visual comparison of infestation prevalence in certain mother plants and/or petri dishes, the decision was made to include mouldering diaspores in further data analysis.

Table 6 summarizes death and mould rates for the twelve treatments.

Treatment	Sample size	Number of dead seeds	Death rate	Number of mouldering seeds	Mould rate
2x2x	363	21	5.79	68	18.37
2x4x	182	58	31.87	67	36.81
2x6x	53	14	26.42	24	45.28
2xSI	92	6	6.52	10	10.87
4x2x	210	26	12.38	52	24.64
4x4x	300	10	3.33	86	28.67
4x6x	285	18	6.32	65	22.81
4xSI	129	9	6.98	54	41.86
6x2x	101	26	25.74	35	34.65
6x4x	365	15	4.11	106	29.04
6x6x	344	9	2.62	109	31.69
6xSI	54	9	16.67	13	24.07
Total	**2478**	**221**	**8.92**	**689**	**27.79**

Table 6: Prevalence of death and mould among the twelve treatments. Sample size is here defined as total number of seeds used in the germination experiment less those that were lost due to manipulation errors. All percentages were calculated using this sample size.

3.1.3 Time to Germination

Among the three intra-cytotype crossings germination duration (defined as the number of days needed from exposure in the climate chamber to the first documentation of the radicle) was shortest for 2x2x seeds (10.68±11.28), intermediate for 6x6x seeds (21.91±14.72) and longest for 4x4x seeds (31.28±14.77). Inter-cytotype treatments and SI treatments needed fewer days to form their radicle than the corresponding intra-cytotype crossing of the maternal cytotype, with 6x4x treatments being the only exception. 6x4x is also the treatment whose germination period was longest (24.60±15.24), whereas 2x6x diaspores display at the same time the shortest germination duration overall and the narrowest time span (7.35±6.85), but note that sample size for this treatment was the smallest of all crossings.

3.1.4 Growth parameters at transplantation state

As supplementary data, four morphological parameters (seedling weight, length of radicle, length of hypocotyl and maximum diameter of cotyledons) were recorded for each seedling at the day of transplantation. Graphical display (Figure 5) revealed a strong positive correlation between ploidy level (i.e. the number of chromosome sets) and weight, roots, cotyledon size and hypocotyl length (7x and 8x seedlings are not considered here as only one seedling was measured for each of those cytotypes). Triploid cotyledons are the only exception to the general pattern as their diameters are on average smaller than those of diploids.

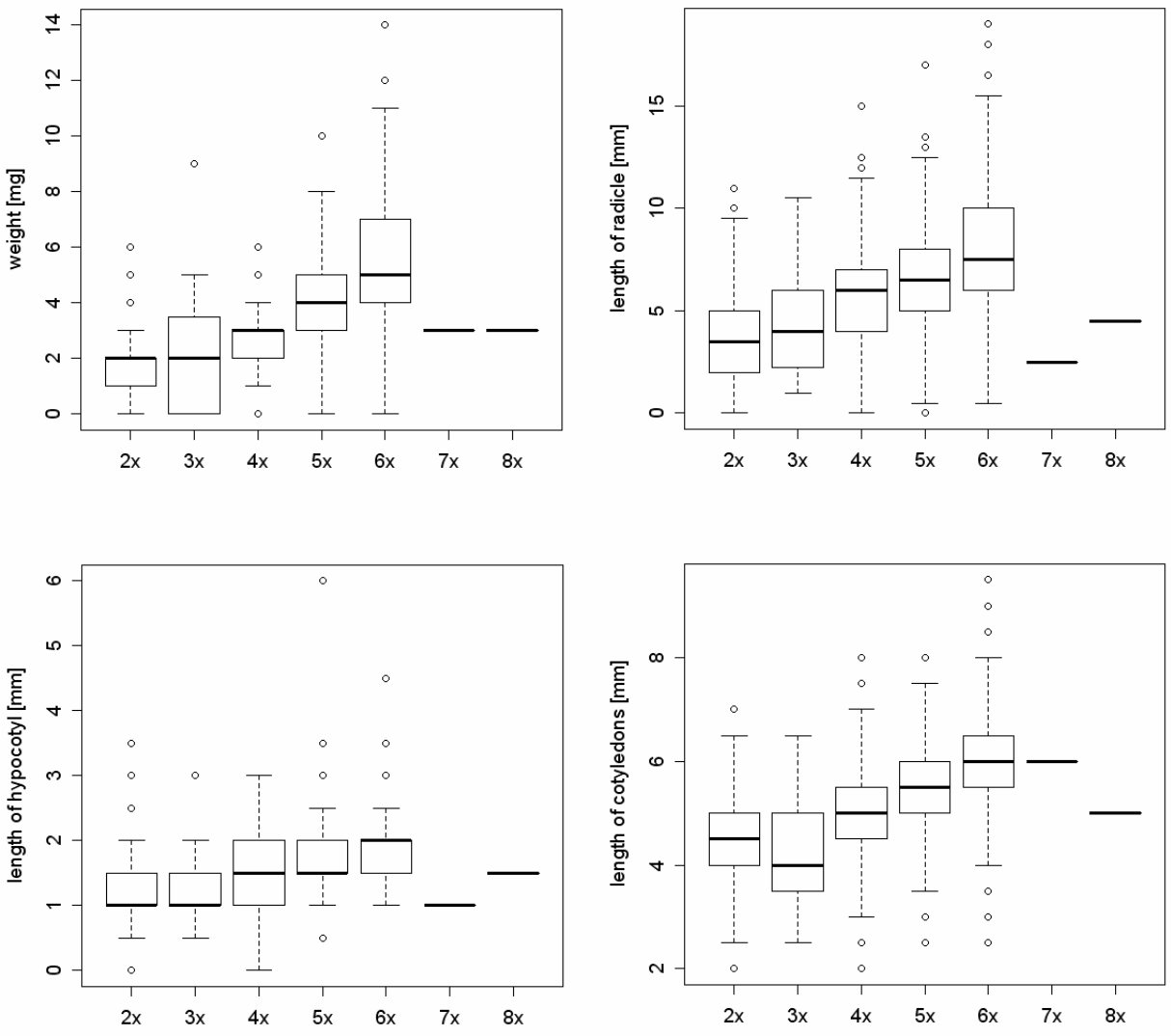


Figure 5: Morphological parameters of seedlings measured at transplantation stage (defined as the full spread of cotyledons). Sample sizes are identical for all four diagrams (2*x*: n=360, 3*x*: n= 72, 4*x*: n=194, 5*x*: n=355, 6*x*: n=315, 7*x*: n=1, 8*x*: n=1). Seeds from intra-cytotype crossings have been included under the implicit assumption that no unreduced gametes are produced and the seedlings always carry the parental cytotype.

3.2 Comparison between performances of hybrid versus intra-cytotype crossings

The six inter-cytotype crossings yielded three classes of progeny: seeds that were produced via selfing, hybrid seeds created through fusion of two properly reduced gametes and hybrid seeds formed under participation of one or two unreduced gametes. Seeds that resulted from selfing or from participation of unreduced gametes were excluded from the analysis of duration but could not be so for analyses of survival (see chapter 4.5 for an explanation). Three meaningful time spans (as described in chapter 2.4) have been chosen for analysis of duration and of survival rates.

For improved legibility we subsequently employ the term “hybrid” synonymous to “inter-cytotype”. “Hybrid seeds” therefore summarizes all diaspores formed in the respective crossing, whereas H_{red} designates only those that were formed through union of two properly reduced gametes and H_{unred} includes all seeds created via fusion of one reduced and one unreduced or of two unreduced gametes. The pooled value of the two respective parental intra-cytotype crossings is hereafter referred to as PPT (pooled parental treatments). The summarized period from exposure to formation of the primary leaf is characterized as “overall”, that means that for example overall survival rates are those calculated for the time span from exposure to primary leaf. The specification “germination” designates the period from exposure to formation of the radicle (e.g. germination duration, germination rates).

3.2.1. Duration

For the analyses of duration, only H_{red} were included.

Overall duration varied between all three intra-cytotype treatments, with 2x2x being very highly significantly faster than 6x6x ($p<0.001$) and 6x6x being very highly significantly faster than 4x4x ($p<0.001$). 2x4x developed very highly significantly faster than expected from the PPT ($p<0.001$), whereas 4x2x seeds took significantly longer ($p=0.050$). This leads to a very highly significant reciprocal difference between the two diploid-tetraploid combinations ($p<0.001$). No results could be obtained for 2x6x treatments as not a single H_{red} produced a seedling.

Regarding germination duration, all relationships between the three intra-cytotype crossings remained the same. Compared to the PPT, 2x4x and 4x6x germinated very highly significantly faster than expected ($p<0.001$ for both treatments). Values for 4x2x, 6x2x and 6x4x seeds did not differ significantly from the PPT. Note that the two treatments germinating faster than expected have higher ploidy levels in their pollen than in their egg cell donor. For the combinations between 2x and 4x

parents and 4x and 6x parents, reciprocal differences were observed, but they were just not big enough to be statistically significant ($p=0.128$ and $p=0.070$, respectively).

The duration from formation of the radicle to formation of the primary leaf revealed differences only between the 2x2x and 6x6x intra-cytotype treatment, with 2x2x being highly significantly slower than 6x6x ($p<0.001$). Hybrid treatments were either slower (4x2x, $p<0.001$; 4x6x, $p<0.001$) or developed at equal speed as their PPT equivalent. 2x4x produced their primary leaf very highly significantly faster than 4x2x ($p<0.001$), and 4x6x very highly significantly slower than 6x4x ($p<0.001$).

Mean germination duration for each treatment is visualized in Figures 6 to 8, and supplementary data obtained from the test statistics can be found in Tables 7 to 18.

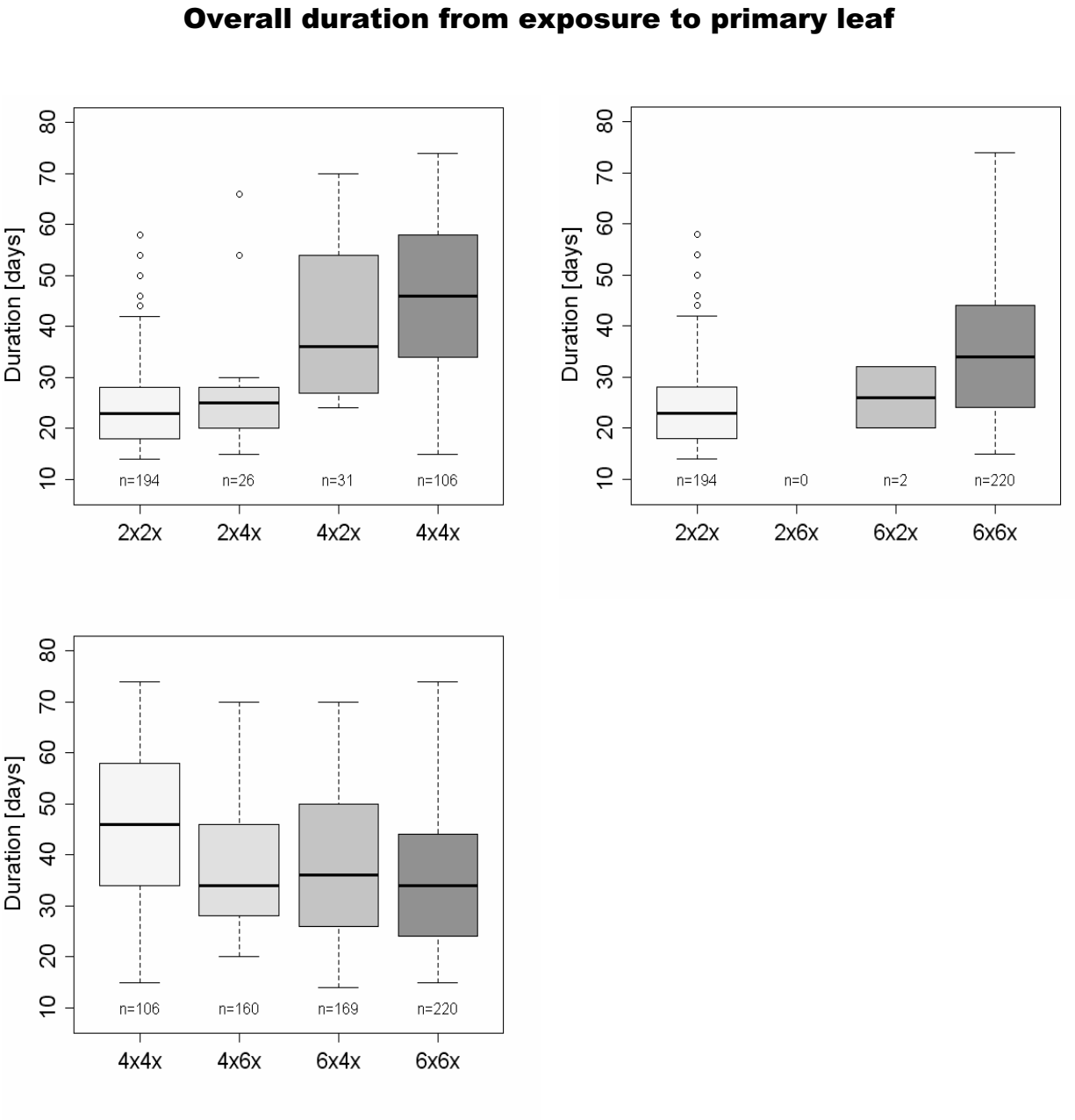


Figure 6: Mean duration from exposure to primary leaf formation. The bold line markes the median, the box represents the interquartile range (i.e. the range including 50% of all values, IQR). The whiskers show the minimum and maximum duration of each treatment within a predefined 1.5fold interquartile range above and below the box. Outliers (open circles) are therefore defined as those data points outside ±1.5*IQR. Sample sizes are given below each box.

	2x4x	4x2x	2x6x	6x2x	4x6x	6x4x
M	0.024± 0.076 p= 0.747	0.132± 0.078 p=0.090	No results	0.306± 0.198 p=0.121	0.175± 0.052 p<0.001	-0.055± 0.054 p=0.305
P	0.564± 0.078 p<0.001	0.408± 0.075 p<0.001	No results	0.010± 0.197 p=0.960	0.068± 0.050 p=0.173	0.188± 0.056 p<0.001

Table 7: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and their maternal and paternal intra-cytotype crossings. Models were applied to the duration from exposure to primary leaf formation. The values for estimated coefficients and standard errors indicate the effect of the treatment given as row caption relative to that given as column caption (i.e. the baseline level of the treatment variable). A negative coefficient therefore indicates that the treatment mentioned on the left takes fewer days to develop primary leaves than the treatment on top of the column. M(Maternal)=Intra-cytotype crossing of the respective egg donator, P(Paternal)=Intra-cytotype crossing of the respective pollen donator.

	Mean of mother and father (PPT)
2x4x	-0.098 ± 0.024; p<0.001
4x2x	0.046 ± 0.024; p=0.050
2x6x	No results
6x2x	-0.053±0.065; p=0.420
4x6x	-0.018±0.015; p=0.230
6x4x	-0.022±0.016; p=0.160

Table 8: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the pooled parental intra-cytotype crossings. Models were applied to the duration from exposure to primary leaf formation. For interpretation see legend to Table 7.

	Reciprocal Crossing
2x4x	-0.434 ± 0.101; p<0.001
2x6x	No results
4x6x	0.013 ± 0.056; p=0.816

Table 9: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the crossing in the reverse direction. Models were applied to the duration from exposure to primary leaf formation. For interpretation see legend to Table 7.

	2x2x	4x4x	6x6x
2x2x	---	-0.270 ± 0.027; p<0.001	-0.148 ± 0.025; p<0.001
4x4x	0.270±0.030; p<0.001	---	0.122 ± 0.025; p<0.001
6x6x	0.148±0.025; p<0.001	-0.122 ± 0.028; p<0.001	---

Table 10: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between all three intra-cytotype treatments. Models were applied to the duration from exposure to primary leaf formation. For interpretation see legend to Table 7.

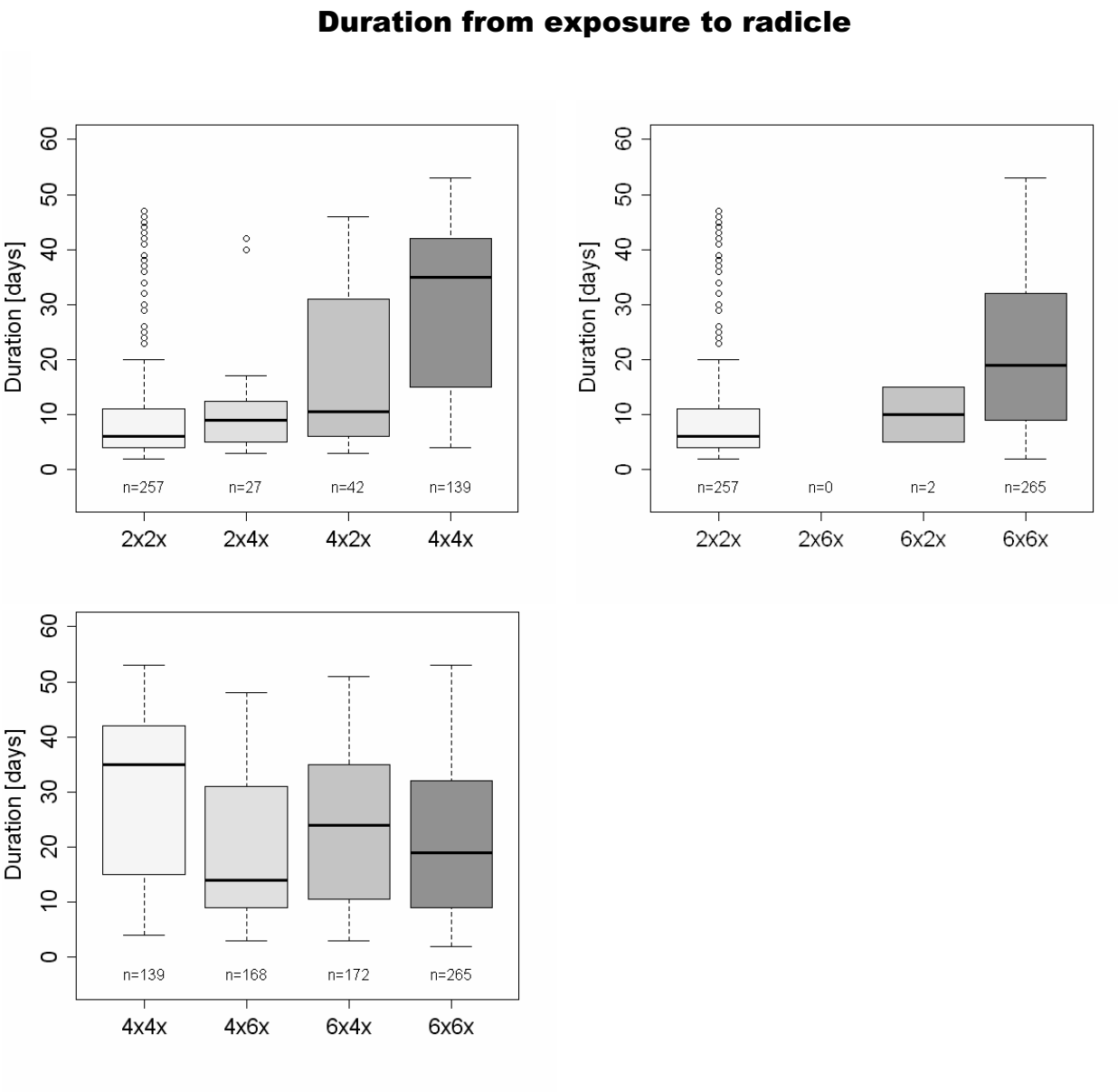


Figure 7: Boxplots for the mean duration from exposure to apparition of the radicle. For further information see legend to Figure 6. Sample sizes are given below each box.

	2x4x	4x2x	2x6x	6x2x	4x6x	6x4x
M	-0.000± 0.165 p=0.999	0.846± 0.165 p<0.001	No results	0.791± 0.454 p=0.816	0.524± 0.113 p<0.001	-0.089± 0.101 p=0.382
P	1.186± 0.167 p<0.001	-0.343± 0.162 p=0.034	No results	0.017± 0.454 p=0.971	0.112± 0.111 p=0.314	0.323± 0.103 p<0.002

Table 11: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and their maternal and paternal intra-cytotype crossings. Models were applied to the duration from exposure to radicle formation. For interpretation see legend to Table 7. M (Maternal)=Intra-cytotype crossing of the respective egg donator, P(Paternal)=Intra-cytotype crossing of the respective pollen donator.

	Mean of mother and father (PPT)
2x4x	-0.198±0.052; p<0.001
4x2x	-0.084±0.049; p=0.090
2x6x	No results
6x2x	-0.134±0.150; p=0.369
4x6x	-0.106±0.033; p=0.001
6x4x	-0.039±0.029; p=0.185

Table 12: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the pooled parental intra-cytotype crossings. Models were applied to the duration from exposure to formation of the radicle. For interpretation see legend to Table 7.

	Reciprocal Crossing
2x4x	-0.347±0.228; p=0.128
2x6x	No results
4x6x	-0.201±0.111; p=0.070

Table 13: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the crossing in the reverse direction (exchange of maternal and paternal cytotype). Models were applied to the duration from exposure to formation of the radicle. For interpretation see legend to Table 7.

	2x2x	4x4x	6x6x
2x2x	---	-0.593±0.061; p<0.001	-0.387±0.064; p<0.001
4x4x	0.594±0.067; p<0.001	---	0.206± 0.054; p<0.001
6x6x	0.387±0.064; p<0.001	-0.206±0.051; p<0.001	---

Table 14: Results of the Generalized Linear Mixed-Effects Model Tests for significant differences between all three intra-cytotype treatments. Models were applied to the duration from exposure to formation of the radicle. For interpretation see legend to Table 7.

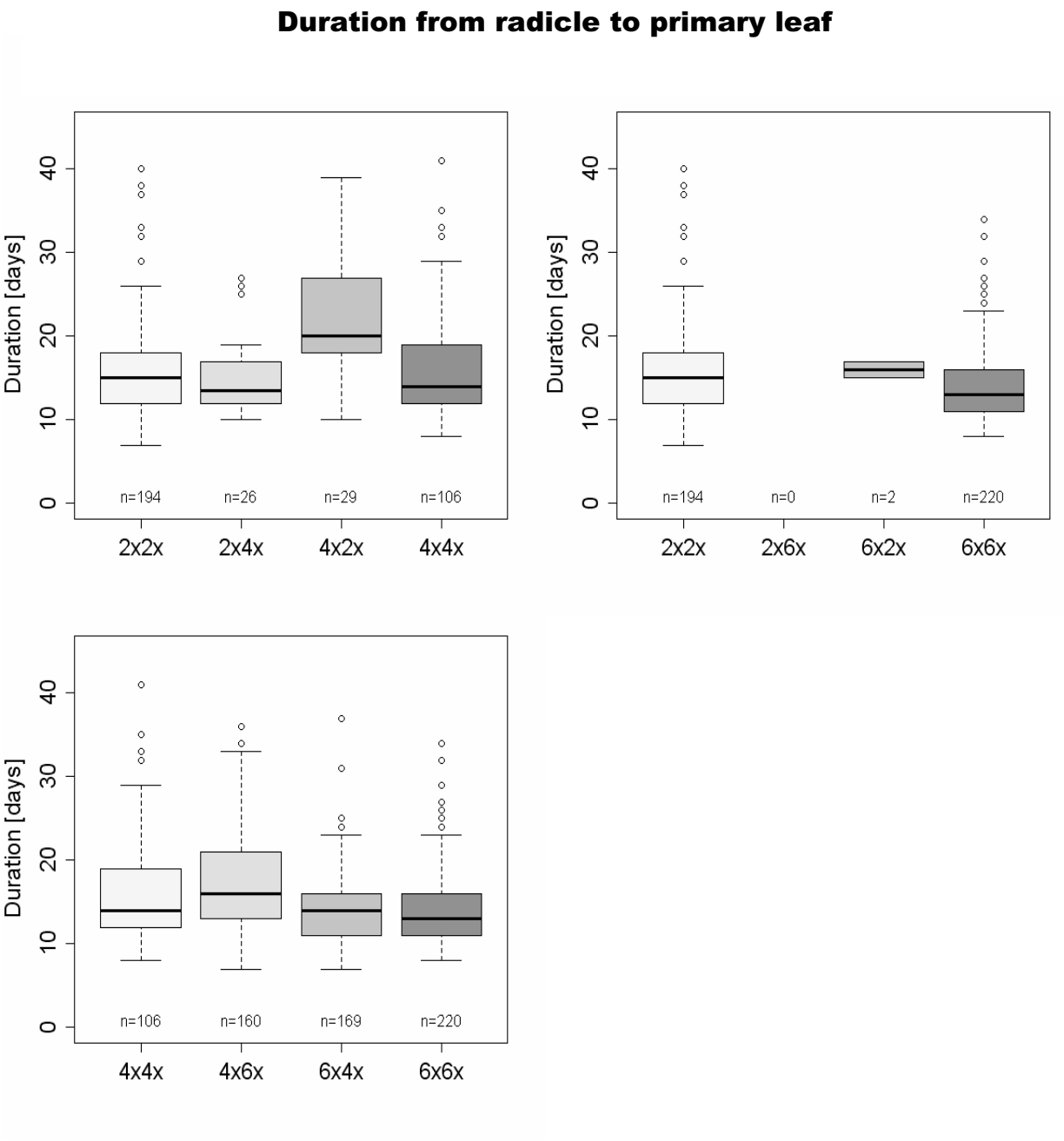


Figure 8: Boxplots for the mean duration from apparition of the radicle to primary leaf formation. For further information see legend to Figure 6. Sample sizes are given below each box.

	2x4x	4x2x	2x6x	6x2x	4x6x	6x4x
M	0.042± 0.077 p=0.583	-0.402± 0.075 p<0.001	No results	-0.121± 0.208 p=0.560	-0.140± 0.056 p<0.013	-0.005± 0.045 p=0.905
P	0.000± 0.081 p=0.996	-0.359± 0.071 p<0.001	No results	-0.004± 0.208 p=0.986	-0.215± 0.052 p<0.001	0.076± 0.049 p=0.124

Table 15: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and their maternal and paternal intra-cytotype crossings. Models were applied to the duration from apparition of the radicle to primary leaf formation. For interpretation see legend to Table 7. M(Maternal)=Intra-cytotype crossing of the respective egg donator, P(Paternal)=Intra-cytotype crossing of the respective pollen donator.

	Mean of mother and father (PPT)
2x4x	-0.007±0.025; p= 0.773
4x2x	0.127±0.022; p<0.001
2x6x	No results
6x2x	0.021± 0.069; p=0.763
4x6x	0.059±0.016; p<0.001
6x4x	-0.012±0.014; p=0.385

Table 16: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the pooled parental intra-cytotype crossings. Models were applied to the duration from apparition of the radicle to primary leaf formation. For interpretation see legend to Table 7.

	Reciprocal Crossing
2x4x	-0.400±0.084; p<0.001
2x6x	No results
4x6x	0.209±0.047; p<0.001

Table 17: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the crossing in the reverse direction. Models were applied to the duration from apparition of the radicle to primary leaf formation. For interpretation see legend to Table 7.

	2x2x	4x4x	6x6x
2x2x	---	0.021±0.027; p=0.438	0.059±0.022; p=0.008
4x4x	-0.022±0.028; p=0.437	---	0.038±0.027; p=0.169
6x6x	-0.059±0.022; p=0.008	-0.041±0.024; p=0.094	---

Table 18: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between all three intra-cytotype treatments. Models were applied to the duration from apparition of the radicle to primary leaf formation. For interpretation see legend to Table 7.

Apart from these three most interesting time spans, cumulative durations for H_{red} from each treatment were also displayed graphically to check for differential acceleration or deceleration of any treatment in any developmental stage or stages (Figure 9). Selfed seeds from the four SI control groups, though not regarded in the Generalized Linear Mixed-Effects Model analysis, were included in this diagram to compare the relative fitness of autogamous and allogamous seeds and the relative fitness of selfed and inter-cytotype hybrid seeds.

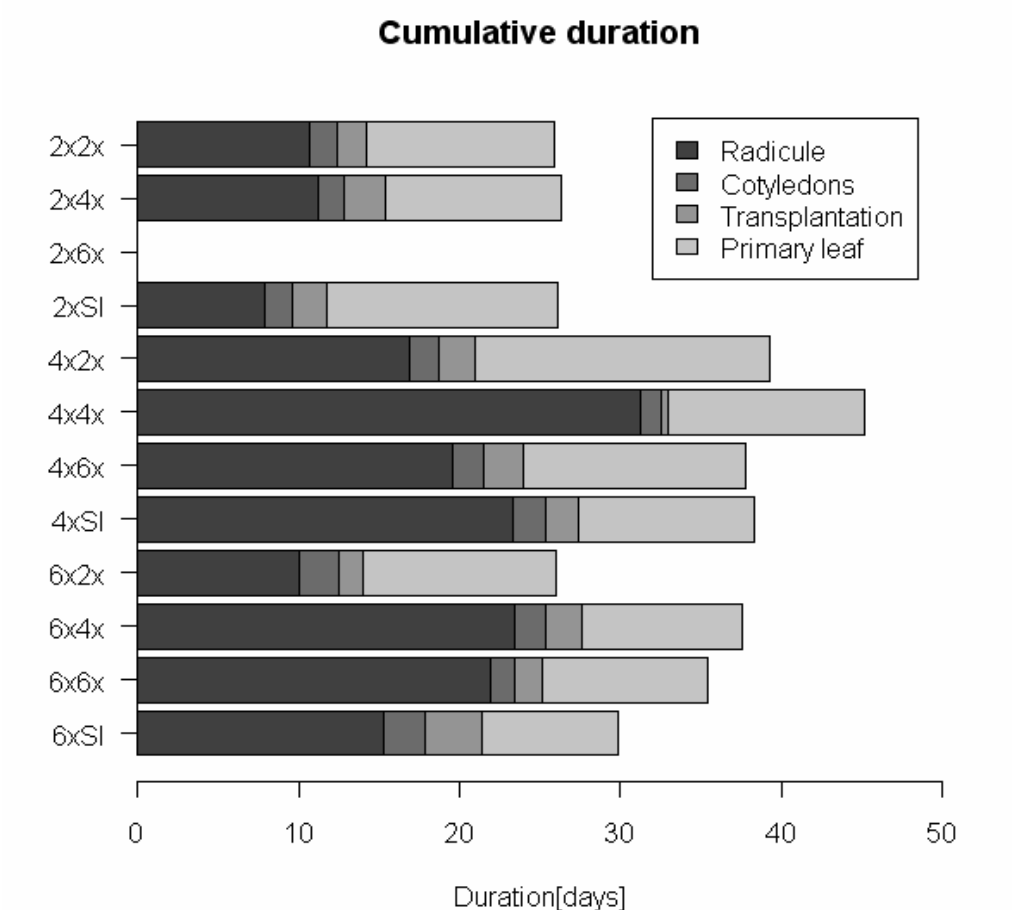


Figure 9: Mean of the days needed to accomplish the given stage (see legend) for all twelve treatments. 2x2x: n=192; 2x4x: n=26; 2x6x: n=0; 2xSI: n=49; 4x2x: n=31; 4x4x: n=103; 4x6x: n=159; 4xSI: n=37; 6x2x: n=2; 6x4x: n=169; 6x6x: n=219; 6xSI: n=31.

As already mentioned in chapter 3.1.3, all three SI-Treatments germinated earlier than the corresponding intra-cytotype crossing. But, examining subsequent stages too, this temporal lead is at least partially compensated by the slower formation and spread of the cotyledons, particularly for tetraploids and hexaploids. Most interestingly, tetraploid seeds from the intra-cytotype crossing which are the last to germinate are by far the fastest to produce and spread their cotyledons.

The time needed from transplantation to formation of the first leaf is more or less the same for all treatments, except for 4x2x hybrids, which take approximately twice as long as the fastest treatment (6xSI). Correlating with germination time, all H_{red} produced with participation of diploid

gametes (pollen and/or eggs), except for 4x2x, accomplished their overall development earlier than the rest, all H_{red} with participation of tetraploid pollen and/or eggs, except for 2x4x, later.

3.2.2. Survival

Survival rates were analysed for all hybrid treatments in the same way, yet the experimental design did not allow for exclusion of selfed seeds and H_{unred} without distorting the results (see chapter 4.5). Survival rates therefore provide only restricted insight in the behaviour of the H_{red} of our interest, but represent all seeds resulting from the respective treatment. Discussion of these data therefore is done in combination with the results given in chapter 3.3.

As to overall survival, 4x6x was the only treatment to survive highly significantly more than its parents ($p= 0.006$). Seeds from the treatments 2x6x and 6x2x where significantly less viable than expected from the PPT ($p=0.018$ and $p=0.047$ respectively). They both highly significantly varied from the 6x6x survival rate ($p=0.002$ and $p=0.005$) but not from the 4x4x one. Comparison of all three intra-cytotype crossings resulted in the finding that 6x6x seeds had the highest survival rate, followed by 2x2x and 4x4x. All differences were highly significant or very highly significant.

Germination rates showed the same pattern for the three intra-cytotype crossings. 4x2x germinated significantly more often than the PPT ($p=0.019$), same as 4x6x seeds ($p<0.001$). Seeds resulting from the 2x6x crossing were the only ones to exhibit germination rates lower than expected ($p=0.027$).

During the time span from formation of the radicle to the first leaf 2x4x, 4x2x and 6x2x seeds showed decreased viability ($p=0.002$, $p<0.001$ and $p=0.041$ respectively). All remaining inter-cytotype crossings did not vary significantly from the PPT.

Reciprocal differences as to the period with highest mortality were found for the diploid-hexaploid combination: Whereas 2x6x seeds perished before forming a radicle, 6x2x seeds rather died before primary leaf formation.

Results for the analysis of survival rates are presented in Figures 10 to 12 and Tables 19 to 27.

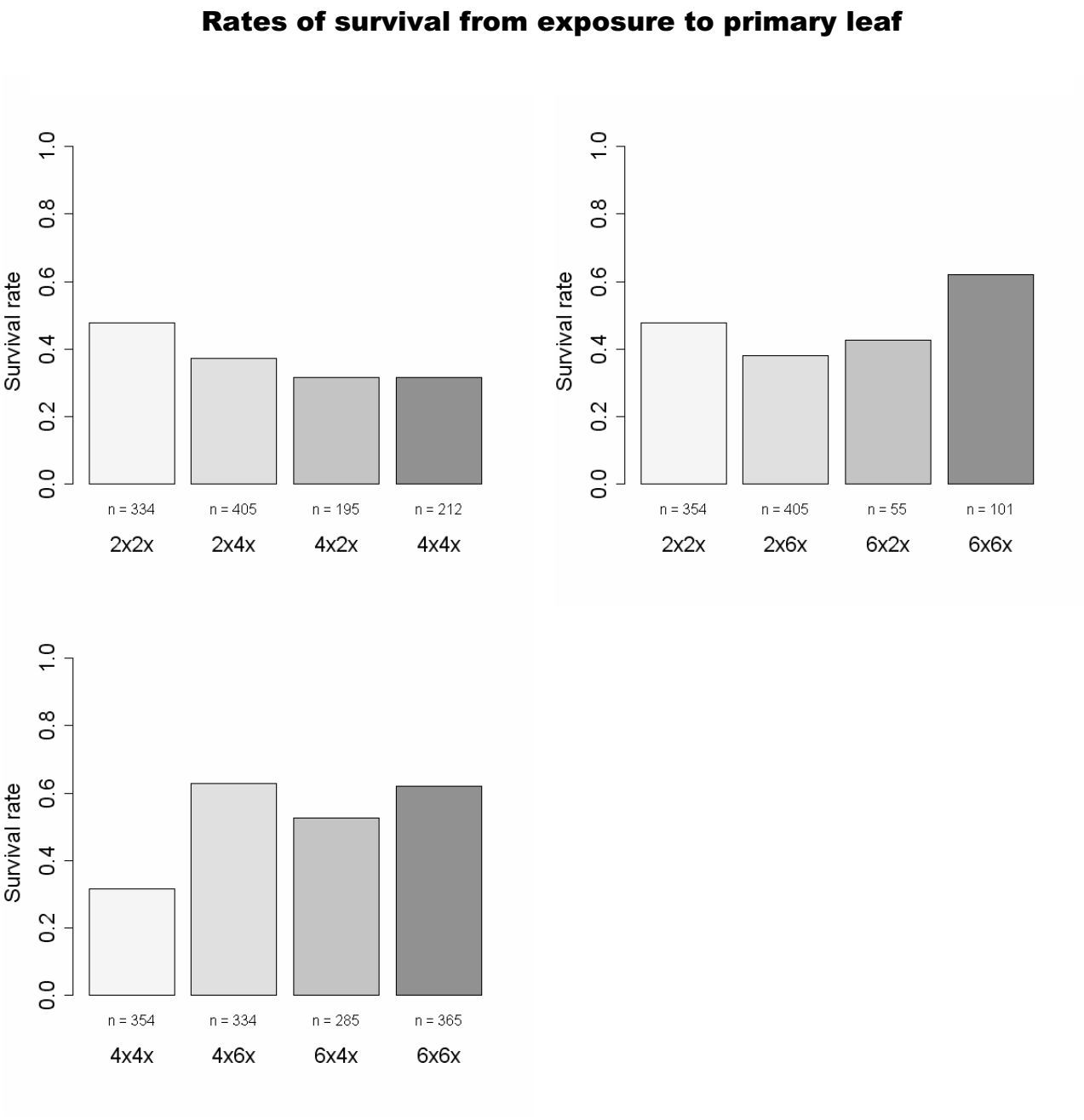


Figure 10: Barplots for overall survival rates of all inter- and intra-cytotype treatments, from exposure to formation of the primary leaf. Sample sizes are given below each bar.

	2x4x	4x2x	2x6x	6x2x	4x6x	6x4x
M	0.390± 0.264 p=0.140	0.025± 0.263 p=0.925	0.519± 0.388 p=0.181	1.026± 0.365 p=0.005	-1.478± 0.302 p<0.001	0.481± 0.275 p=0.080
P	-0.366± 0.277 p=0.186	0.770± 0.250 p=0.002	1.236± 0.397 p=0.002	0.299± 0.354 p=0.399	0.041± 0.301 p=0.890	-1.024± 0.276 p<0.001

Table 19: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and their maternal and paternal intra-cytotype crossings. Models were applied to the duration from exposure to primary leaf formation. The values for estimated coefficients and their standard errors indicate the effect of the treatment given as row caption relative to that given as column caption (i.e. the baseline level of the treatment variable). A negative coefficient therefore indicates that the treatment mentioned on the left survives less during the period from exposure to primary leaf formation than the treatment mentioned on top of the column. M(Maternal)=Intra-cytotype crossing of the respective egg donator, P(Paternal)=Intra-cytotype crossing of the respective pollen donator.

	Mean of mother and father (PPT)
2x4x	-0.004±0.081; p=0.961
4x2x	-0.132±0.077; p=0.085
2x6x	-0.292±0.124; p=0.018
6x2x	-0.221±0.111; p=0.047
4x6x	0.239±0.088; p=0.006
6x4x	0.091±0.079; p=0.250

Table 20: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the hypothetical mean of maternal and paternal intra-cytotype crossings. Models were applied to the survival rates from exposure to formation of the primary leaf. For interpretation see legend to Table 19.

	2x2x	4x4x	6x6x
2x2x	---	0.378±0.120; p=0.002	-0.358±0.128; p=0.005
4x4x	-0.373±0.113; p<0.001	---	-0.760±0.147; p<0.001
6x6x	0.364±0.133; p=0.006	0.753±0.142; p<0.001	---

Table 21: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between all three intra-cytotype treatments. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19.

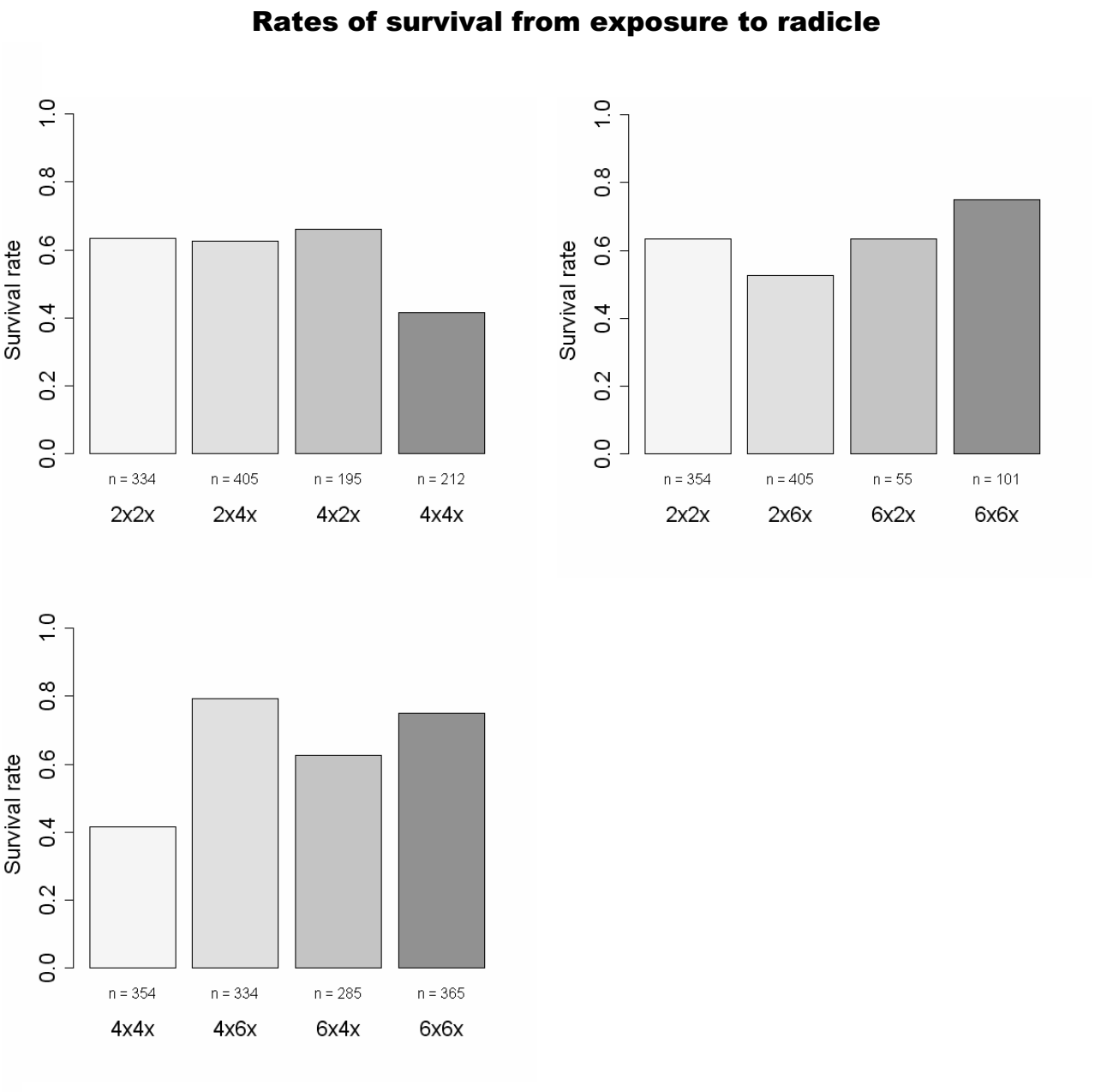


Figure 11: Barplots for survival rates of all inter- and intra-cytotype treatments, from exposure to formation of the radicle. Sample sizes are given below each bar.

	2x4x	4x2x	2x6x	6x2x	4x6x	6x4x
M	0.005± 0.302 p=0.988	-1.142± 0.302 p<0.001	0.536± 0.421 p=0.203	0.724± 0.400 p=0.070	-1.975± 0.315 p<0.001	0.666± 0.292 p=0.023
P	-1.037± 0.311 p<0.001	-0.107± 0.293 p=0.716	1.229± 0.436 p=0.005	0.035± 0.384 p=0.927	-0.250± 0.324 p=0.440	-1.050± 0.283 p<0.001

Table 22: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and their maternal and paternal intra-cytotype crossings. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19. M(Maternal)=Intra-cytotype crossing of the respective egg donator, P(Paternal)=Intra-cytotype crossing of the respective pollen donator.

	Mean of mother and father (PPT)
2x4x	0.172±0.092; p=0.060
4x2x	0.208±0.089;p=0.019
2x6x	-0.294±0.133; p=0.027
6x2x	-0.127±0.121; p=0.294
4x6x	0.371±0.094; p<0.001
6x4x	0.064±0.082; p=0.437

Table 23: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the hypothetical mean of maternal and paternal intra-cytotype crossings. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19.

	2x2x	4x4x	6x6x
2x2x	---	0.521±0.137; p<0.001	-0.346±0.154; p=0.024
4x4x	-0.517±0.133; p<0.001	---	-0.862±0.150; p<0.001
6x6x	0.344±0.152; p=0.023	0.858±0.148; p<0.001	---

Table 24: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between all three intra-cytotype treatments. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19.

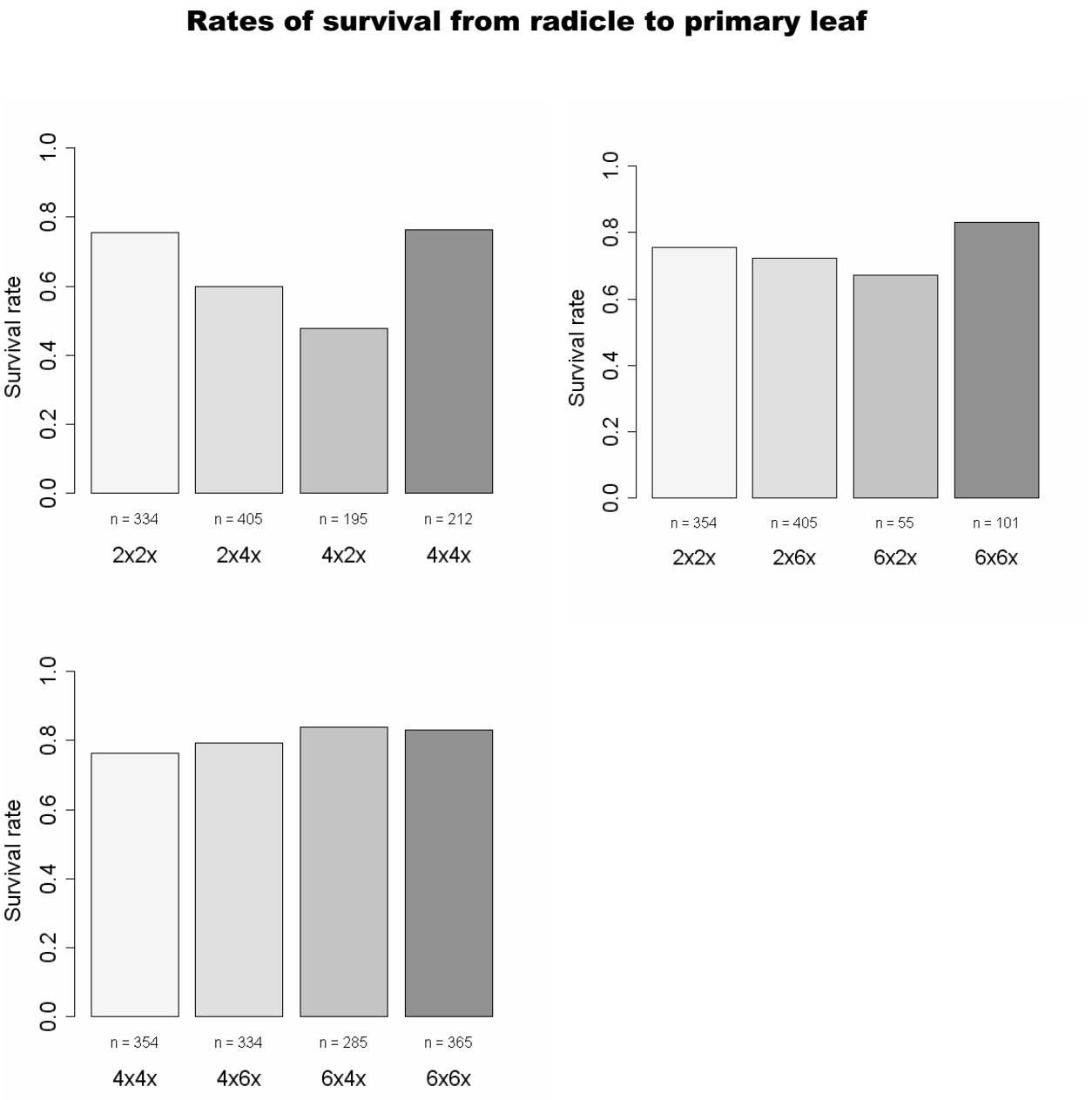


Figure 12: Barplots for survival rates of all inter- and intra-cytotype treatments, from appartion of the radicle to formation of the primary leaf. Sample sizes are given below each bar.

	2x4x	4x2x	2x6x	6x2x	4x6x	6x4x
M	0.728± 0.254 p<0.001	1.304± 0.283 p<0.001	0.211± 0.506 p=0.676	1.124± 0.430 p=0.009	0.005± 0.411 p=0.990	0.131± 0.369 p=0.723
P	0.785± 0.291 p=0.007	1.246± 0.244 p<0.001	0.825± 0.518 p=0.111	0.473± 0.414 p=0.253	0.553± 0.392 p=0.158	-0.408± 0.388 p=0.294

Table 25: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and their maternal and paternal intra-cytotype crossings. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19. M(Maternal)=Intra-cytotype crossing of the respective egg donator, P(Paternal)=Intra-cytotype crossing of the respective pollen donator.

	Mean of mother and father (PPT)
2x4x	-0.252±0.080; p=0.002
4x2x	-0.425±0.076; p<0.001
2x6x	-0.173±0.163; p=0.291
6x2x	-0.266±0.131; p=0.041
4x6x	-0.093±0.114; p=0.416
6x4x	0.046±0.108; p=0.670

Table 26: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between inter-cytotype hybrids and the hypothetical mean of maternal and paternal intra-cytotype crossings. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19.

	2x2x	4x4x	6x6x
2x2x	---	-0.028±0.132; p=0.831	-0.307±0.147; p=0.037
4x4x	0.029±0.133; p=0.828	---	-0.274±0.209; p=0.190
6x6x	0.325±0.158; p=0.039	0.269±0.195; p=0.168	---

Table 27: Results of the Generalized Linear Mixed-Effects Models testing for significant differences between all three intra-cytotype treatments. Models were applied to the survival rates from exposure to formation of the radicle. For interpretation see legend to Table 19.

3.2.3 Mouldering diaspores

It was clear from the Chi-Square test and the analysis of fungal infestation incidence among certain mother plants and petri dishes that mouldering had different effects among the treatments. Assuming that mouldering would distribute similarly in the field we did not remove these diaspores from the analysis in order not to distort the results. Nevertheless were Generalized Linear Mixed-Effects Models repeated without infested seeds to find out which crossing combinations were particularly effected by mould.

When mouldering diaspores were excluded from the analysis, the results remained the same in large parts. Mouldering seemed to have almost no effect at all on the developmental speed, whereas some minor changes occurred when mouldering was excluded from survival analysis (data not shown).

In detail, the analysis of duration for uninfected seeds only revealed a slightly higher success of 4x4x seeds. In other words, 4x4x seeds infested by fungi were more restrained in growth speed than all other treatments.

In the analysis of overall survival, significances for the differences between 6x6x seeds and from the PPT decreased for 2x6x and 6x2x diaspores. In other words, mouldering seemed to be disproportionately abundant among these two treatments. On the contrary, the very successful crossing combination 4x6x did not perform significantly better than expected when mouldering was excluded, but highly so when all diaspores were considered. In other words, 4x6x seeds seemed to be disproportionately resistant to fungal infestation.

Concluding, these analyses go along with the basic calculation of mould rates (presented in chapter 3.1.3.), in the sense that high mould rates correlated with high mortality, yet there are exceptions (e.g. seeds from 2x4x).

3.3 Distribution of cytotypes resulting from inter-cytotype crossings

DNA ploidy level was determined flow-cytometrically for half of the seeds. The results obtained from these seeds were compared to the FCM data of the seedlings at the end of the experiment to see if ploidy level distribution was the same in seeds and seedlings, in other words if there was any selective mortality of H_{red}, H_{unred} and selfed seeds. The distribution of the resulting ploidy levels is shown in Figures 13 to 15; differences in their proportions in seeds versus in seedlings are given in Table 28.

Significant differences in H_{red} frequencies of seeds and seedlings were found for the treatments 2x4x, 2x6x and 6x2x. Among all seeds produced via pollination of diploid flowers with pollen from tetraploid plants (2x4x) those that were created via selfing survived to transplantation disproportionately well. H_{unred} germinated less than expected from their prevalence in seeds, but still experienced fewer decrease in frequency than H_{red}. This pattern remains the same for 2x6x and 6x2x seeds: Selfed diaspores displayed disproportional high viability, followed by H_{unred}. H_{red} were the least successful cytotype. For the 2x6x crossing, the disadvantage of H_{red} is so grave that not a single one of them yielded a transplantable seedling despite the fact that more than every fifth seed (23.26%) originally produced is tetraploid. For 6x2x seeds, two hybrids detected at the end of the experiment (5.13%) face eighteen hybrids detected in advance of the germination experiment (31.58%). Most interestingly, for the two combinations including a diploid and a hexaploid parent, the share of seeds leading to no result because of bad embryo material was by far the highest. These most probably belonged to hybrids that perished even before the embryo was properly formed.

The three remaining treatments (4x2x, 4x6x and 6x4x) show near-identical distributions for seeds and seedlings. In the combination between tetraploids and hexaploids almost 100% of all seeds produced were H_{red}, and this did not alter when seedling FCM data was regarded. Among 4x6x seeds those created via selfing were at a slight disadvantage, yet this could also be an artefact caused by the restriction of the germination time (which was particularly grave for tetraploid intra-cytotype crossings) and does not necessarily mean that selfed diaspores are less viable than hybrid ones.

Crossings between tetraploids and hexaploids were highly successful, and all embryos for this treatment were in sufficient condition to be cytometrized.

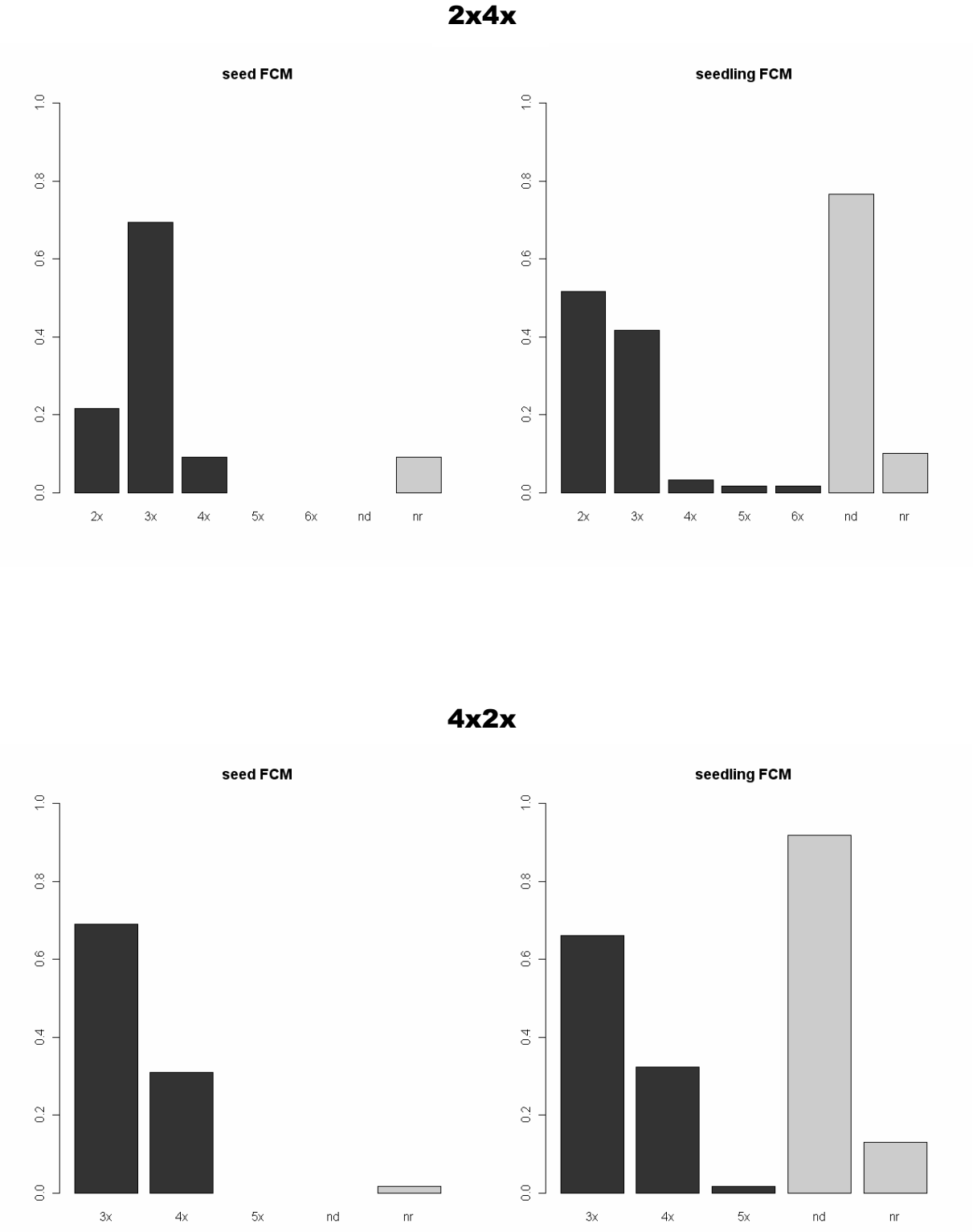


Figure 13: Cytotype distribution of seed and seedlings in the crossing treatments between diploids and tetraploids. The black bars indicate the percentage of each designated cytotype, and so per definitionem, the values for all black bars taken together equal 1. The "nd" bar (not determined) is the percentage of all seeds that did not yield measurable seedlings, either because they did not germinate at all or because they perished before flow cytometry (FCM). Its height is relative to the absolute number of results obtained. "Nr" (No result) is the percentage of seedlings that gave no result in FCM, when the plant material was in too bad condition at to moment of sampling.

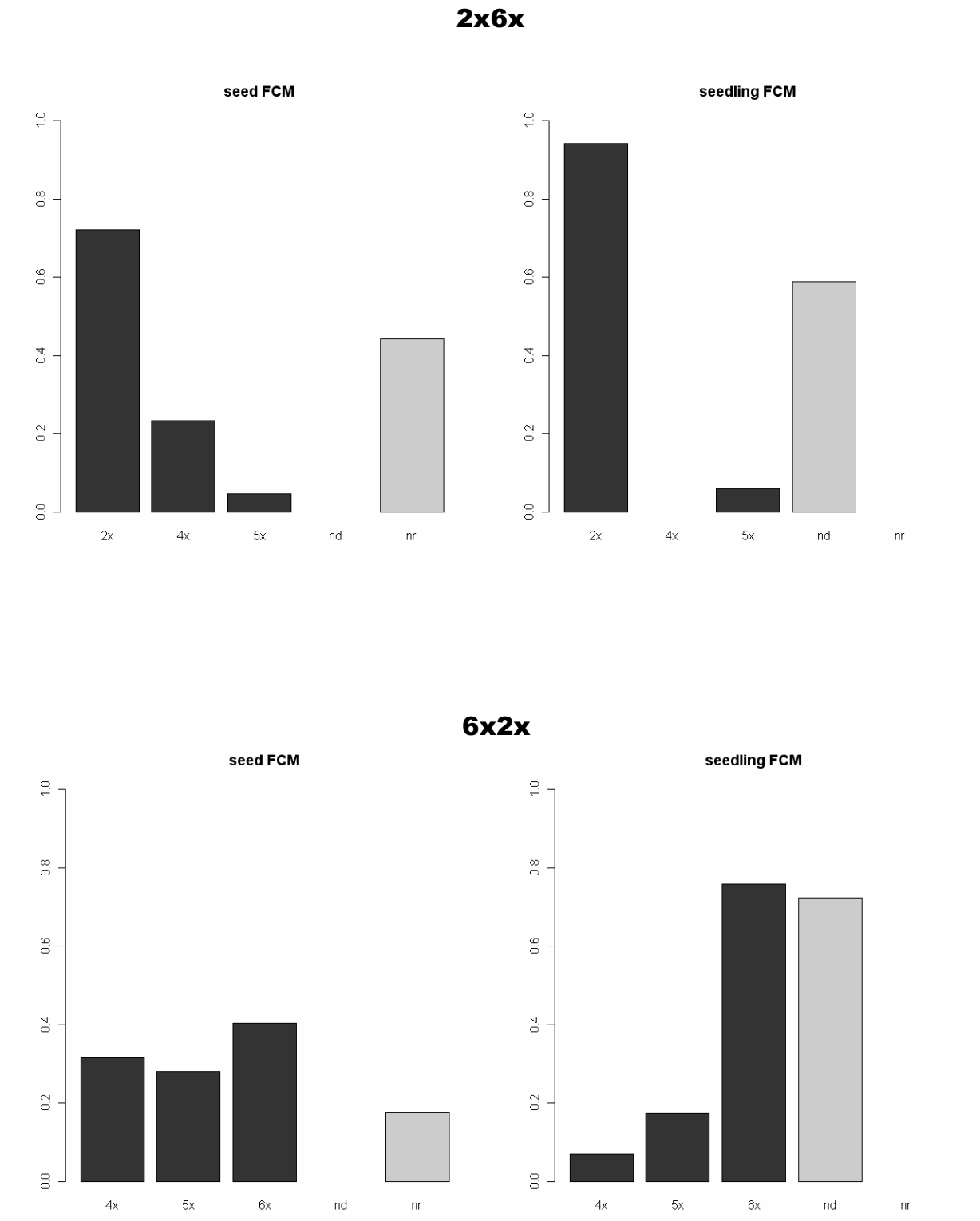


Figure 14: Cytotype distribution of seed and seedlings, crossings between diploids and hexaploids. For further explantations see legend to Figure 13.

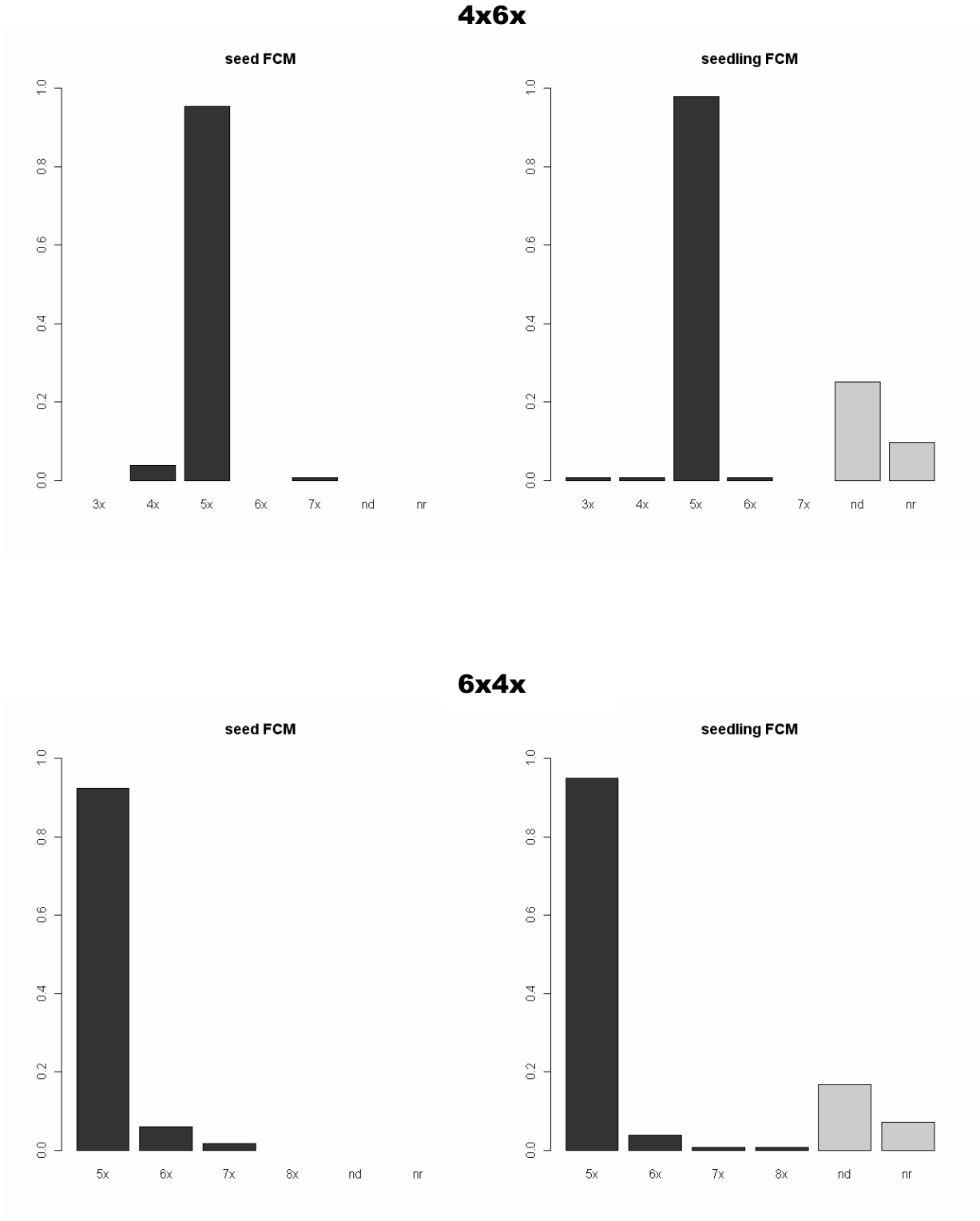


Figure 15: Cytotype distribution of seed and seedlings, crossings between tetraploids and hexaploids. For further explanation see legend to Figure 13.

Treat-ment	Cyto-type	Interpretation	N seeds	N seed-lings	Deviation (%)	
2x4x	2x	Selfing	26	33	+ 29.53	Chi-Square-Test for equal distribution of cytotypes: $\chi^2 = 11.766$ $p < 0.001$
	3x	Hybrid	81	27	- 26.45	
	4x	Hybrid, unred. egg	11	2	- 6.19	
	5x	Hybrid, unred. pollen	0	1	+ 1.56	
	6x	Hybrid, unred. egg and pollen	0	1	+ 1.56	
	Nd	-	0	51		
	Nr	-	11	7		
4x2x	3x	Hybrid	85	44	- 4.40	$\chi^2 = 0.059$ $p = 0.808$
	4x	Selfing	38	22	+ 1.46	
	5x	Hybrid, unred. egg	0	2	+ 2.94	
	Nd	-	0	63		
	Nr	-	2	8		
2x6x	2x	Selfing	31	17	+ 22.35	$\chi^2 = 3.218$ $p = 0.073$
	4x	Hybrid	10	0	- 23.26	
	5x	Hybrid, unred. pollen	2	1	+ 0.91	
	Nd	-	0	11		
	Nr	-	19	0		
6x2x	4x	Hybrid	18	2	- 26.45	$\chi^2 = 5.251$ $p = 0.022$
	5x	Hybrid, unred. pollen	16	11	+ 0.14	
	6x	Selfing	23	26	+ 26.32	
	Nd	-	0	26		
	Nr	-	10	0		
4x6x	3x	unclear	0	1	+ 0.58	$\chi^2 = 0.735$ $p = 0.391$
	4x	Selfing	6	2	- 2.74	
	5x	Hybrid	147	168	+ 1.66	
	6x	unclear	0	2	+ 1.16	
	7x	Hybrid, unred. egg	1	0	- 0.65	
	Nd	-	0	37		
	Nr	-	0	16		
6x4x	5x	Hybrid	172	172	+ 2.56	$\chi^2 = 0.462$ $p = 0.497$
	6x	Selfing	11	7	- 2.04	
	7x	Hybrid, unred. pollen	3	1	- 1.06	
	8x	Hybrid, unred. egg	0	1	+ 0.55	
	Nd	-	0	35		
	Nr	-	0	13		

Table 28: Distribution of cytotypes in the progeny of the six inter-cytotype crossings: comparison of data from seed and seedling FCM. Deviation is defined as the difference between the proportion of a cytotype in the seed and seedling sample. Deviations above zero indicate a higher proportion of the concerned cytotype in seedlings compared to seeds and hence a higher germination and survival rate. A p-value of less than 0.05 indicates an unequal distribution of the H_{red} frequency in seeds and seedlings. N seeds=Number of seeds sent to FCM, N seedlings=Number of seedlings sent to FCM, nd=not determined; nr=no results. For calculations of the deviation, individuals without cytotype determination (nr, nd) were excluded.

4. DISCUSSION

4.1 General outcome of the experiment

In the light of the fact that the three main cytotypes of *Senecio carniolicus* will probably soon be described as proper species (see chapter 1.2.2), our crossings and the subsequent germination experiment revealed unexpected results: Crossings yielded at least some fructified seeds in all combinations of parental cytotypes (see chapter 4.4), and for each treatment a smaller or larger proportion of these seeds did actually germinated and produce at least visually healthy seedlings. At a closer look slight performance disadvantages of hybrid seeds (seeds from inter-cytotype crossings) can be made out, especially for those crossings involving diploid, still we must conclude that the postzygotic isolation that we investigated is a lot weaker than expected.

As presented in chapter 3.1.4, we observed that seedlings with higher ploidy level were larger and heavier, and that this correlation was approximately linear. This underlines the findings previously made by many scientists working on polyploidy (Stebbins, 1979; Otto and Whitton, 2000; but see Comai (2005) for a different view on the cell size of autopolyploids). Larger cells and organs have also often been associated with the broader ecological tolerances found in polyploids (e.g. Otto and Whitton, 2000). Their evolutionary significance remains highly questionable, as increased cell size is supposed to be mainly reached through elevated turgor pressure than through mobilization of supplementary nutrient resources. Larger DNA contents need more time to be replicated and are therefore not necessarily advantageous (Bennett et al., 1977; Large Genome Constraint Hypothesis: Knight et al., 2005). As they were only measured once and their significance for hybrid survival (and consequently for postzygotic isolation) is unclear, these performance data were not regarded in further analysis and are not going to be subjected to further discussion.

4.2 Seedling performance

We know that heterozygosity is able to confer huge evolutionary advantages to its bearer by combining two or more alleles that are highly beneficial when inherited together. For example, they can confer superior viability and fertility to a hybrid in a certain environment (hybrid vigour, Bomblies et al., 2007; Chen, 2010). For autopolyploids like *Senecio carniolicus* presumably is, hybrid vigour is not expected as the genetic divergence between the parents is supposed to be rather low (Parisod et al., 2010).

Indeed, neither treatment of the crosses among the ploidy levels of *Senecio carniolicus* did display any transgressive characters (heterosis effects, e.g. Bomblies et al., 2007), neither positive nor negative: All results obtained for developmental speed and survival rates lay within the parental ranges.

4.2.1 Comparison of developmental speed of inter- and intra-cytotype crossings

When analyses of duration were done, only seeds created via properly reduced gametes (H_{red}) were regarded.

For germination duration, inter-cytotype crossings did not show identical patterns in both directions: In combinations where the mother is of lower ploidy than the father, the seeds germinated significantly faster than the pooled value of the two parental intra-cytotype crossings (PPT), whereas in the other direction, no significant deviation of the H_{red} values from the PPT could be observed. Yet, the difference is just not big enough to reveal significant reciprocal differences in the respective test.

Even though some scientists claim that members of the Asteraceae family produce seeds with no endosperm once mature (Ramsey and Schemske, 1998), FCM samples of *Senecio carniolicus* produce two distinct peaks which can only be the result of at least remnants of an endosperm. Therefore genomic imprinting is very likely to also play a role in our study species. In that case an excess of paternal genome above the target ratio of 2:1 (see chapter 1.1.4) would lead to an accelerated nutrient acquisition of the embryo in early stages. This is very likely the reason for the temporal advantage of H_{red} resulting from 2x4x and 4x6x crossings over those from 4x2x and 6x4x treatments, respectively. As the endosperm is poorly developed the available nutrients are restricted and the growth potential of these diaspores is supposed to be exhausted in later stages. In fact, this can be seen in the analyses for later stages, when performance of hybrid seeds generally becomes worse than those of intra-cytotype crossings, concerning duration and survival alike.

The duration from radicle to primary leaf formation is longer or not different from the parental intra-cytotype crossings for all hybrid treatments, with 4x2x and 4x6x showing the worst performance in relation to the PPT. No general pattern was found in this stage. A possible negative effect of diploid egg cells produced by tetraploid mother plants which would explain these findings can be neglected as in the mere comparison of the intra-cytotype crossings 4x4x seeds developed faster than 2x2x ones. Also, the ratio of maternal to paternal genome participation in formation of the endosperm does not have an effect at this stage any more.

Despite the fact that 4x4x seeds were able to gain speed after their delayed germination the analysis of overall duration did not change the relations for the intra-cytotype crossings: Diploids were still the fastest, followed by tetra- and hexaploids. 2x4x H_{red} were the only ones that still show faster development than PPT in terms of overall duration; all remaining treatments that show deviations from the expectation have their advantage or disadvantage compensated in a later life stage (4x2x and 4x6x). Again no general pattern can be found and no general explanation for the behaviour of seedlings can be given.

For the combination between diploid and tetraploid parents, it seems that the cytotype of the maternal plant has a larger effect on the developmental speed of the progeny, yet as this pattern was not confirmed for crossings between tetraploid and hexaploid parents, this finding ought not to be generalized.

Concerning the analysis of cumulative duration for the twelve treatments, no real conclusions can be drawn from this aspect. It is evident that tetraploid intra-cytotype crossings developed faster between the formation of the radicle and the primary leaf, but this does not compensate for the later germination. Total durations provide in large parts the same pattern as germination duration. This may indicate that the early developmental stages up to the appearance of the radicle are the ecologically most relevant ones, later stages being less important and thus under lower selective pressure. Alternatively, it might be that the genetic constitution does allow adaptations only in those early developmental stages.

Theoretically, the growth speed of a polyploid plant should be lower compared to its diploid progeny due to slower cell cycles in the presence of larger DNA amounts (e.g. Bennett et al., 1977; but see Bretagnolle et al. (1995) who showed that in *Dactylis glomerata* the heavier seeds of the tetraploid individuals display higher germination rates and developmental speed alike). The magnitude of the decrease in speed is correlated with ploidy level (though not linearly as consequence of gene loss following polyploidization, Wendel, 2000; Otto and Whitton, 2000) and there is no evidence whatsoever that this should not be valid for seedlings as we observed them. In *Senecio carniolicus* slower development of higher ploidy levels could not be verified, so even if 2x2x seeds happened to

be the first to germinate, their growth speed decreased in later stages and they are soon overtaken by the tetraploid seedlings from 4x4x crossings. It is therefore likely that ecology and different biotic and abiotic factors have a bigger influence on growth speed than cell size, and that diploid seedlings could potentially grow faster than their higher ploidy counterparts unless environmental conditions select for individuals with slower growth rates. On the other hand, hexaploid plants usually growing in dense *Carex curvula*-meadows and *Loiseleuria* shrubs on the other hand could be forced to exhaust their potential and produce leaves as fast as possible to maximize solar input for photosynthesis. Environmental constraints possibly cover up different cell cycle durations correlated with developmental speed.

Lower temperature at the habitats of diploids and tetraploids could also be a possible explanation for their slower growth, but only in case that the plants' responses were not of modificative nature as abiotic factors were exactly the same for all treatments in the climate chamber.

The typical habitat of diploid plants is rocky and exposed, so that snow cover is removed by wind and afterwards the surface dried by solar radiation early in spring. Consequently, the period in which diploid seeds can successfully germinate is very short. Known to display pioneer character, diploids are supposed to follow the r-strategy: Due to low competition at these sites they can afford the loss of some individuals due to possible late frost events which is inevitably connected with early germination.

On the other hand, tetraploids growing on cool slopes which do not get much solar energy at all are probably profit from delayed germination in periods of higher temperatures instead of early producing tiny seedlings which are then doomed to developmental standstill and slow-motion growth.

For hexaploids the exact timing of germination does probably not play an important role as in their habitat there is no danger of drying up and in case of wintergreen *Loiseleuria* shrubs equally shaded during the whole year. So adaptation to their habitat also offers a plausible explanation for the high variation for the germination duration in hexaploid intra-cytotype crossings.

Under the light of this scenario we need to ask about the consequences that these differences in developmental speed as an adaptation to the respective growth site provide for the hybrid seeds of our interest. *Senecio carniolicus*' pollen grains are effectively dispersed by insects (entomophily), its mature fruits possess a pappus and are dispersed by wind (anemochory). Unfortunately, no data on the covered distances for either of the two means of dispersal for *Senecio carniolicus* is available. Yet it is probable that even diaspores adapted to wind dispersal remain within a radius of a few meters around the mother plant (studies on dispersal distances of Asteraceae: Sheldon and Burrows, 1973). Therefore, hybrid progeny is more likely to germinate under biotic and abiotic conditions similar to those of its mother's cytotype's typical habitat than to those of its fathers. In this case we would expect inter-cytotype crossings to be more successful when they possess characteristics similar to their

maternal cytotype and less successful when they resemble their paternal intra-cytotype crossing. Yet, if inside a contact zone the habitat is not patchy but intermediate, a hybrid bearing a balanced mixture of the features of both parents is expected to be adapted best.

This means that under the assumption that the duration from soaking in spring to germination plays the biggest ecological role, 2x4x hybrids and 6x4x hybrids are expected to be at advantage in patchy contact zones and 4x2x, 6x2x and 6x4x hybrids in intermediate contact zones. Further study is needed on the nature of contact zones and the frequencies of the involved cytotypes which determine over the probability of a certain crossing to happen, to get a profound idea of whether this fact could participate to postzygotic reproductive isolation in the polyploid complex.

Easily combinable with the discussed scenario and further curtailing the effect of Bennett‘s nucleotype hypothesis (Bennett, 1971), developmental speed for seedlings of *Senecio carniolicus* does depend more on the egg and pollen donor than on actual cytotype: There were large differences between the durations needed for those tetraploids that resulted from intra-cytotype crossings and those from the diploid-hexaploid combination (which is not at all surprising under the light of genomic imprinting, see chapter 4.2.2). Pentaploids were slightly slower than hexaploids. Summarized, the time hybrids need for their development is very likely determined by their gamete donors’ respective ecological adaptations and not their actual cytotype.

According to its current distribution and prevalence, the hexaploid cytotype is the most successful in the Eastern Alpine habitat of *Senecio carniolicus*. Yet it is not the earliest to germinate, and so the assumption that faster germination provides evolutionary advantages remains dubious. The fact that 2x4x, 2x6x and 4x6x inter-cytotype crossings do not really succeed in the field despite their significantly shorter germination time further supports the assumption that germination duration is far more expression of their habitat constraints and therefore subject to strong selection than of the physiology and cytology of the respective cytotype. Moreover, Baack and Stanton (2005) state in their study on *Ranunculus adoneus* that for an alpine plant a two days lead does not make a big difference in an environment where production and biomass increase are really low and therefore is unlikely to positively influence competitive force.

4.2.2 Comparison of survival of inter- and intra-cytotype crossings

Cytotype determination of perished soaked seeds or seedlings is not possible. Hence survival rates can only be defined for the whole seed yield of a treatment, but not for H_{red}, H_{unred} or selfed diaspores separately (see chapter 4.5). Therefore, survival rates need to be interpreted in combination with the results of the cytotype distribution of seeds and seedlings.

The survival rates of all inter-cytotype treatments except of 4x6x were either lower than or laid within the expected range given by the parental cytotypes. In all cases where cytotype distribution differed between seeds and seedlings (2x4x, 2x6x and 6x2x, see chapter 3.3), it was H_{red} that displayed decreased viability compared to the seeds produced via selfing. Therefore it is clear that survival rates for H_{red} can only be lower than calculated for the whole set of seeds from the respective treatment.

Survival rates were largely congruent with the duration needed for accomplishment of the respective state: In the beginning, H_{red} seem to have no significant disadvantage (2x6x is the only exception), but once the radicle has emerged, their survival rates fall below those of their corresponding intra-cytotype crossings. At a closer look, for the germination period of the treatments containing eggs from tetraploid individuals (i.e. 4x2x and 4x6x) had higher survival rates higher than the PPT. This might, however, be an experimental artefact because of the probably distorted survival rate for 4x4x seeds when their germination time is restricted to 55 days.

The two crossing combinations between a diploid and a tetraploid parent showed considerable mortality rates between formation of radicle and of primary leaf. This goes along with the finding that crossings involving diploids as any partner showed elevated mortality in early life stages and especially after transplantation (personal observation, see Figure 5 for the smaller size of diploids compared to polyploids, which could be a key reason for their elevated mortality). The crossings between diploids and hexaploids were equally concerned, but due to embryo mortality and high selfing rates, this effect remained disguised for 2x6x seedlings.

The crossing between tetraploid and hexaploid plants did not show a significant disadvantage in any of the three analysed periods, neither had their survival rates to be considered as overestimate because of differences between cytotype frequency in seeds and seedlings, in other words selective mortality of any type of seeds resulting from this crossing. On the contrary, 4x6x seeds showed in two out of three periods survivability even higher than the PPT. At first sight this seems contradictory to the fact that only few pentaploids were found in the field during the comprehensive sampling of the Eastern Alpine populations (Sonnleitner et al., 2010), but note that only average adult plants were collected and no information is available on the cytotype distribution of the seedlings. Besides, mixed populations of tetraploid and hexaploids are not quite as common as those of diploids and hexaploids (13% vs. 36% of all sample sites, according to Sonnleitner et al., 2010). Despite this asymmetry, pentaploids were the most commonly found odd-ploid type, reflecting the finding that crossing between tetra- and hexaploids is the most successful inter-cytotype crossing in terms of survival.

Survival rates are a direct expression of crossing ability. The most important factors that influence crossing abilities (or, negatively spoken, the strengths of crossing barriers) are discussed in the following chapter. As for the crossings we only investigated plants from the Eastern Alpine area

we can be sure that all diploids used belong to the eastern lineage. Polyploids have been proven through genetic investigations to be more closely related to the eastern lineage (Schönswetter, unpubl.). This close relationship is certainly one of the key features of the unexpectedly high crossing success observed for *Senecio carniolicus* inter-cytotype crossings.

4.3 The nature of the postzygotic isolation

We observed strong postzygotic isolation between the combinations containing diploid and hexaploid *Senecio carniolicus* while tetraploids were isolated least.

We could therefore assume that tetraploids, the possible result of diploid-hexaploid crossings, were less divergent from either of the two other cytotypes. Evidence for a secondary origin would be provided by the current geographical distribution of tetraploids being restricted to putative glacial refugia (Schönswetter et al., 2005), where tetraploids conceivably have been formed when di- and hexaploids gained contact during the last glacial maximum, and could have occupied an ecological niche not yet occupied by the two other cytotypes. This would explain their poor (because unnecessary) dispersal ability but their being adapted to the roughest habitats. But, in the light of this scenario we would then need to ask why diploids are a lot better isolated from tetraploids than tetraploids are from hexaploids. Genetic analyses using AFLP and ribosomal ITS regions (Escobar-Garcia et al., unpublished data) revealed the fact that the evolutionary distance between (eastern) diploids and tetraploids equals that between tetraploids and hexaploids. It therefore seems more likely – also under the light of constraints of polyploid formation and the relative importance of the triploid bridge to tetraploid formation - that tetraploid establishment preceded that of hexaploids.

In a second more realistic scenario we therefore take hexaploids for the youngest cytotype and assume that hybrid inviability and infertility is the consequence of problems in endosperm development caused by imprinting inbalances. Indeed, this mechanism of reproductive isolation has been shown to be of vital importance for speciation in many investigated polyploidy complexes due to its potential to create an immediately acting isolation barrier (Köhler et al., 2009).

We would expect that in large parts the success of any hybrid between parents of different ploidy levels would decrease linearly with the absolute deviation from the ratio (ploidy level of maternal/paternal genome) fulfilled in intra-cytotype crossings. We consent to the most common view and assume that the ratio of maternal to paternal genome contributing to the endosperm plays a more important role than the ratio of embryo to endosperm ploidy (e.g. Haig and Westoby, 1991; Ortiz and Ehlenfeldt, 1992; Scott et al., 1998 and references therein; Bushell et al., 2003; but see Sobel et al., 2009 for a different position).

Indeed, almost all observations made in the course of our experimental study can be explained in view of deviations of the ratio of maternal to paternal genome contribution to endosperm, assuming that deviations in negative direction (disproportional high paternal share) are more noxious than in positive direction. The effects of paternalization, discussed in chapter 1.1.4, include dramatic acceleration of the normal developmental speed of embryo at the expense of the endosperm, the

nutrients of which are easily mobilized in case of paternal excess. It is evident that for an Asteraceae bearing only very small amounts of endosperm early consummation of the nutrients reserves is particularly fatal. Besides, as we mainly examined development in early life stages, any extant influence of unbalanced genome ratio is much more likely to be detected when it shows up early.

An additional virtue of this scenario is that different strengths of postzygotic reproductive barriers are also explainable when we ignore the origins of the polyploid complex and assume that all cytotypes are of approximately the same age.

Table 29 gives an overview of the expected ratios and the deviations from those fulfilled in standard intra-cytotype crossings for all performed crossing combinations as well as for all hybrid cytotype combinations involving unreduced gametes that were identified through FCM of the seedlings. It was shown that treatments involving paternalization (2x4x, 2x6x and 4x6x) germinated early. The treatment with the largest absolute deviation, 6x2x, has been shown to perform worse than the PPT both in terms of duration and survival, but note that only for duration can we be sure to cope with H_{red}. 2x6x seeds performed worse than 4x2x ones despite smaller absolute deviation, however 2x6x "suffers" from paternalization whereas 4x2x has an excess of maternal genome contributing to the endosperm formation.

Even stronger evidence for the correlation of endosperm balance and successful seed formation is provided when percentages of fully developed seeds among the total seed sets of each treatment are regarded (see chapter 4.4)

Crossing	Hybrid	%	Emb.	Endo.	Ratio1	Dev1.	Mat.	Pat.	Ratio2	Dev2.
Intra-cytotype			2	3	0.67		2	1	2	
2x4x		42.19	3	4	0.75	0.08	2	2	1.00	-1.00
	unred egg	3.13	4	6	0.67	0.00	4	2	2.00	0.00
	unred pollen	1.56	5	6	0.83	0.17	2	4	0.50	-1.50
	unred egg and pollen	1.56	6	8	0.75	0.08	4	4	1.00	-1.00
4x2x		63.77	3	5	0.60	-0.07	4	1	4.00	2.00
	unred egg	2.90	5	9	0.56	-0.11	8	1	8.00	6.00
	unred pollen	?	4	6	0.67	0.00	4	2	2.00	0.00
2x6x		0	4	5	0.80	0.13	2	3	0.67	-1.33
	unred egg	5.56	5	7	0.71	0.05	4	3	1.33	-0.67
	unred pollen	0.00	7	8	0.88	0.21	2	6	0.33	-1.67
6x2x		5.13	4	7	0.57	-0.10	6	1	6.00	4.00
	unred egg	0.00	7	13	0.54	-0.13	12	1	12.00	10.00
	unred pollen	28.21	5	8	0.63	-0.04	6	2	3.00	1.00
4x6x		97.11	5	7	0.71	0.05	4	3	1.33	-0.67
	unred egg	0.00	7	11	0.64	-0.03	8	3	2.67	0.67
	unred pollen	0.00	8	10	0.80	0.13	4	6	0.67	-1.33
6x4x		95.03	5	8	0.63	-0.04	6	2	3.00	1.00
	unred egg	0.55	8	14	0.57	-0.10	12	2	6.00	4.00
	unred pollen	0.55	7	10	0.70	0.03	6	4	1.50	-0.50

Table 29: Overview of expected ploidy levels of embryo and endosperm and numbers of maternal and paternal genome contributing to formation of the endosperm. Percentages of seedlings base on FCM measurements at the end of the study and can therefore be regarded as a surrogate for the germination ability of the respective hybrid. The question mark for 4x2x hybrids including unreduced pollen had to be put because knowing their cytotype only we cannot refer to their origin (selfing or H_{unred}). Emb.=Embryo ploidy, Endo.=Endosperm ploidy, Ratio1=Ratio of embryo to endosperm ploidy, Dev1.=Deviation of Ratio1 from the target ratio for embryo to endosperm ploidy, Mat.=Number of genomes from the egg donor contributing to the endosperm, Pat.=Number of genomes from the pollen donor contributing to the endosperm, Ratio2=Ratio of maternal to paternal genomes in endosperm, Dev2.=Deviation of Ratio2 from the target ratio for endosperm genomes. Target ratios are given in the first line.

Last but not least, the fact that we encountered quite many hybrids including unreduced pollen despite their low prevalence (0%, 0.5% and 0.3% for diploids, tetraploids and hexaploids respectively) when studied microscopically by Fössinger (2010) confirms the assumption that balanced endosperm ratios play a fundamental role in hybrid success. No data has yet been obtained on the frequency of unreduced eggs but we assume that the situation there is similar. Also note that whenever there is more than one possible hybrid including unreduced gametes, the one that leads to better balanced seeds is more frequently formed. We would expect that for 4x2x a considerable share of tetraploids result from fusion of the reduced diploid egg and an unreduced diploid pollen grain, because this would imitate to the original situation. For many inter-ploidy crosses a disproportional success of a likewise cross has already been confirmed (*Solanum sp.* - Ortiz and Ehlenfeldt, 1992; *Dactylis glomerata* - Sato et al., 1993; *Arabidopsis thaliana* - Bushell et al., 2003; *Primula rosea* x *denticulata* - Hayashi et al., 2009). Ultimate proof could be obtained through scanning the seeds that did not germinate for their ploidy levels: According to their postulated disproportional high germination success, we would expect that

nearly no hybrids containing unreduced gametes would be found here. Unfortunately, soaked diaspores can not be used for FCM anymore.

Concerning to the second major reproductive isolation mechanism for inter-ploidy crossings, F1-breakdown caused by meiotic irregularities that impede the formation of fertile gametes, we cannot make any reliable statement on its importance for *Senecio carniolicus*, as we only analysed seedlings (also see the following chapter).

4.4 Relevance of postzygotic isolation for total reproductive isolation

From our results it becomes evident that postzygotic isolation alone can not suffice to keep apart the three major cytotypes as we observe it in the field. Despite the fact that it is usually believed that for polyploid speciation postzygotic reproductive barriers ought to precede prezygotic ones (Sobel et al., 2009, and many others who emphasize on the immediate incompatibility between a neopolyploid and its diploid progenitor), there also is evidence for polyploid complexes where newly established polyploids are immediately ecologically differentiated from their ancestors (e.g. when Stebbins (1979) synthesized a polyploid variant of the North-American wild grass *Ehrharta erecta*). This is a probable explanation for the weak postzygotic barriers acting in tetraploid-hexaploid combinations. Even if contact zones between tetraploids and hexaploids are currently known (Sonnleitner et al., 2010), clustering of the two cytotypes according to spatial heterogeneity of the habitat on microscale seems quite likely and could result in highly assortative mating. Additionally, partial phenological separation among the three main cytotypes combined with pollinator fidelity and their tendency to minimize the distances of their foraging flights this can contribute highly to total reproductive isolation (as observed for *Chamerion angustifolium* by Husband and Schemske, 2000).

For the combinations between diploid and tetraploid and diploid and hexaploid plants, even without any prezygotic barriers hybrids are unlikely to successfully develop in the field. Triploid seedlings resulting from a cross between a diploid and a tetraploid plant may perform almost equally well than the PPT, but they are produced in just very low amounts: The percentage of fully developed seeds produced capable of germination was considerably low for all plants subjected to the treatments 2x4x and 4x2x. This pattern is even stronger developed for the combination between diploid and hexaploid plants that additionally produced hybrids that are not viable at all.

Figure 16 is a synopsis of seed sets for the twelve treatments and cytotype distribution among the seeds formed. Including seed sets for the twelve tested treatments in our analysis of reproductive barriers - even though we can not determine whether a small seed set is the result of seed abortion after zygote formation or of a reduced ability of gametes to fuse whenever their ploidy levels are too divergent (gametic isolation sensu Sobel et al., 2009), and so ignore whether its nature is post-pollination prezygotic or postzygotic – allows to get an idea of the relative importance of the investigated parts of postzygotic isolation for the six hybrid crossings. With exception of plants from the treatments 4x6x and 6x4x, that even produce seed sets higher than those of one of their parents (6x6x) and with seeds of almost exclusively hybridogenic origin, all remaining inter-cytotype hybrid treatments had seed sets significantly lower than those of the parental intra-cytotype crossings. Therefore, low seed sets for diploid-tetraploid and diploid-hexaploid hybrids combined with the slight

disadvantages in seedling performance might be enough to keep the percentages of inter-cytotype hybrids at the level observed in natural populations.

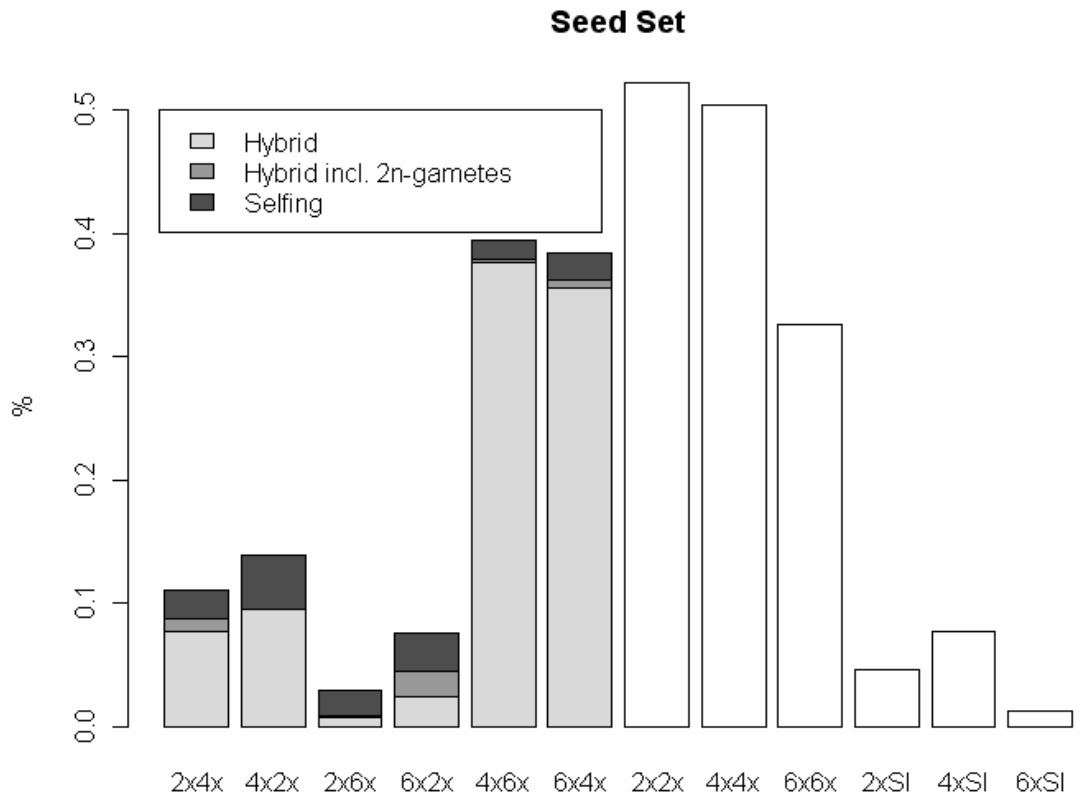


Figure 16: Combined illustration of seed set data and cytotype distribution in the seeds sent to FCM. Seed set hereby is defined as percentage of fructified seeds in relation to the total number of achenes. The sum of fructified and empty seeds did only differ for treatments with diploid mother plants, which had slightly lower seed sets (data not shown). Seed set data was kindly provided by Michaela Sonnleitner.

Finally, assuming similar rates of death and mouldering in the field, these two aspects would further raise the strength of the postzygotic isolation (not included in Figure 16). As we were working under more or less sterile conditions and did not introduce infestations with our working material this is very likely the case. To be completely sure it would be necessary to check if spores were ubiquitous on all testae or can only be found on those containing seeds judged to be less fit and viable (e.g. 2x6x and 6x2x).

A last word ought to be said concerning selfing rates. In seedling performance, as far as we studied them, seeds from the three control treatments which have not been artificially crossed but were only exposed to their proper pollen to test for selfing rates (2xSI, 4xSI, 6xSI) did not have any significant disadvantage, neither in duration nor in survival. Only selfed hexaploid plants showed a slightly shorter time needed from exposure to germination. As *Senecio carniolicus*, like most Asteraceae, is proterandrous and therefore has a built-in mechanism to keep selfing rates low, we would not expect proportion of selfed seed in open-pollinated plants to be any higher than recorded for

the SI-treatments. Despite the previously discussed advantages that mentor effects (breakdown of self-incompatibility systems under the presence of pollen from different cytotypes) can have for polyploid establishment (see chapter 1.1.3), no considerable rise in selfing has been observed when the SI-treatments and the percentages of selfed seeds in inter-cytotype crossings are juxtaposed. Again, proterandry is a good means to explain why for *Senecio carniolicus* this way of facilitating polyploid reproduction is partly blocked. This goes well along with the recent finding that a positive correlation exists between polyploidy and selfing when analysed throughout all angiosperms, but not when Asterids were analysed solely (Barringer, 2007).

Concluding, postzygotic barriers for *Senecio carniolicus* (in this case we particularly examined intrinsic postzygotic isolation sensu Sobel et al., 2009) do not play an exorbitant role: As long as a set of different barriers act in common between two lineages – and this certainly is the case for our research species – cumulative isolation can be very high even when the strengths of each single barrier are fairly low (Husband and Sabara, 2003; Sobel et al., 2009; Martin and Willis, 2010 and references therein).

4.5 Problems and errors in the experimental design

Viewed collectively, the germination experiment itself as well as the needed equipment and manipulation worked well and no major problems occurred during the experiment, nor during the period of care of the plants in the experimental garden.

Concerning the observation of the seedlings, apart from the rather difficult determination of the appropriate moment for transplantation for the remarkable number of seedlings that did not spread their cotyledons at all or formed just one cotyledon, the assessment of the seed and seedling developmental states proved to be easy and reliable.

The single most severe problem that occurred during designing the experiment is of methodical nature and can not really be overcome with the currently available machinery and techniques: Seeds can not be taken for cytometry once soaked, so as soon as a seed was destined to be used for germination, we would only know its cytotype if it successfully produces at least one healthy cotyledon to be dried in silica gel. Therefore, analysis of survival was suboptimal and only partly reliable (i.e. for those treatments that did not show any deviation in cytotype distribution between seeds and seedlings). Only in combination with the data received from the comparison of seed and seedling cytotype distribution could at least a rough estimation of the relative survival of H_{red} and intra-cytotype crossings be made.

A second major negligence concerned selfing rates in intra-cytotype crossings. In spite of the fact that many polyploid complexes have been reported to have elevated selfing rates (Rausch and Morgan 2005; Barringer, 2007; Husband et al., 2008), *Senecio carniolicus* is widely considered a presumably outcrossing species (e.g. Schönswetter et al., 2007). The only empirical evidence for this assumption is the provided by the seed set data for unpollinated (exposed to proper pollen only) obtained during the artificial crossings in the field in 2009. Yet, it is not sure that stigmas react in the same way when they are exposed to a mixture of foreign and proper pollen and when exposed to proper pollen only. Moreover, an effect of the mere amount of pollen, which is probably a lot higher in our artificial pollinations than it would be in open-pollination situation via insects, is also plausible (Baack, 2005).

To draw incontestable conclusions on the existence and degree of mentor effects we would have to know about the prevalence of selfing in the intra-cytotype crossing control groups. Yet again, this experimental inaccuracy could only be avoided by applying molecular markers, which would be far beyond the scope of this thesis. The decision to use unpollinated control groups to get a rough idea about selfing rates is therefore admissible, but note that these offer no real insight on selfing prevalence among intra-cytotype crossings.

The necessity to grow seeds in petri dishes first (for both higher germination rates and handling reasons) introduced to our experiment an unnatural disturbance due to the need of transplantation from the dishes into substrate in the early development of the seedlings. Even if this disturbance had similar effects on all treatments, we do not know whether cytotypes differ with respect to their tolerance of disturbance. We saw that a higher percentage of diploid plants and of all treatments involving any gamete of them died after transplantation. This of course influences survival rates for these treatments and, for diploid-tetraploid and diploid-hexaploid combinations, also the calculated PPT which was basis for our Generalized Linear Mixed-Effects Model analyses. We are unable to make any statement on whether these seedlings would also have died in this stage if we had not by experimental design introduced this selection criterion non-existent in nature.

In the autopolyploid complex of *Ranunculus adoneus* (Baack and Stanton, 2005) one cytotype (tetraploid) had a distinct performance advantage under the controlled conditions in the climate chamber that could not be observed in the field. Although no evidence was found for an equal situation in *Senecio carniolicus* we must be aware of its possibility.

The exclusion of hybrids formed under participation of unreduced gametes (H_{unred}) was dubious because of our missing understanding of their prevalence and relevance in this polyploid complex. Fössinger (2010) found that a negligible number of pollen was unreduced, yet the artificial crossings definitely contained an unexpectedly high number of unreduced pollen (and egg cells). The pollen counts were done using plants that had been removed from their original mountain habitats before the beginning of meiosis in the anthers. But as many previous studies (reviewed by Ramsey and Schemske, 1998) ascertained, the frequency of unreduced gametes correlates with temperature and other environmental factors. There are plans to repeat pollen scan for unreduced gametes to test for the reliability of the values from Fössinger (2010) when meiosis had taken place entirely in alpine environment.

Apart from that, possible explanations for the disproportionate success of unreduced gametes are increased siring ability of unreduced pollen (comparable to the discovery made by Husband et al. (2002) that pollen from polyploid plants sired better on diploid and polyploid stigmas alike) or improved development of H_{unred} seeds (see chapter 4.3). Sample sizes for H_{unred} were too low to analyse them separately in the Generalized Linear Mixed-Effects Models, and inclusion of them did not alter the results. As indicated in chapter 4.3 it would have been interesting to cytometrize seeds from intra-cytotype crossings and compare abundance of unreduced gametes in them and in inter-cytotype crossings.

H_{unred} seeds were strongly restricted to few plant individuals (Karl Hülber, unpubl.; personal observation) suggesting that individual tendencies to produce unreduced gametes do exist (as it is the case for some other investigated polyploid complexes, see Ramsey (2007) and references therein). The

significance that this fact can have on neopolyploid formation and on establishment of mating barriers (e.g. possibility of decreased genetic divergence and therefore higher inbreeding depression – though the latter is not applying to predominantly outcrossing *Senecio carniolicus*) remains elusive.

The only grave self-inflicted error was to break up the germination experiment after 55 days even for those treatments who did not meet the finishing criterion. Although sample sizes were large enough for all treatments when the experiment was finished, we had to encounter a biased rate of survival for the treatments at the end of the experiment (4x2x, 4x4x, 4x6x, 6x4x and 6x6x) as we do not know to what value their rates could have raised when the time for the germination would not have been limited. Furthermore, we do not know whether survival rates for all treatments are equally biased when time is restricted to 55 days.

4.6 Future directions

Several interesting conclusions were achieved from the analysis of the germination experiment to better understand the reproductive isolation that currently exists between the three main cytotypes of *Senecio carniolicus*. Yet, a lot of aspects in this fascinating polyploid complex are still elusive and offer interesting possibilities of future research.

Our analysis of postzygotic isolation repeated the common consent that in most taxa more than one reproductive barrier are at work to keep diverging lineages apart. For *Senecio carniolicus*, many of the supposed components of total isolation have not yet been studied, e.g. there is still insufficient data on flowering phenology, just as none exists for pollinator preferences. If we talk about postzygotic isolation only, it would be highly interesting to also investigate extrinsic post-zygotic isolation (which may be especially important in early stages of speciation: Wolf et al., 2010 and references therein), as well as intrinsic postzygotic isolation that goes beyond the formation of the first primary leaf. For example, there is no consensus on whether pentaploid individuals are able to flower or not (Michaela Sonnleitner, personal communication). No study has been made on eventual F1- or F2-breakdown despite the fact that it is absolutely plausible that the disproportionately high mortality rates for hybrid seeds persist in later life stages.

Finally, as comprehensive as the last sampling of the Eastern Alpine population was, only average adult plants have been taken. Furthermore, we had no information on the pollination partners for these plants and therefore could not differentiate between intra-cytotype crossings and selfed plants from hybrid crossings and in case of diploid – tetraploid combinations not even recognize H_{unred} seeds. Yet these are just the ones that display disproportionate survival rates in our germination study. Maybe the prevalence of hybrids in the field after all is higher than expected.

In any case, *Senecio carniolicus* offers numerous interesting possibilities of study to get a more detailed image of the population and speciation dynamics currently acting in this polyploidy complex, as well as of the age and origin of the polyploid complex, in order to better understand the mechanisms of reproductive isolation in this taxon.

LITERATURE CITED

Ainouche ML, Jenczewski E. 2010. Focus on Polyploidy. *New Phytologist*, **186**: 1-4.

Ainouche ML, Fortune PM, Salmon A, Parisod C, Grandbastien M-A, Fukunaga K, Ricou M, Misset M-T. 2009. Hybridization, polyploidy and invasion: lessons from *Spartina* (Poaceae). *Biological Invasions*, **11**: 1159-1173.

Baack EJ. 2005. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): Minority cytotype exclusion and barriers to triploid formation. *American Journal Of Botany*, **92**: 1827-1835.

Baack EJ, Rieseberg LH. 2007. A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics and Development*, **17**: 513-518.

Baack EJ, Stanton ML. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adoneus*): niche differentiation and tetraploid establishment. *Evolution*, **59**: 1936–1944.

Barringer BC. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany*, **94**: 1527-1533.

Bates D, Sarkar D. 2008. lme4: Linear mixed-effects models using S4 classes. URL http://CRAN. R-project. org/package= lme4, R package version 0.999375-28 (16 March 2010).

Bennett MD. 1971. The duration of meiosis. *Proceedings of the Royal Society London B - Biological Sciences*, **178**: 277-299.

Bennett MD, Lewis KR, Harberd DJ. 1977. The time and duration of meiosis. *Philosophical Transactions of the Royal Society*, **277**: 201-226.

Bomblies K, Lampe J, Epple P, Warthmann N, Lanz C, Dangl JL, Weigel D. 2007. Autoimmune Response as a Mechanism for a Dobzhansky-Muller-Type Incompatibility Syndrome in Plants. *PLOS Biology*, **5**: 1962-1972.

Bretagnolle F, Thompson JD. 1995. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytologist*, **129**: 1-22.

Bretagnolle F, Thompson JD, Lumaret R. 1995. The influence of seed size variation on seed-germination and seedling vigor in diploid and tetraploid *Dactylis glomerata* L. *Annals Of Botany*, **76**: 607-615.

Brown JD, O'Neill RJ. 2010. Chromosomes, Conflict, and Epigenetics: Chromosomal Speciation Revisited. *Annual Review of Genomics and Human Genetics*, **11**: 291-316.

Buggs RJA, Soltis PS, Soltis DE. 2009. Does hybridization between divergent progenitors drive whole-genome duplication? *Molecular Ecology*, **18**: 3334-3339.

Burton TL, Husband BC. 2001. Fecundity and offspring ploidy in matings among diploid, triploid and tetraploid *Chamerion angustifolium* (Onagraceae): consequences for tetraploid establishment. *Heredity,* **87**: 573–582.

Bushell C, Spielman M, Scott RJ. 2003. The Basis of Natural and Artificial Postzygotic Hybridization Barriers in Arabidopsis Species. *The Plant Cell,* **15**: 1430-1442.

Chen ZJ. 2010. Molecular mechanisms of polyploidy and hybrid vigor. *Trends In Plant Science,* **15**: 57-71.

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nature,* **6**: 836-846.

Cui L, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, Doyle JJ, Soltis PS, Carlson JE, Arumuganathan K, Barakat A, Albert VA, Ma H, DePamphilis CW. 2006. Widespread genome duplications throughout the history of flowering plants. *Genome Research,* **16**: 738-749.

Doležel J, Bartoš J. 2005. Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size. *Annals of Botany,* **95**: 99-110.

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF. 2008. Evolutionary genetics of genome merger and doubling in plants. *Annual Review Of Genetics,* **42**: 443-461.

Duchoslav M, Safarova L, Krahulec F. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals Of Botany,* **105**: 719-735.

Dušková E, Kolár F, Sklenár P, Rauchová J, Kubešová M, Fér T, Suda J, Marhold K. 2010. Genome size correlates with growth form, habitat and phylogeny in the Andean genus *Lasiocephalus* (Asteraceae). *Preslia,* **82**: 127-148.

Eggenberg S, Möhl A. 2007. *Flora vegetativa: ein Bestimmungsbuch für Pflanzen der Schweiz im blütenlosen Zustand,* Bern, Wien, Haupt.

Felber F. 1991. Establishment of a tetraploid cytotype in a diploid population: Effect of relative fitness of the cytotypes. *Journal of Evolution Biology,* **4**: 195–207.

Fischer MA, Oswald K, Adler W. 2008. *Exkursionsflora für Österreich, Liechtenstein und Südtirol,* Linz, Biologiezentrum der Oberösterreichischen Landesregierung.

Fössinger S. 2010. *Senecio carniolicus als Modellorganismus für Arten mit sympatrisch verbreiteten Zytotypen.* Diplomarbeit, Universität Wien, Wien.

Fowler NL, Levin DA. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *American Naturalist,* **124**: 703-711.

Götzenberger L. 2008. *Comparative biology of the pollen-ovule ratio,* Dissertation, Martin-Luther Universität Halle-Wittenberg.

Haig D, Westoby M. 1991. Genomic imprinting in endosperm: its effect on seed developement in crosses between species, and between different ploidies of the same species, and its

implications for the evolution of apomixis. *Philosophical Transactions of the Royal Society,* **333**: 1-13.

Hayashi M, Kato J, Ohashi H, Mii M. 2009. Unreduced 3x gamete formation of allotriploid hybrid derived from the cross of *Primula denticulata* (4x) x *P. rosea* (2x) as a causal factor for producing pentaploid hybrids in the backcross with pollen of tetraploid *P. denticulata*. *Euphytica,* **169**: 123-131.

Heberling O, Gamperer S, Leist M, Stöhr O, Weinberger M. 2006. Biotopkartierung Gemeinde Vorderstoder. In: Rußmann K ed. *Naturraumkartierung Oberösterreich.* Kirchberg an der Krems, Lochen, Land Oberösterreich.

Hörandl E, Temsch EM. 2009. Introgression of apomixis into sexual species is inhibited by mentor effects and ploidy barriers in the *Ranunculus auricomus* complex. *Annals of Botany*, **104**: 81-89.

Hülber K, Sonnleitner M, Flatscher R, Berger A, Dobrovsky R, Niessner S, Nigl T, Schneeweiss GM, Kubesova M, Rauchova J, Suda J, Schoenswetter P. 2009. Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. *Preslia,* **81**: 309-319.

Husband BC. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society of London - Series B: Biological Sciences,* **267**: 217–223.

Husband BC. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal Of The Linnean Society,* **82**: 537-546.

Husband BC, Ozimec B, Martin SL, Pollock L. 2008. Mating consequences of polyploid evolution in flowering plants: current trends and insights from synthetic polyploids. *International Journal of Plant Sciences,* **169**: 195-206.

Husband BC, Sabara HA. 2003. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist,* **161**: 703–713.

Husband BC, Schemske DW. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *Journal of Ecology,* **88**: 689–701.

Husband BC, Schemske DW, Burton TL, Goodwillie C. 2002. Pollen competition as a unilateral reproductive barrier between sympatric diploid and tetraploid *Chamerion angustifolium*. *Proceedings of the Royal Society London B,* **269**: 2565-2571.

Knight CA, Molinari NA, Petrov DA. 2005. The Large Genome Constraint Hypothesis: Evolution, Ecology and Phenotype. *Annals of Botany,* **95**: 177-190.

Köhler C, Mittelsten Scheid O, Erilova A. 2009. The impact of the triploid block on the origin and evolution of polyploid plants. *Trends in Genetics,* **26**: 142–148.

Kondrashov AS. 2003. Accumulation of Dobzhansky-Muller incompatibilities within a spatially structered population. *Evolution,* **57**: 151-153.

Leitch IJ, Bennett MD. 1997. Polyploidy in angiosperms. *Trends In Plant Science,* **2**: 470-476.

Leitch IJ, Bennett MD. 2004. Genome downsizing in polyploid plants. *Biological Journal Of The Linnean Society,* **82**: 651-663.

Levin DA. 1975. Minority cytotype exclusion in local plant populations. *Taxon,* **24**: 35–43.

Liu S-L, Adams KL. 2010. Dramatic Change in Function and Expression Pattern of a Gene Duplicated by Polyploidy Created a Paternal Effect Gene in the Brassicaceae. *Molecular Biology and Evolution,* **27**: 2817-1818.

Lumaret R, Guillerm JL, Delay J, Loutfi AAL, Izco J, Jay M. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L from Galicia (Spain). *Oecologia,* **73**: 436–446.

Mable BK. 2003. Breaking down taxonomic barriers in polyploidy research. *Trends In Plant Science,* **8**: 582–590.

Mable BK. 2004. Why polyploidy is rarer in animals than in plants´: myths and mechanisms. *Biological Journal Of The Linnean Society,* **82**: 453-466.

Martin NH, Willis JH. 2010. Geographical variation in postzygotic isolation and its genetic basis within and between two *Mimulus* species. *Philosophical Transactions of the Royal Society,* **365**: 2469-2478.

Meyers LA, Levin DA. 2006. On the abundance of polyploids in flowering plants. *Evolution,* **60**: 1198-1206.

Mráz P, Paule J. 2006. Experimental hybridization in the genus *Hieracium* s. str.: crosses between diploid taxa. *Preslia,* **78**: 1-26.

Oberdorfer E. 2001. *Pflanzensoziologische Exkursionsflora für Deutschland und angrenzende Gebiete,* Stuttgart, Eugen Ulmer Verlag.

Orr HA, Presgraves DC. 2000. Speciation by postzygotic isolation: forces, genes and molecules. *Bioessays,* **22**: 1085–1094.

Ortiz R, Ehlenfeldt MK. 1992. The importance of Endosperm Balance Number in potato breeding and the evolution of tuber-bearing *Solanum* species. *Euphytica,* **60**: 105-113.

Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics,* **34**: 401–437.

Parisod C, Holderegger R, Brochmann C. 2010. Evolutionary consequences of autopolyploidy. *New Phytologist,* **186**: 5-17.

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

Pitschmann H, Reisigl H. 1965. *Flora der Südalpen,* Stuttgart, Fischer

R Development Core Team. 2008. R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Ramsey J. 2007. Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity,* **98**: 143–150.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics,* **29**: 467–501.

Rausch JH, Morgan MT. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishement. *Evolution,* **59**: 1867-1875.

Reisigl H. 1978. *Blumenwelt der Alpen,* München, Bertelsmann, Gütersloh Prisma-Verlag.

Reisigl H, Keller R. 1987. *Alpenpflanzen im Lebensraum. Alpine Rasen, Schutt- und Felsvegetation.,* Stutgart, Gustav Fischer Verlag.

Rieseberg LH, Willis JH. 2007. Plant speciation. *Science,* **317**: 910–914.

Sato T, Maceira N, Lumaret R, Jacquard P. 1993. Flowering characteristics and fertility of interploidy progeny from normal and 2n gametes in *Dactylis glomerata* L. *New Phytologist,* **124**: 309-319.

Scott RJ, Spielman M, Bailey J, Dickinson HG. 1998. Parent-of-origin effects on seed development in *Arabidopsis thaliana*. *Development,* **125**: 3329-3341.

Sheldon JC, Burrows FM. 1973. The dispersal effectiveness of the achene-pappus units of selected compositae in steady winds with convection *New Phytologist,* **72**: 665-675.

Schönswetter P. 2007. Origin and maintenance of intrapopulational cytotype mixture in an alpine plant species (*Senecio carniolicus, Asteraceae*). Vienna, Department of Biogeography.

Schönswetter P, Lachmayer M, Lettner C, Prehsler D, Rechnitzer S, Reich DS, Sonnleitner M, Wagner I, Hülber K, Schneeweiss GM, Trávníček P, Suda J. 2007. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research,* **120**: 721–725.

Schönswetter P, Stehlik I, Hoolderegger R, Tribsch A. 2005. Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology,* **14**: 3547–3555.

Sobel JM, Chen GF, Watt LR, Schemske DW. 2009. The Biology of Speciation. *Evolution,* **64**: 295-315.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng CF, Sankoff D, dePamphilis CW, Wall PK, Soltis PS. 2009. Polyploidy and angiosperm diversification *American Journal of Botany,* **96**: 336–348.

Soltis DE. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon,* **56**: 13-30.

Sonnleitner M, Flatscher R, Escobar-García P, Rauchová J, Suda J, Schneeweiss GM, 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. *Annals of Botany,* **106**: 967-977.

Stebbins GL. 1947. Types of polyploids: Their classification and significance. *Advances in Genetics,* **1**: 403-429.

Stebbins GL. 1966. Chromosomal variation and evolution - polyploidy and chromosome size and number shed light on evolutionary processes in higher plants. *Science,* **152**: 1463-1469.

Stebbins GL. 1968. *Evolutionsprozesse. Einzelvorgänge im Wandel der Organismen. Aus dem Amerikanischen übersetzt von Jutta Querner,* Jena, VEB Gustav Fischer Verlag.

Stebbins GL. 1979. Polyploidy in plants: unsolved problems and prospects. *Basic Life Sciences,* **13**: 495-520.

Suda J, Pyšek P. 2010. Flow cytometry in botanical research: introduction. *Preslia,* **82**: 1-2.

Suda J, Weiss-Schneeweiss H, Tribsch A, Schneeweiss GM, Trávníček P, Schönswetter P. 2007. Complex distribution patterns of di-, tetra-, and hexaploid cytotypes in the European high mountain plant *Senecio carniolicus* (Asteraceae). *American Journal of Botany,* **94**: 1391–1401.

Tate JA, Joshi P, Soltis KA, Soltis PS, Soltis DE. 2009. On the road to diploidization? Homoeolog loss in independently formed populations of the allopolyploid *Tragopogon miscellus* (*Asteraceae*). *BMC Plant Biology,* **9**

Welch JJ. 2004. Accumulating Dobzhansky-Muller Incompatibilities: Reconciling theory and data. *Evolution,* **58**: 1145-1156.

Wendel JF. 2000. Genome evolution in polyploids. *Plant Molecular Biology,* **42**: 225–249.

Wolf JBW, Lindell J, Backström N. 2010. Speciation genetics: Current status and evolving approaches. *Philosophical Transactions of the Royal Society,* **365**: 1717-1733.

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. 2009. The frequency of polyploid speciation in vascular plants. *PNAS,* **106**: 13875-13879.

Woodhouse M, Burkart-Waco D, Comai L. 2009. Polyploidy. *Nature Education,* **2**.

Yamauchi A, Hosokawa A, Nagata H, Shimoda M. 2004. Triploid Bridge and Role of Parthenogenesis in the Evolution of Autopolyploidy. *The American Naturalist,* **164**: 101-112.

APPENDIX

Abstract

Senecio carniolicus Willd is a common, abundant high mountain plant of the Eastern Alps and Carpathians that forms a polyploid complex with three main cytotypes (diploid, tetraploid and hexaploid). Recent sampling of individuals over the whole Eastern Alpine range showed that despite a large number of mixed populations odd-ploidy individuals are largely missing. Prezygotic isolation, although certainly existent, does not suffice to explain this absence, thus a germination experiment using seeds from artificial crossings was designed to uncover the nature and strength of the postulated postzygotic reproductive barriers. Crossing success of all inter-cytotype combinations, but especially for tetraploid-hexaploid ones, was unexpectedly high. Analysed over the whole observation period (until formation of the primary leaf), there were slight disadvantages for inter-ploidy hybrids compared to cytotype-uniform crossings - neither in terms of developmental speed, nor in terms of survival rates.

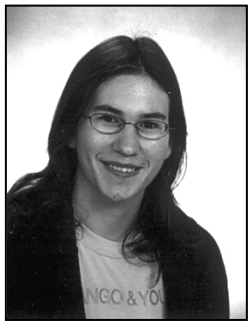
Furthermore, our results suggest that for *Senecio carniolicus* inter-cytotype hybrids imbalances in genomic imprinting of the endosperm (caused by altered ratios of maternal and paternal genomes contributing to its formation) are the prevailing reason for selective mortality. Seedlings from crossings between tetraploid and hexaploid plants, which are the most common both in the field and in our experiment thus exhibit the smallest deviation of the target ratio for cytotype-uniform crossings. The effects of paternalization (excess of paternal over maternal genome) are more disastrous than those of maternalization, at least in the early life stages that we investigated in the course of the germination study.

Zusammenfassung

Senecio carniolicus ist eine häufige und verbreitete Hochgebirgspflanze der Ostalpen und Karpaten, die einen Polyploidkomplex aus drei Haupt-Zytotypen (diploid, tetraploid und hexaploid) bildet. Die letzten umfangreichen Beprobungen der Art, die sich über das gesamte ostalpine Verbreitungsgebiet erstreckten, ergaben, dass trotz eines nachgewiesen hohen Anteils an gemischten Populationen intermediäre Ploidiestufen weitgehend fehlen. Zwar gibt es verschiedene präzygotische Isolationsmechanismen, diese reichen aber nicht aus, um dieses Fehlen vollständig zu erklären. Um Art und Stärke der postzygotischen Isolation zu untersuchen, wurde ein Kreuzungsexperiment mit Samen aus künstlichen Bestäubungen durchgeführt. Der Samenansatz war bei allen Kombinationen der Kreuzbestäubung zwischen Eltern unterschiedlicher Zytotypen unerwartet hoch, besonders aber wenn Tetraploide mit Hexaploiden gekreuzt wurden. Bei der Analyse der Entwicklung vom Samen bis zur Bildung des ersten Laubblatts waren Keimlinge, die aus den gemischten Kreuzungen hervorgingen, sowohl was die Entwicklungsgeschwindigkeit als auch was die Überlebensraten betrifft generell nur leicht im Nachteil gegenüber Keimlingen aus homoploiden Kreuzungen.

Die gewonnenen Ergebnisse zeigen, dass praktisch ausschließlich die Unausgeglichenheit von paternalem und maternalem Anteil am Endosperm (Genomic Imprinting Hypothese) für die Sterblichkeitsrate der Hybriden verantwortlich ist. So weisen die Hybriden zwischen tetraploiden und hexaploiden Individuen, die im Freiland am häufigsten zu finden sind und auch im Experiment den höchsten Samenansatz und die beste Performance zeigten, die geringsten Abweichungen zum Verhältnis von Nicht-Hybriden auf. Paternalisierung (Überschuss an väterlichem Genom) wirkte sich, in den im Rahmen des Keimversuchs untersuchten Lebensstadien, negativer auf die Vitalität der Pflanzen aus als Maternalisierung.

Lebenslauf



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