Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of Senecio carniolicus (Asteraceae) in the Eastern Alps

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• Background and Aims The spatial distribution of cytotypes can provide valuable insights into evolutionary patterns of polyploid complexes. In a previous study the macro-scale distribution of the three main cytotypes in Senecio carniolicus (Asteraceae) within the Eastern Alps was characterized. Employing a roughly 12-fold extended sampling, the present study focuses on unravelling patterns of cytotype distribution on the meso- and microscale and on correlating those with ecological properties of the growing sites.

• Methods DAPI flow cytometry of dried samples was used to determine DNA ploidy level in 5033 individuals from 100 populations spread over the entire Eastern Alpine distribution area of S. carniolicus. Descriptors of microhabitats as well as spatial data were recorded in the field, and analysed with a mixed-effects ANOVA.

• Key Results Extensive variation in DNA ploidy levels (2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x) was detected. Of the main cytotypes, diploids and hexaploids were widespread and had strongly overlapping distributions resulting in the frequent occurrence of cytotype mixtures (half of the investigated populations), whereas tetraploids were distinctively distributed and occurred in the south-west and the east of the species’ distribution area. In spite of the frequent co-occurrence of cytotypes, only 1% of the samples belonged to secondary cytotypes (3x, 5x, 7x, 8x, 9x). Diploids, tetraploids and hexaploids were altitudinally segregated, but with broad overlap. Similarly, highly significant differences in vegetation and rock cover as well as microhabitat exposure were found between the main cytotypes.

• Conclusions Senecio carniolicus shows a remarkable diversity of cytotypes. The distribution of the three main cytotypes (2x, 4x, 6x) has been shaped by Pleistocene glaciations to different extents. Whereas tetraploids are nearly entirely restricted to refugia, hexaploids colonized areas that were extensively glaciated. Diploid and hexaploid individuals often co-occur in mixed populations, whereas they are spatially and ecologically segregated at both the meso-scale and micro-scale. With regard to the ecological parameters investigated, the tetraploid cytotype occupies an intermediate position. The rareness of secondary cytotypes suggests the presence of strong pre- or post-zygotic mating barriers.

Key words: Contact zones, cytotype mixture, Eastern Alps, flow cytometry, habitat segregation, ploidy level, polyploidy, refugia, Senecio carniolicus.

INTRODUCTION

Polyploidization, the multiplication of complete chromosome sets, has played a fundamental role in the evolution and diversification of angiosperms (Soltis et al., 2003). Estimates of its frequency have experienced a steady upward trend, although current discussions are more focused on how many rounds of polyploidization various lineages might have undergone rather than on estimating the percentage of polyploid angiosperm species (Soltis et al., 2003). Major radiations in the angiosperm tree of life have been temporally and perhaps causally linked to key polyploidization events (Fawcett et al., 2009; Soltis et al., 2009). Formerly believed to be ‘a hindrance to the evolutionary success of higher plants’ (Stebbins, 1971), polyploidy is now recognized as an important diversifying force in evolutionary history, and one of the important mechanisms of sympatric speciation in land plants (Otto and Whittton, 2000).

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Successful long-term establishment of a newly arisen polyploid results in cytotype mixtures, which may either be a transitional state or stable through time. In the latter case, they may form narrow contact zones, eventually comprising only a few populations, as evidenced in Chamaenerion angustifolium (Husband and Schemske, 2000), Knautia arvensis (Kolář et al., 2009), Melampodium (Stuessy et al., 2004) and Ranunculus adoneus (Baack, 2004). Cytotype mixtures extending over large areas have been less frequently reported, for example for Galax urceolata (Burton and Husband, 1999) or Solidago altissima (Halverson et al., 2008), and sometimes involved apomixis as in Arnica cordifolia (Kao, 2008). They are expected to be maintained by different means of reproductive isolation that prevent the minority cytotype from losing too many gametes in crosses with the majority cytotype, which eventually would lead to its extirpation (minority cytotype exclusion principle; Levin, 1975; Husband, 2000). Reproductive isolation is often conferred by the interaction of several mechanisms, which reduce or even inhibit gene flow between potentially interbreeding populations (Widmer et al., 2009). Pre-zygotic isolating mechanisms such as adaptation to different pollinators, flowering time divergence, ecological or habitat differentiation, as well as the predominance of selfing or apomixis prevent pollination or fertilization and thus the formation of hybrid zygotes (Petit et al., 1999). In contrast, post-zygotic isolating mechanisms concern the viability and reproductive success of hybrid offspring (Orr and Presgraves, 2000; Husband and Sabara, 2004; Rieseberg and Willis, 2007).

Theoretical considerations and empirical studies suggest that the role of inter-cytotype hybrids in mixed stands of different cytotypes is ambiguous. On the one hand, offspring from inter-cytotype crosses are often sterile, producing non-functional, unbalanced gametes, especially when hybridization results in odd ploidy levels (Levin, 1975). Additionally, back-crossing of polyploid plants with either parent most often leads to non-viable progeny due to endosperm malfunction (Köhler et al., 2010). This is often referred to as ‘triploid block’ (Felber, 1991), although comparable outcomes may also be expected for higher-ploidy level crosses. In this case hybridization weakens both parental lineages and potentially drives the rarer cytotype to extinction (Levin, 1975; Husband, 2000). On the other hand, odd-ploid hybrids may not only produce functional gametes with complete chromosome complements (Felber and Bever, 1997; Burton and Husband, 2001), but are also likely to generate an enhanced number of unreduced gametes. Backcrosses with diploid parental lineages may act as additional sources for the recurrent formation of polyploids (Burton and Husband, 2001). Under these conditions, hybrids may mediate the coexistence and stabilize the equilibrium between co-occurring cytotypes (Felber and Bever, 1997).

A good system for studying various aspects of stable cytotype mixtures and incipient speciation processes is Senecio carniolicus. This common and abundant high mountain species of the Eastern Alps and the Carpathians was long considered to be uniformly hexaploid, but recent investigations (Suda et al., 2007) revealed a complex pattern with three main cytotypes (di-, tetra- and hexaploid). Although a considerable number of the investigated sample sites contained cytotype mixtures, only very few odd-ploid individuals (pentaploids, heptaploids) were found, indicating that there are effective mechanisms preventing the formation of inter-cytotype hybrids. Further support for this hypothesis arises from the absence of tetraploids – the potential hybridogenic offspring – from many sample sites containing mixtures of both diploid and hexaploid individuals including Mt Hoher Sadnig, Austria, where roughly 500 individuals have been investigated (Schönswetter et al., 2007; Hübler et al., 2009). To date, mechanisms maintaining cytotype mixtures in S. carniolicus are not fully understood, but there is evidence for habitat segregation between diploid and hexaploid cytotypes. Whereas hexaploids are linked to communities with denser vegetation, diploids are growing in open, rocky habitats (Hübler et al., 2009), the greater abundance of which at higher altitudes is probably responsible for the previously found altitudinal separation of cytotypes (Schönswetter et al., 2007). As these hypotheses were based on data collected from a single mountain with diploids and hexaploids only, they need to be tested on a wider geographical scale and also including tetraploids, the third main cytotype. Here, we explore the cytotype distribution of S. carniolicus sensu lato in the Eastern Alps on different spatial scales, i.e. macro-scale (entire Eastern Alps), meso-scale (within each of the 100 investigated single mountains) and micro-scale (immediate environment of each of the roughly 3000 individuals). Employing a moderately increased number of populations and a roughly 12-fold extended sampling of individuals as compared with Suda et al. (2007), we test for spatial segregation on all three spatial scales across the entire Eastern Alpine distribution area also including the tetraploid cytotype. Geographical and environmental descriptors representing the meso-scale position (altitude, geographical coordinates) and the ecological micro-site conditions (e.g. rock and vegetation cover) were recorded to address the following questions: (1) Does the macro-scale pattern of the distribution of the main cytotypes (2x, 4x, 6x) change with an increased number of investigated individuals, i.e. are areas which were previously thought to be cytologically uniform in fact heterogeneous? (2) Can the hypothesis of habitat segregation between diploids and hexaploids on the meso- (altitude) and on the micro-scale (dense vegetation and high rock cover, respectively), as established from an in-depth study on a single mountain (Schönswetter et al., 2007; Hübler et al., 2009), be corroborated on a range-wide scale? If at all, how are tetraploids segregated from diploids and hexaploids? (3) Which secondary cytotypes (3x, 5x, >6x) exist, how frequent are they and does the combination of associated main cytotypes give an indication for their origin (hybridogenic or via unreduced gametes)? The results presented form the basis for future molecular investigations exploring the evolution of the intricate polyploid complex of S. carniolicus.

MATERIAL AND METHODS

Study species

Senecio carniolicus Wild. (Asteraceae) is a common mountain species endemic to the Eastern European Alps and the Carpathians. It is a herbaceous perennial that inhabits a variety of habitats on siliceous bedrock, such as grasslands,
dwarf shrub communities, stable screes, moraines, rock crevices and fellfields, ranging from the treeline up to altitudes of 3300 m a.s.l. (Reisigl and Pitschmann, 1958).

Plant material and recording of environmental descriptors

In summer 2008, 100 sampling sites were visited in Switzerland, Italy, Austria and Slovenia, covering the entire distribution of S. carniolicus in the Eastern Alps (Fig. 1, Appendix). The Carpathian distribution was not included because Suda et al. (2007) found exclusively hexaploid populations in that area. Leaves of approx. 30 individuals per sampling site were collected spanning the entire local altitudinal range and habitat types and were dried in silica gel. We aimed to collect samples as representative as possible for the population, i.e. areas with high densities of individuals were sampled more intensively than areas with less frequent occurrences. Additionally, we did not favour specific individuals (flowering or particularly big plants) but chose individuals at random. This strategy combined with our inability to distinguish cytotypes in the field at the time of collecting ensured an unbiased sample. Herbarium specimens for each sampling site were deposited in the herbarium of the University of Vienna (WU).

We recorded environmental parameters describing the micro-site, namely exposure and inclination, as well as percentage coverage of rock (>1 cm) and flowering plants (in the following termed vegetation cover) within a distance of 0.2 m from each sampled individual (in the following referred to as central individuals). Geographical coordinates were recorded with a GPS system, and GPS-corrected barometric calibration was used for altitude. Furthermore, we determined the number of additional S. carniolicus individuals in the same plot (referred to as additional individuals) and sampled – where available – two individuals for ploidy-level estimation.

Flow cytometry

DNA ploidy levels of silica-dried leaf tissue were determined using DAPI flow cytometry as described by Suda et al. (2007), but using Pisum sativum as the sole internal reference standard. Mean fluorescence values of standard and sample never differed more than 3.4-fold, thus being well within the range deemed acceptable by Suda and Leitch (2010). Vicia faba was not suitable for flow cytometric analysis of 9x plants because the similarity in genome size with the standard resulted in overlapping peaks. Pooled samples of three Senecio individuals were usually analysed. In the case of mixed-ploidy samples or low-quality histograms (i.e. coefficients of variation, CVs, of G0/G1 Senecio peaks >5 %, high background or a low number of intact nuclei forming the peaks), each individual was re-analysed separately. Analyses of all minority cytotypes were repeated 2–5 times on different days to minimize potential instrument instability.

Statistical analyses

The following environmental descriptors were used in the analyses: altitude, inclination, exposure, as well as cover of
RESULTS

Occurrence, distribution and frequency of cytotypes

DNA ploidy levels were estimated from 5033 plants (2914 central individuals and 2119 additional individuals; Appendix, and Supplementary Data Table S1, available online). Flow cytometric analyses mostly yielded high-resolution histograms, with average sample CV of 3.37\% (range 1.08–7.71\%) and average standard CV of 2.63\% (range 1.03–6.46\%). The arbitrary threshold of 5.0\% was achieved in 92.6 and 98.0\% of sample and standard runs, respectively. Only small between-day variation (4.1\% maximum) was observed when the same sample was re-analysed. Collectively, these measures of quality indicate that the recorded fluorescence values are stable and allow a reliable estimation of DNA ploidy levels.

Eight distinct groups of fluorescence intensities were obtained (see Supplementary Data Table S2, Figs S2 and S3). Most samples matched the fluorescence values reported by Suda et al. (2007) for karyologically counted diploid (2n = 2x = 40), tetraploid (2n = 4x = 80) and hexaploid (2n = 6x = 120) individuals. Five additional groups corresponded to DNA-pentaploids, DNA-heptaploids and previously unknown DNA-triploids, putative DNA-octoploids, and DNAenneaploids. The relative monoploid genome size varied only weakly among the ploidy levels (see Supplementary Data Table S2). Slightly lower fluorescence values per monoploid genome observed in DNA-octoploids together with their rarity (only two individuals found in one population) leave room for some uncertainty in the inferred ploidy. Most plants belonged to DNA-hexaploids (49.6\%), whereas DNA-diploids and DNA-tetraploids made up 33-8 and 15-6\% of the samples, respectively. Secondary cytotypes were found in 27 sample sites: six DNA-triploids, 37 DNA-pentaploids, four DNA-heptaploids, two DNA-octoploids and five DNAenneaploids, totalling 54 individuals (1.07\%). For simplicity, we will refer to the different cytotypes from here on without using the prefix ‘DNA’.

The distribution of the three main cytotypes of *S. carniolicus* is complex, showing some degree of large-scale spatial segregation with large areas of overlap and hence frequent cytotype mixture (Fig. 1). Di- and hexaploid individuals are widespread throughout most of the distribution range. Hexaploids are absent from the western-most Hohe Tauern and from the south-western and south-eastern distribution margin around Lago di Como and in the Karawanken/Karavanke, respectively, where only diploids occur. The Central Alps around the upper Inn valley are exclusively populated by hexaploids and correspond to a large break in the distribution of diploids. Tetraploids were found in two disjunct areas, whose centres are located in the Niedere Tauern in the East and in the Ortler and Adamello massifs in the west. Roughly half of the sample sites (44\%) contained more than one main cytotype. All possible combinations of cytotypes were found with 2x/6x mixtures being most frequent (28\% of sample sites), whereas 2x/4x and 4x/6x sample sites were rare (3 and 5\%, respectively). In 8\% of the sample sites, all three main cytotypes occurred. Spatial co-occurrence patterns of cytotypes within selected mixed populations are shown in the Supplementary Data (Fig. S1).

Many sampled individuals, irrespective of ploidy level, had no conspecific neighbours within a 0.2-m radius, but in sample plots with open habitats or vegetation gaps numerous additional individuals were sometimes encountered. The number of additional individuals did not differ (P = 0.371) between tetraploids and hexaploids (both with median 0 additional individuals), whereas diploids had significantly more neighbours (1 individual as median) compared with other main cytotypes (P < 0.001 for both).

Secondary cytotypes were scattered over the distribution area both in mixed and in otherwise pure sample sites. Their occurrence depended on the composition of main cytotypes within sample sites (Table 1). Among secondary cytotypes, pentaploids were most frequent (37 individuals in 14 populations) and occurred with the exception of a single population (PLE; for details see Appendix) exclusively in populations comprising tetraploids. More than one-quarter of all pentaploids were found in otherwise purely tetraploid populations. Triploids were considerably rarer (six individuals in four populations) and were only found in sample sites where diploid individuals were present, either in pure diploid populations.
or in mixed populations comprising all three main cytotypes. Hepta-, octo- and enneaploid plants were exclusively found in association with hexaploids.

Close relationships of each secondary cytotype to a single main cytotype were also encountered on the micro-scale. Within a distance of 0.2 m, triploids were exclusively associated with diploids (three individuals) and pentaploids grew close to themselves (ten individuals) and/or to tetraploids (20 individuals) with the exception of a single individual located close to a diploid. Similarly, hepta-, octo- or enneaploids occurred next to individuals with a ploidy level equal to or higher than hexaploid (seven individuals).

Ecological differentiation among cytotypes

Cytotypes were spatially clustered within mixed sampling sites. While 99.98% of all pairwise distances < 0.2 m involved individuals of the same ploidy level \((n = 2088\) comparisons), this proportion decreased to 93.6% \((n = 513)\) in the distance class \(< 10\) m, 78.5% \((n = 2401)\) for 10–100 m, 58.3% \((n = 12,358)\) for 100–1000 m and 37.0% \((n = 4879)\) for distances >1000 m.

Pairwise comparisons among the three main cytotypes revealed highly significant differences for most of the environmental descriptors investigated (Fig. 2). Diploids, tetraploids and hexaploids differed significantly in vegetation and rock cover as well as in the relative altitude \((P < 0.001\) in all cases) while nothing discriminated between tetraploids and the other cytotypes but not between diploids and hexaploids. Local inclination, cryptogam cover and East/West exposure had no significant explanatory value. Cover of rock and of vegetation displayed an inverse relationship and revealed largely clear differences between diploid and hexaploid individuals (Fig. 3). Most diploids occupied rocky micro-sites with low vegetation cover, whereas hexaploids were generally associated with high vegetation cover. Tetraploids displayed an intermediate behaviour with regard to rock cover, while vegetation cover was similar to those of hexaploids. Both diploids and hexaploids covered a large altitudinal range with hexaploids centred at lower altitudes than diploids which ascended to the highest relative altitudes sampled. Tetraploids showed a narrower vertical distribution but highest average values. Furthermore, tetraploids were more frequent on northerly exposed slopes, whereas di- and hexaploids appeared to be more indifferent with respect to exposure.

## DISCUSSION

Diploids, tetraploids and hexaploids, the main cytotypes encountered in *Senecio carniolicus*, are partially segregated on the macro-scale, i.e. throughout the whole Alpine range, but overlap strongly. The resulting frequent occurrence of cytotype mixtures enables us to explore patterns and mechanisms of spatial and ecological segregation within roughly half of the 100 investigated sample sites (meso-scale) as well as with respect to the immediate vicinity of about 3000 investigated individuals (micro-scale).

### The macro-scale: cytogeography across the Eastern Alps

The distribution pattern of diploid, tetraploid and hexaploid cytotypes throughout the Eastern Alps was found to be remarkably complex \((Suda et al., 2007)\). The approximately 12-fold increase of overall sample size here compared with the previous study resulted only in minor changes in the large-scale pattern of cytotype distribution \((e.g.\) in the Niedere Tauern, thought to be dominated by tetraploids, diploids are widespread, but occur nearly exclusively south of the main chain), but the proportion of mixed sample sites, mostly di- and hexaploids, rose from about one-third to nearly a half (Fig. 1). Range disruption and survival of at least the last cold stage of the Pleistocene in disjoint refugia appear to have played an important role in shaping the current cytotype distribution. Areas previously suggested to be glacial refugia based on molecular data as well as on species distribution patterns \((Tribsch and Schönswetter, 2003; Schönswetter et al., 2005)\) match regions with high main cytotype diversity. Additionally, the two main regions comprising only diploids \((populations 1–4 and 6 from the south-western part and population 78 from Karawanken/Karavanke; Fig. 1)\) overlap with presumed refugia, a pattern in line with early concepts of presumably relicual diploids being mostly found in unglaciated areas \((Ehrendorfer, 1958)\). The seemingly contradictory exclusive occurrence of hexaploids in the only weakly glaciated \((van Husen, 1987)\) refugial area of the easternmost margin of the Alpine distribution \((populations 98–100)\) fits well with the early recognized poverty of its alpine flora \((Scharfetter, 1909)\) and is probably related to the current scarcity of suitable habitats for the high-altitude adapted (see below) diploid and tetraploid cytotypes, which might have become extirpated due to habitat loss as vegetation zones ascended after the
last glaciation. Furthermore, both disjoint distribution areas of the tetraploid cytotype, separated by a gap of >180 km, correspond to putative glacial refugia (Scho¨ nswetter et al., 2003, 2005; Tribsch and Scho¨ nswetter, 2003) and are characterized by a large number of rare or endemic plant taxa (Schneeweiss and Scho ¨ nswetter, 1999; Tribsch, 2004).

Consequently, the distribution pattern of this cytotype was probably shaped by extensive habitat loss during cold stages of the Pleistocene and weak colonization ability after the last glacial period. This contradicts widespread assumptions about colonizing and competitive success of polyploids as compared with diploids (Stebbins, 1984, 1985; Otto and Whitton, 2000; Otto et al., 2007). It is unclear which traits of the tetraploids are limiting their success in dispersal and population establishment. Ongoing experiments suggest delayed germination and lower seedling viability of tetraploids (M. Sonnleitner and R. Flatscher, unpubl. res.), which may at least in part be responsible for their weaker colonizing ability.

In contrast to tetraploids, the hexaploid cytotype is the most widespread and the one exclusively found in the north-western part of the distribution range, which was the most intensively glaciated area of the Eastern Alps during the Last Glacial Maximum (van Husen, 1987), in agreement with the above-mentioned hypotheses of polyploids being superior colonizers of novel habitats as compared with diploids. Underlying factors include greater plasticity and adaptation potential due to gene redundancy and the massive genomic restructuring processes triggered by whole-genome duplication (reviewed by Otto and Whitton, 2000; Wendel, 2000; Hegarty and Hiscock, 2008; Leitch and Leitch, 2008), but the precise nature of the factors responsible for the higher colonization ability of the hexaploids, such as better dispersal capabilities or higher competitiveness, remains to be established.

Segregation on meso- and microscales

The high frequency of mixed-cytotype populations offers the possibility to test for spatial patterns on smaller geographical scales. Previous studies within a single model population in the Austrian Alps have provided evidence for spatial segregation of diploid and hexaploid cytotypes on meso- as well as microscales (Schönswetter et al., 2007; Hülber et al., 2009). The present study allows a generalization of these patterns for the entire Eastern Alps and provides a first characterization of the habitat requirements of the tetraploid cytotype.

Individuals sharing the same cytotype are spatially strongly aggregated in mixed sampling sites. Whereas virtually no cytotype mixture was encountered within a radius of 0.2 m, variation increased with distance and in the distance class of >1000 m only slightly more than one-third of all pairwise
distances involved individuals of the same ploidy level. This pattern may be explained by a correlation between spatial proximity and microhabitat similarity, i.e. neighbouring individuals are more likely to share a similar environment than more distant individuals. Alternatively, clustering of individuals may be caused by dispersal limitation. Although the fruits of *S. carniolicus* are anemochorous, dense flocks of seedlings around adult plants are frequently observed in the field (M. Sonnleitner and R. Flatscher, pers. obs.). This is in line with dispersal kernels of plant diaspores usually reaching their greatest density in the immediate surroundings of the mother plant (Nathan, 2006).

Di- and hexaploid cytotypes of *S. carniolicus* were suggested to be adapted to open, rocky microhabitats and to dense grass swards or dwarf shrub communities, respectively (Scho¨ nswetter et al., 2007; Hülber et al., 2009). Our results confirm this as a general pattern over the entire distribution area (Fig. 2). The low-growing diploids are mainly found in rocky habitats with sparse and prostrate vegetation or single grass tussocks and in open, heavily cryoerupted fellfields (as described by Ellenberg, 1996). In contrast, the larger and potentially more competitive hexaploids populate habitats characterized by high vegetation and low rock cover. These habitat preferences correlate with distinct characteristics of the two cytotypes, especially differences in plant height and leaf length (R. Flatscher et al., unpubl. res.). Our results are in line with other case studies reporting higher productivity and competition ability of polyploids as compared with their diploid progenitors (Lumaret et al., 1987; Lindner and Garcia, 1997; Petit and Thompson, 1997). Consequently, polyploids were often shown to inhabit more nutrient-rich communities with dense vegetation, whereas diploids tend towards more open habitats (Stählberg, 2009) where competition for essential resources is reduced and abiotic stress becomes the limiting factor.

Diploid and hexaploid cytotypes are centred at different altitudes, albeit with considerable overlap (Figs 2 and 3) and, in accordance with previous findings (Scho¨ nswetter et al., 2007), diploids generally ascend higher than hexaploids (see next paragraph for a discussion on the tetraploids). The observed altitudinal differentiation could either be a direct consequence of altitude and related changes in abiotic parameters such as lower temperatures, lower CO₂ partial pressure, higher irradiation and UV stress (Körner, 2003). Alternatively, it may reflect that, as a general rule, the extent of open habitats with sparse vegetation increases with altitude while dense grasslands and dwarf shrub vegetation decline (Körner, 2003). This correlation probably reconciles the two competing hypothesis for segregation of di- and hexaploid cytotypes of *S. carniolicus*, i.e. altitude (Scho¨ nswetter et al., 2007) and openness of the vegetation (Hülber et al., 2009).

Definition of the ecological niche of the tetraploids is not straightforward. Their altitudinal amplitude was narrower and significantly higher than those of the other two cytotypes (Figs 2 and 3). With regard to rock and to vegetation cover, tetraploid *S. carniolicus* occupies an intermediate position between di- and hexaploids. Vegetation cover is similarly high, but still statistically significantly smaller than that found in hexaploids, and rock cover is significantly lower than in diploids (Figs 2 and 3). However, tetraploids are more frequent on or sometimes even restricted to north-facing

![Fig. 3. Comparison of the main cytotypes of Senecio carniolicus with respect to rock cover, vegetation cover, altitudinal distance to the local tree line and north/south exposure of the microhabitat. Boxes span the range between the 25th and 75th percentile with indicated median, and whiskers extend to 1.5-fold the interquartile range. Outliers are represented by open circles.](http://aob.oxfordjournals.org/)

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slopes, whereas di- and hexaploids seemed to be more indifferent towards exposure direction (Figs 2 and 3). Furthermore, tetraploids have a tendency towards slightly base-rich soils (P. Escobar García et al., pers. obs.) whereas diploids and hexaploids are strictly acidophilic. The single exception is the disjunct diploid population 78 in the Karawanken/Karavanke range, where S. carniolicus grows on dolomite bedrock, which may be a product of local adaptation processes facilitated by its isolated position.

Secondary cytotypes: occurrence and possible evolutionary implications

Secondary cytotypes, i.e. tri-, penta-, hepta-, octo- and enneaploids, were encountered in low frequencies only (approx. 1% altogether). This number is comparable with pre-tetraploids, were encountered in low frequencies only (2

CONCLUSIONS AND OUTLOOK

Even if in S. carniolicus the 12-fold increase in the number of investigated individuals does not significantly affect the cytogeographical pattern described previously (Suda et al., 2007), only large data sets such as that presented here with a sampling scheme which is both intensive (many plants per site) and extensive (many sites throughout the whole distribution area; Halverson et al., 2008) allow for a synthetic approach addressing questions about polyploid origin, their morphological, ecological or reproductive differentiation as well as the mechanisms of polyploid speciation.

We have shown that the three main cytotypes of S. carniolicus (2x, 4x, 6x) exhibit a complex distribution pattern with an unusually high proportion of contact areas with varying spatial extent. As causes for this distribution pattern we suggest historical processes connected to Pleistocene climatic fluctuations on the macro-scale and ecological divergence on the meso- and micro-scales. As habitat segregation does not prevent the occurrence of different cytotypes in close spatial proximity, other pre- or post-zygotic isolating mechanisms need to be invoked to explain the obviously strong reproductive isolation. These are the subject of current ongoing research that combines molecular data, to enable the reconstruction of spatio-temporal evolutionary diversification of S. carniolicus, with experimental approaches, to explore the mechanisms maintaining cytotype mixtures.

SUPPLEMENTARY DATA

Supplementary Data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: Relative fluorescence intensities of DAPI-stained nuclei of 5033 silica gel-dried leaf samples of Senecio carniolicus from 100 sample localities. Table S2: Mean relative fluorescence intensities (per monoploid genome) of individual ploidy levels of Senecio carniolicus in the present study and in previous work (Suda et al., 2007). Fig. S1: Spatial distribution of individuals of Senecio carniolicus within selected mixed populations. Fig. S2: Mean relative fluorescence intensities of DNA-diploid, DNA-triploid, DNA-tetraploid, DNA-pentaploid and DNA-hexaploid samples of Senecio carniolicus. Fig. S3: Mean relative fluorescence intensities of DNA-hexaploid, DNA-heptaploid, DNA-octoploid and DNA-enneaploid samples of Senecio carniolicus.

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Italy: Autonome Provinz Bozen – Südtirol/Provincia Autonoma di Bolzano – Alto Adige (63.01.05/298143); Switzerland: Amt für Natur und Umwelt – Kanton Graubünden (La 7·73/5807). We thank Hanna Weiss-Schneeeweiß for helpful discussions, and Daniela Stawik, Christian Gilli, Bozo Frijman and Manfred Schmucker for help with fieldwork.

LITERATURE CITED


APPENDIX

Overview of the 100 sample sites where material of *Senecio carniolicus* was collected for the present study. Geographical position (centroid of the individual coordinates) and altitudinal range of sampled individuals are given as well as DNA ploidy levels with the number of individuals per ploidy level (*n*) and the total number (*N*) of individuals investigated per sample site. Herbarium vouchers stored in WU are labelled with the population code. Country abbreviations: A, Austria; CH, Switzerland; IT, Italy; SLO, Slovenia.

<table>
<thead>
<tr>
<th>Code</th>
<th>Locality (Country)</th>
<th>Latitude (°N)</th>
<th>Longitude (°E)</th>
<th>Altitudinal range (m a.s.l.)</th>
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