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Reciprocal transplantations reveal strong niche differentiation among ploidy-differentiated species of the *Senecio carniolicus* aggregate (Asteraceae) in the easternmost Alps

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Abstract

Phenotypic changes conferred by polyploidisation likely alter the ecological niche of polyploids, coming along with differences in performance compared to their diploid ancestors. However, it is largely unknown whether these performance differences remain constant during the life history of plants. Diploid *Senecio noricus*, tetraploid *S. disjunctus*, and hexaploid *Senecio carniolicus* s. str. of the autopolyploid species complex of *S. carniolicus* (Asteraceae) from the easternmost Alps were reciprocally transplanted to address the following questions: Are there differences in species performances indicating niche differentiation? If so, does the resident or the higher ploid species—which is taller growing and possibly more tolerant against challenging abiotic and biotic conditions—show higher performance? Are performance differences consistent between the early and late life-history stages? Our data suggest that the hierarchy of species performance depends on the life-history stage. For early life-history stages (seeds and seedlings), we mainly observed superior performance of *S. carniolicus* s. str. even on resident sites of the two other species. In contrast, vital rates of adults were predominantly highest for the resident species. The resident site of *S. carniolicus* s. str., which was expected to be environmentally least stressful (i.e., longest growing period, least affected by frost), turned out to be the most selective one, with high mortality and (nearly) no reproduction of the foreign species most likely due to top soil desiccation. Our study illustrates that there may be no clear-cut answer to the question if the resident or the higher ploid species shows superior performance in polyploid complexes, but rather suggests that relative performance may depend on life-history stage.

Keywords Niche differentiation · Polyploidy · *Senecio carniolicus* agg. · Vital rates

Karl Hülber and Michaela Sonnleitner have contributed equally to this manuscript.

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Introduction

Polyploidisation plays a fundamental role in the evolution and diversification of angiosperms (Soltis et al. 2009). Polyploids and their diploid or lower ploid ancestors frequently differ in morphological and physiological traits (Weiss-Schneeweiss et al. 2013; Kolář et al. 2017). Their divergence may arise as a direct consequence of genome duplication (Otto and Whitton 2000) and/or be due to subsequent

adaptive evolution (Petit et al. 1999; Ramsey and Schemske 2002). Polyploids often have larger cells and are typically larger than diploids (Sagarin and Gaines 2002), potentially increasing their competitive abilities (Maceira et al. 1993) despite their frequently slower development (Otto 2007). However, the generality of this pattern is debated (Buggs and Pannell 2007; Collins et al. 2011). Furthermore, polyploids have been suggested to exhibit greater tolerance against challenging abiotic and biotic conditions due to higher genetic and biochemical diversity (Comai 2005; Weiss-Schneeweiss et al. 2013) or higher phenotypic plasticity (Hahn et al. 2012).

Phenotypic changes conferred by polyploidisation likely help polyploids to overcome the ecological limits of their diploid progenitors and allow them to colonize new habitats, resulting in an altered ecological niche of polyploids (Stebbins 1971). Thereby, two principle types of niche differentiation between diploids and their polyploid descendants, both coming along with performance differences in particular habitats, can be distinguished. The first are niche shifts, i.e., changes in environmental optima. They imply that adaptive differentiation at the ploidy-level results in the superiority of the resident (i.e., better adapted) cytotype. Niche shifts promote spatial separation and are, hence, a prerequisite for the long-term co-existence of many ploidy-level differentiated taxa (Kolář et al. 2017). Second, polyploidisation may cause an increased ecological amplitude allowing polyploids to occupy a broader range of habitats than diploids (Levin 1983, 2002; but see McIntyre 2012). Such a scenario might support the often hypothesised general superiority of polyploids compared to their di- or lower ploid congeners (e.g., Maceira et al. 1993; Comai 2005; Weiss-Schneeweiss et al. 2013). Thus, polyploids might have distinct and/or broader niches compared to diploids (McIntyre and Strauss 2017), resulting in strongly varying degrees of ecological differentiation ranging from the restriction of cytotypes to different habitats (Lumaret et al. 1987), which evidently promotes spatial segregation (Parisod et al. 2010; te Beest et al. 2012) to differences in the relative abundance of accompanying species (Johnson et al. 2003).

Niche differentiation in heteroploid species has been inferred by observations of natural populations based on sampling in different habitats (Lumaret et al. 1987; Duchoslav et al. 2010), collecting environmental parameters for individuals of the focal species (Mráz et al. 2012), comparing associated vegetation (Felber-Girard et al. 1996; Johnson et al. 2003; Ståhlberg and Hedrén 2009; Sonnleitner et al. 2016) or performance of plants in common garden experiments (Bretagnolle and Thompson 2001; Manzaneda et al. 2012). Recently, species distribution modelling was applied to compare the climatic niches of cytotypes (McIntyre 2012; Godsoe et al. 2013; Glennon et al. 2014; Kirchheimer et al. 2016). Undoubtedly, these approaches have their merits, but

they may also face serious caveats. For instance, observational studies might confound competitive displacement with niche differentiation (Mitchell et al. 2009), and common garden experiments as well as modelling approaches cannot cover all potentially relevant environmental conditions of the cytotypes' native habitats (Baack 2005). In that light, only reciprocal transplantations provide direct evidence for differences in the fundamental niche (Soltis et al. 2010) by comparing the local performance. To date, reciprocal transplants have been applied to test for niche differentiation in di-/tetraploid (Flegrová and Krahulec 1999; Baack 2005; Martin and Husband 2013) or di-/hexaploid model systems (Buggs and Pannell 2007; Raabová et al. 2008), but they have rarely been used in polyploid complexes comprising more than two cytotypes allowing for comparisons among polyploid levels (see also Duchoslav et al. 2017; McIntyre and Strauss 2017). In addition, we are not aware of any reciprocal transplantation experiment covering the full life history (from germination to reproduction) of a heteroploid model system.

The *Senecio carniolicus* Willd. (syn. *Jacobaea carniolica* (Willd.) Schrank) aggregate comprises the silicicolous alpine perennials *Senecio noricus* Flatscher, Schneew. & Schönsw. (diploid), *S. disjunctus* Flatscher, Schneew. & Schönsw. (tetraploid), and *S. carniolicus* s. str. (hexaploid), which occur sympatrically in the easternmost Eastern Alps (Suda et al. 2007; Sonnleitner et al. 2010). The fourth species of this group, the diploid *S. insubricus* (Chenevard) Flatscher, Schneew. & Schönsw., is not present in this region (Flatscher et al. 2015). Molecular genetic evidence suggests that the polyploid species are autopolyploid derivatives of *S. noricus* (Winkler et al. 2017) and that recurrent formation is highly unlikely, which is in line with consistent morphological differentiation (Flatscher et al. 2015) among the species. Consequently, co-occurrence of species—which was frequently observed in various combinations in close spatial proximity (less than one meter; Hülber et al. 2009, 2015; Sonnleitner et al. 2010)—is likely due to secondary contact in the course of the postglacial colonization of the Alps. Reconstruction of ecological niches based on proxies such as Landolt indicator values of the surrounding vegetation (Schönswetter et al. 2007; Hülber et al. 2009, 2015) provided indirect evidence for ecological differentiation between pairs of species. A large-scale survey (Sonnleitner et al. 2016) additionally revealed differences in the realised niches between pure and sympatric populations of all three species, i.e., niche displacement, which is likely due to competitive effects.

Here we assess differences in performance—measured via two estimates of overall fitness and six single vital rates—of diploid *S. noricus*, tetraploid *S. disjunctus*, and hexaploid *S. carniolicus* s. str. by reciprocally transplanting seeds and adult individuals among natural populations.

We focus on the following questions: (1) Are there ploidy-dependent differences in species performances indicating niche differentiation? (2) Does the resident or the higher ploid species show higher performance refuting or supporting the often hypothesised general superiority of polyploids? (3) Are performance differences consistent between early (seeds, seedlings) and late (adult) life-history stages?

Materials and methods

Study design

The study was conducted east of Turracher Höhe (Gurktaler Alpen, Austria) in areas of exclusive occurrence, in the following referred to as resident sites, of diploid *S. noricus*, tetraploid *S. disjunctus*, and hexaploid *S. carniolicus* s. str. The resident site of *S. noricus* was situated on a flat, gravelly, windswept plateau to the West of Mt Kaserhöhe (N 46.91162°, E 13.91403°; 2283 m a.s.l.), that of *S. disjunctus* on the northern slope of Mt Bretthöhe (N 46.91087°, E 13.92445°; 2308 m a.s.l.), and that of *S. carniolicus* s. str. on the southwestern slope of Mt Schoberriegel (N 46.90967, E 13.89500; 2174 m a.s.l.); all three resident sites are within a maximal distance of 3 km. In a reciprocal design, we transplanted cypselas (termed "seeds" in the following for simplicity) and adult individuals of the three species to each resident site to assess six vital rates (seed germination, seedling establishment, and seedling growth, as well as adult survival, adult growth, and reproduction) and two indices of overall fitness.

Transplantation of seeds

Mature seeds were collected in late summer 2010 at each resident site as a pooled sample from about 100 mother plants. Because we expected low survival, we did not consider analysing seed families separately.

Germination experiments were conducted in six 1 m × 1 m plots per resident site. Plots were subdivided by a removable grid consisting of 100 cells of 0.1 m × 0.1 m. For each species, 25 seeds were sown into each of 15 randomly selected cells in each plot in autumn of 2010 (3 resident sites × 6 plots × 3 species × 15 cells × 25 seeds = 20,250 seeds). In addition, we randomly chose 15 cells in each plot to observe natural background germination. The number of emerged seedlings was recorded in autumn 2011, 2012 and 2013. After the last observation above-ground biomass of living individuals was harvested, oven dried at 60 °C for 1 week, and the combined dry weight of seedlings of a cell was measured to the nearest 0.1 mg. Separate measurements of individuals were not feasible as the seedlings were rarely bigger than 15 mm in diameter. Tracing the fate of individual seedlings was impossible, because tagging would have entailed a high risk of damaging the tiny plants frequently growing close to each other. The relatively high proportion of germinating seeds together with massive substrate movement (particularly debris and dead biomass) caused oscillations in the number of recorded seedlings in individual cells in consecutive years. Thus, the number of recorded seedlings in cells within a plot was summed up for each species to balance errors in the detection and re-detection of plants. In addition, the slow development of seedlings—the majority possessed only 2–3 leaves even after 3 years of observation—prevented the discrimination of newly emerged plants from older seedlings.

Seed germination

Because seedlings emerged in the previous years cannot be differentiated from recently germinated ones, seed germination was defined as the number of seedlings divided by the number of sown seeds only in the first observation after sowing. To test for differences in germination between the species in their resident sites on one hand and the foreign species or the control cells on the other hand, Poisson Generalised Linear Models (function "glm" from the library "stats") were used.

Seedling establishment

Seedling establishment was defined as the temporal trend in the number of seedlings across observations. To test for differences in seedling establishment, Poisson Generalised Equation Estimations (function "geeglm" from the library "geepack", Halekoh et al. 2006) with an auto-regressive correlation structure among annual observations of the same plot relating the number of seedlings to species, the year of observation, and the species × year of observation interaction were applied.

Seedling growth

Differences in seedling growth were calculated by means of log-transformed mean dry weights of seedlings within a cell. To this end, Gaussian Generalised Equation Estimations assuming identical correlation among all cells of a plot (i.e., correlation structure "exchangeable" in the geeglm-function) and weighting cells by the number of seedlings were applied to compare the species. Model selection was done with the function "step".

Transplantation of adults

In autumn 2008, 150 adult individuals per species were dug out from their natural populations, 50 of these were transplanted to each resident site of the two other species, and 50 adult individuals were transplanted within their resident site. The ploidy of all transplanted individuals was determined via flow cytometry (FCM) of silica-dried leaf material following Sonnleitner et al. (2010). All transplanted plants were tagged and observed at annual intervals until autumn 2013. Individuals that were dead at the first observation in 2009 were excluded from all analyses to minimise confounding effects of transplantation. At each observation, (1) the survival status (dead/alive), (2) variables describing plant size (i.e., number of rosettes, number of leaves, length of the longest leaf, largest diameter of the biggest rosette, and diameter perpendicular to it) to model the biomass in earlier years for subsequent growth analysis, and (3) the number of hyperinflorescences, (i.e., shoots bearing flower heads, termed "inflorescences" in the following for simplicity) as a measure for reproduction were recorded. At the end of the experiment, the above-ground biomass of all surviving transplanted individuals was harvested, oven dried at 60 °C for 2 weeks, and the dry weight measured to the nearest 0.1 mg.

Adult survival

To compare the survival rates of adults among species, Cox Proportional Hazards regression models (function "coxph" in the library "survival", Therneau 2015) were applied, which relate the time between transplantation and death of individuals (in years) to species. Thereby, individuals still alive in 2013 were included as right-censored data.

Adult growth

To obtain predictions of the biomass in the previous years, the relationship between the measured size variables and dry weight of adults was estimated by least-squares regression using species, the number of leaves and rosettes, the interaction of number of leaves and rosettes with species, first- and second-order polynomials of both diameters, and the length of the longest leaf amended by a presence/absence transformation of the number of inflorescences as predictors. Variable selection omitted the interaction of number of rosettes with species resulting in a model appropriately fitting the data ($R^2 = 0.93$, $F_{13,298} = 311.5$, $p < 0.001$). Prior to running the model, dry weight and the number of leaves and rosettes were log-transformed. Fitted regression models were then applied to predict the dry weight of all individuals at each observation. These predictions were scaled to zero mean and unit variance separately for each species in each

resident site to be used as measure of adult growth. This was necessary to balance differences in average plant size among species (hexaploid *S. carniolicus* s. str. >> tetraploid *S. disjunctus* > diploid *S. noricus*, Flatscher et al. 2015). An a priori log-transformation ensured the approximate normal distribution of data. Gaussian Generalised Equation Estimations with an auto-regressive correlation structure among annual observations of the same individual (i.e., "ar1" in the "geeglm"-function of the library "geepack", Halekoh et al. 2006) were applied. These models relate dry weight to species, year of observation, and their interaction. Coefficients and marginal p values of the interaction term indicate whether the changes in dry weight across time differ between species.

Reproduction

The number of inflorescences in 2009 was about three times higher than that summed up for all subsequent years indicating direct effects of the transplantation. Thus, we omitted data of this year from analyses. To compare the reproduction among the species, we applied Poisson Generalised Equation Estimations (function "geeglm") with an auto-regressive correlation structure among annual observations of the same individual. We fitted models with species, year of observation, and their interaction to compare the temporal trend in flowering frequency (indicated by the coefficients of the interaction term). Comparisons including *S. noricus* and *S. disjunctus* in the resident site of *S. carniolicus* s. str. as well as of *S. disjunctus* in the resident site of *S. noricus* were not feasible because of the low number (0, 1, 1) of inflorescences.

Overall fitness

As a measure of overall success of a species transplanted (adults) or sown (seeds) to a particular resident site, two fitness indices were calculated. Fitness$_{veg}$ relates to establishment success and encompasses seed germination as well as seedling and adult survival, and is calculated as: (number of germinated seedlings 2011/number of seeds sown 2010) × (sum of number of seedlings 2010–2013/sum of number of seedlings 2009–2012) × (sum of number of surviving adults 2010–2013/sum of number of adults 2009–2012). Fitness$_{rep}$ relates to reproductive success and includes also reproduction, and is calculated as fitness$_{veg}$ × (sum of number of inflorescences 2010–2013).

All analyses and figures were done using the statistical computing environment R 3.3.2 (R Development Core Team 2016). For an extension of the experiment including plants from other mountain ranges, see Supplementary Material 1. Data are available from the Dryad Digital Repository: https ://doi.org/10.5061/dryad.cq98678.

Results

Both indices of overall fitness, fitness$_{veg}$ and fitness$_{rep}$, delivered qualitatively identical results (Table 1). Resident species showed the highest overall fitness, except for the resident site of *S. disjunctus*, where *S. carniolicus* s. str. was the best-performing species. Fitness of foreign species was particularly low at the resident site of *S. carniolicus* s. str., and *S. disjunctus* had a low fitness, particularly at foreign sites.

The relative performance of the three investigated species strongly depended on the vital rate considered. Concerning seed germination and seedling growth, *S. carniolicus* s. str. performed best at all sites: i.e., this species showed the highest number of seedlings in the first year after sowing (Fig. 1, Table 2) and the highest dry weight of emerged seedlings at the end of the observation period (Fig. 2, Table 2). However, at the resident site of *S. carniolicus* s. str., mean seedling weight of *S. noricus* and *S. carniolicus* s. str. was not significantly different. Mean germination rate at the resident site of *S. noricus, S. disjunctus*, and *S. carniolicus* s. str. was 12.7, 26.3, and 10.5%, respectively. Seed germination in control cells was significantly lower than in sown-in cells for all resident species (*S. noricus*: coef $= -2.00 \pm 0.18$, z value $= -11.24$, $p < 0.001$; *S. disjunctus*: coef $= -2.28 \pm 0.14$, z value $= -16.29$, $p < 0.001$; *S. carniolicus* s. str.: coef $= -2.91 \pm 0.22$, z value $= -13.28$, $p < 0.001$). In general, the number of seedlings decreased throughout the observation period (Fig. 1). This reduction was generally lowest (i.e., seedling establishment was highest) for the resident species, except for *S. noricus* performing better than *S. disjunctus* on the resident site of the latter

Table 1 Overall fitness of species of the *Senecio carniolicus* agg. transplanted or sown to resident sites of diploid *Senecio noricus*, tetraploid *S. disjunctus* and hexaploid *S. carniolicus* s. str. (a) fitness$_{veg}$, index based on seed germination, seedling and adult survival only; (b) fitness$_{rep}$, index based on seed germination, seedling and adult survival and reproduction

Transplanted species	Resident species		
	S. noricus	*S. disjunctus*	*S. carniolicus* s. str.
(a) Fitness$_{veg}$			
S. noricus	**0.108**	0.168	0.023
S. disjunctus	0.055	0.190	0.019
S. carniolicus s. str	0.085	**0.230**	**0.115**
(b) Fitness$_{rep}$			
S. noricus	**2.164**	2.685	0.000
S. disjunctus	0.000	2.087	0.019
S. carniolicus s. str	1.189	**3.443**	**1.265**

Data from several years were pooled for analysis. The best-performing species at a given transplantation site is highlighted in bold

Fig. 1 Seed germination (i.e., number of seedlings in 2011) and seedling establishment (change in number of seedlings from 2011 to 2013) of species of the *Senecio carniolicus* agg. observed at resident sites of diploid *S. noricus*, tetraploid *S. disjunctus*, and hexaploid *S. carniolicus* s. str. in 2011 (light grey), 2012 (medium grey), and 2013 (dark grey). Underlined names indicate the species to whose resident site individuals were transplanted. The number of seedlings is summed up for each plot
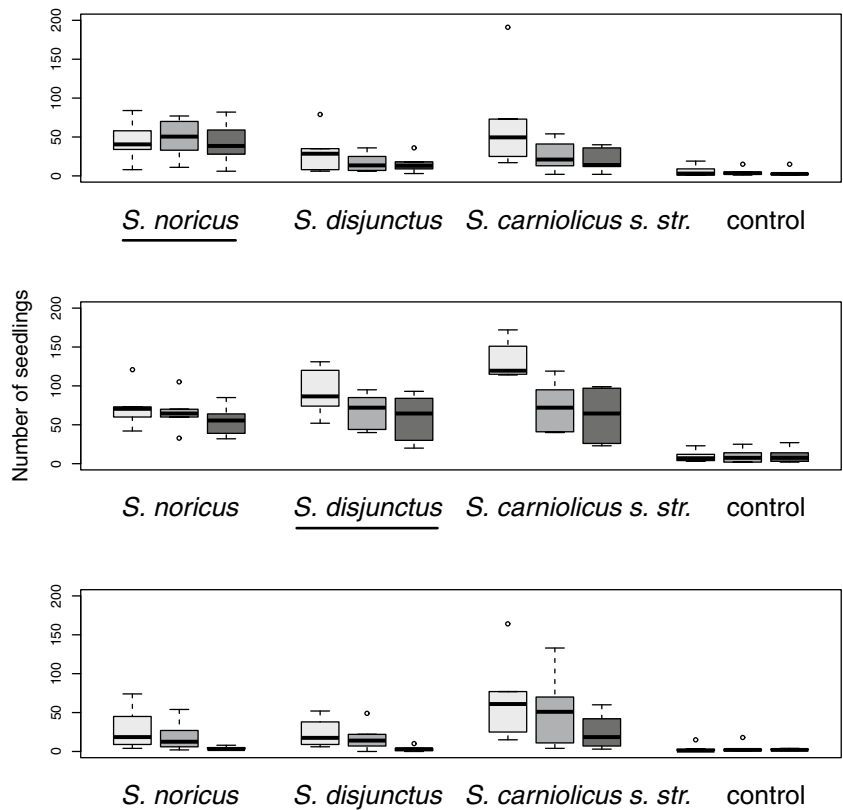
Table 2 Comparison of six vital rates between resident and foreign species of the *Senecio carniolicus* agg. derived from reciprocal transplantations of seeds and adult individuals into resident sites of diploid *S. noricus*, tetraploid *S. disjunctus*, and hexaploid *S. carniolicus* s. str. and subsequent annual observations (2009–2013)

	Seed germination			Seedling establishment			Seedling growth		
	Coef ± SE	z value	p value	Coef ± SE	Wald	p value	Coef ± SE	Wald	p value
Resident *S. noricus*				Scale = 16.50 ± 7.34			Scale = 0.32 ± 0.05		
S. disjunctus	−0.36 ± 0.10	−3.75	<0.001	−0.37 ± 0.06	38.24	<0.001	0.09 ± 0.11	0.59	0.443
S. carniolicus s. str	0.42 ± 0.08	5.37	<0.001	−0.70 ± 0.15	21.21	<0.001	0.47 ± 0.06	51.76	<0.001
Resident *S. disjunctus*				Scale = 9.24 ± 0.85			Scale = 0.21 ± 0.03		
S. noricus	−0.23 ± 0.06	−3.59	<0.001	0.12 ± 0.05	6.79	0.009	0.10 ± 0.11	0.93	0.335
S. carniolicus s. str	0.36 ± 0.06	6.55	<0.001	−0.19 ± 0.08	6.63	0.010	0.27 ± 0.11	5.55	0.018
Resident *S. carniolicus* s. str				Scale = 20.80 ± 10.30			Scale = 0.39 ± 0.13		
S. noricus	−0.87 ± 0.09	−9.48	<0.001	−0.58 ± 0.11	27.12	<0.001	−0.07 ± 0.23	0.10	0.746
S. disjunctus	−1.05 ± 0.10	−10.78	<0.001	−0.33 ± 0.12	7.58	0.006	−0.64 ± 0.24	7.21	0.007

	Adult survival			Adult growth[a]			Reproduction[b]		
	Coef ± SE	z value	p value	Coef ± SE	Wald	p value	Coef ± SE	Wald	p value
Resident *S. noricus*; n = 147	Deaths = 9			Scale = 0.95 ± 0.10			Scale = 1.44 ± 1.35 (n = 96)		
S. disjunctus	−0.01 ± 1.00	−0.01	0.994	−0.12 ± 0.05	5.48	0.019	–	–	–
S. carniolicus s. str	0.95 ± 0.84	1.14	0.256	−0.18 ± 0.05	11.87	<0.001	−1.03 ± 0.33	9.93	0.002
Resident *S. disjunctus*; n = 139	Deaths = 9			Scale = 0.95 ± 0.08			Scale = 1.04 ± 1.15 (n = 138)		
S. noricus	−0.96 ± 0.84	−1.15	0.249	−0.10 ± 0.06	2.89	0.089	−0.91 ± 0.37	5.90	0.015
S. carniolicus s. str	−0.78 ± 0.84	−0.94	0.349	−0.23 ± 0.06	17.81	<0.001	−1.06 ± 0.45	5.50	0.019
Resident *S. carniolicus* s. str.; n = 123	Deaths = 71			Scale = 0.89 ± 0.17			–		
S. noricus	4.30 ± 0.74	5.85	<0.001	−0.69 ± 0.11	38.84	<0.001	–	–	–
S. disjunctus	4.12 ± 0.74	5.60	<0.001	−0.58 ± 0.08	52.91	<0.001	–	–	–

Seed germination, seedling establishment, and seedling growth represent the number of seedlings emerged in 2011, the temporal trend in the number of seedlings from 2011 to 2013, and the mean dry weight of seedlings, respectively. Survival, growth, and reproduction of adult transplants represent temporal trends in the number of living individuals, predicted above-ground biomass, and the number of inflorescences, respectively. Marginal coefficients were derived from Generalised Linear Models (seed germination), Cox Proportional Hazards regression models (adult survival), and Generalised Estimation Equations (other vital rates), and represent either the species term (adult survival, seed germination, and seedling growth) to illustrate differences among species or the year of observation × species interaction (adult growth, reproduction, and seedling establishment) to illustrate differences in the temporal trend among species. Main effect coefficients for species and year in the latter and the intercept in all models are not presented to enhance clarity. $N = 6$ plots per species at each site for all seed and seedling models

[a] Adult weight for annual observations was obtained from a linear regression relating non-destructively measured variables of plant size to plant weight measured after harvest in 2013

[b] Missing values for reproduction are due to low numbers of inflorescences (zero or one)

(Table 2). Seedling survival of foreign species was lowest at the resident site of *S. carniolicus* s. str., with only 24 and 21 seedlings of *S. noricus* and *S. disjunctus*, respectively, surviving until the end of the observation period (Fig. 1). Thus, the low numbers of seedlings in cells sown-in with *S. noricus* and *S. disjunctus* seeds are in the range of the background germination of *S. carniolicus* s. str.

Resident species outperformed their foreign counterparts in terms of adult growth and reproduction (Table 2). Predicted dry weight of foreign species consistently decreased during the observation period, while that of the resident species remained approximately at the same level as at the time of transplantation (Fig. 3). Similarly, the mean number of inflorescences was either close to zero throughout the observation period or decreased between 2010 and 2013 for foreign species. In contrast, the number of inflorescences increased over time (*S. noricus*) or remained approximately constant (*S. disjunctus, S. carniolicus* s. str.) for resident species (Fig. 4). Survival of adults (Fig. 5, Table 2) did not differ among species at the resident sites of *S. noricus* and *S. disjunctus* as survival rates of all three species were ≥ 90%. In contrast, *S. carniolicus* s. str. strongly outperformed foreign species at its resident site, where not a single and only
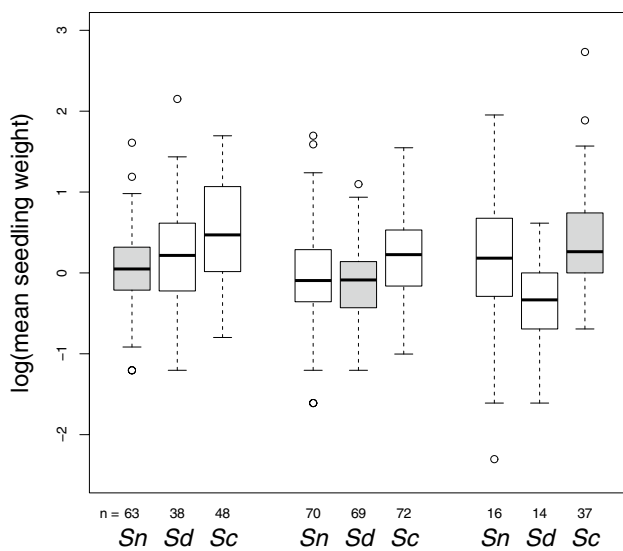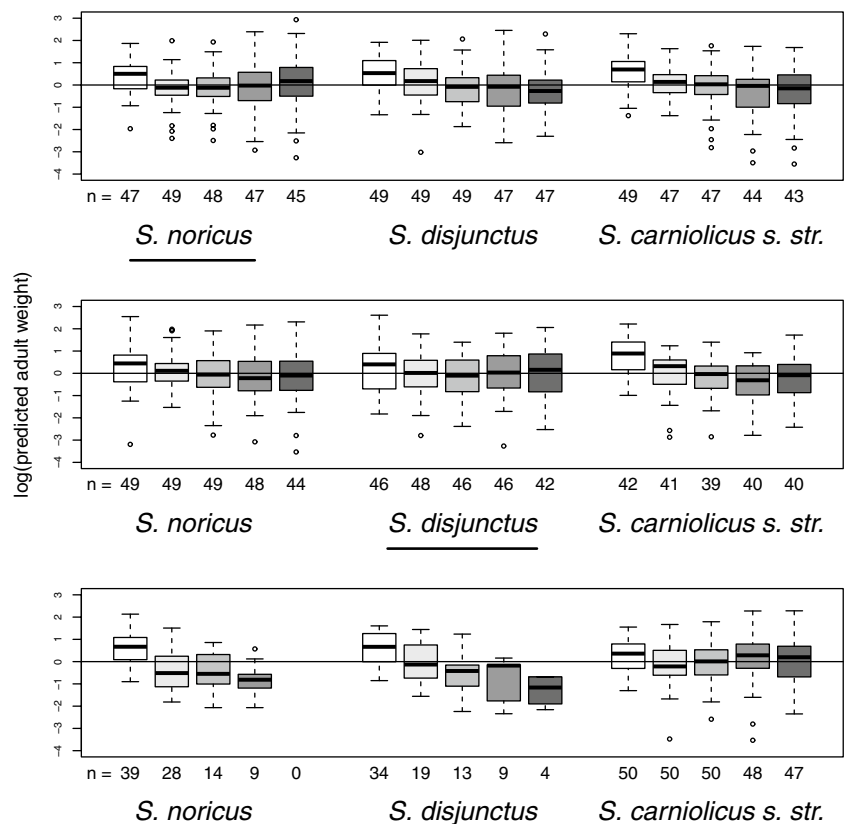
Fig. 2 Log-transformed mean weight of seedlings of species of the *Senecio carniolicus* agg., indicating the growth 4 years after sowing in resident sites of diploid *S. noricus* (*Sn*), tetraploid *S. disjunctus* (*Sd*), and hexaploid *S. carniolicus* s. str. (*Sc*). Grey boxes indicate the species to whose resident site individuals were transplanted. *N* represents the number of seedlings

Fig. 3 Growth of adult individuals of species of the *Senecio carniolicus* agg. reciprocally transplanted to resident sites of diploid *S. noricus* (above), tetraploid *S. disjunctus* (centre), and hexaploid *S. carniolicus* s. str. (below). Underlined names indicate the species to whose resident site individuals were transplanted. *N* represents the number of individuals from 2009 to 2013 (white to dark grey). The *y*-axes represent dry weight of individuals predicted by means of linear regressions from individuals harvested and weighed at 2013 based on annual, non-destructive measurements of size parameters. Dry weight was log-transformed and standardised to zero mean and unit variance separately for each species in each resident site to balance different plants sizes of species. Increases in the number of individuals were caused by missed individuals (overlooked or without aboveground organs in a particular year)

four (8%) individuals of *S. noricus* and *S. disjunctus*, respectively, survived until the end of the experiment.

Not a single individual of *S. disjunctus* flowered in the resident site of *S. noricus*. *S. carniolicus* s. str. initially performed well there but showed a strong decrease in reproduction between 2011 and 2013 (Fig. 4). Conversely, in the resident site of *S. disjunctus*, the average number of inflorescences of *S. noricus* was high, but decreased from year to year. The resident species, in contrast, showed an approximately doubled reproduction in 2013 compared to the previous years. Reflecting the low survival rates, reproduction of *S. disjunctus* and *S. noricus* was zero in the resident site of *S. carniolicus* s. str. during the last 3 years of the observation period (Fig. 4).

Discussion

Spatial segregation, as observed within the *S. carniolicus* aggregate (Schönswetter et al. 2007; Sonnleitner et al. 2010, 2016; Hülber et al. 2015) and many other polyploid systems, may result from frequency-dependent selection against rare cytotypes (Levin 1975), the migration history in combination with dispersal limitation (Thompson and Lumaret 1992; Baack 2004), or niche differentiation among species. To test for the latter, we performed reciprocal transplantations

Fig. 4 Reproduction of individuals of species of the *Senecio carniolicus* agg. reciprocally transplanted to resident sites of diploid *S. noricus* (above), tetraploid *S. disjunctus* (centre), and hexaploid *S. carniolicus* s. str. (below). Underlined names indicate the species to whose resident site individuals were transplanted. *N* represents the number of observed individuals from 2010 to 2013
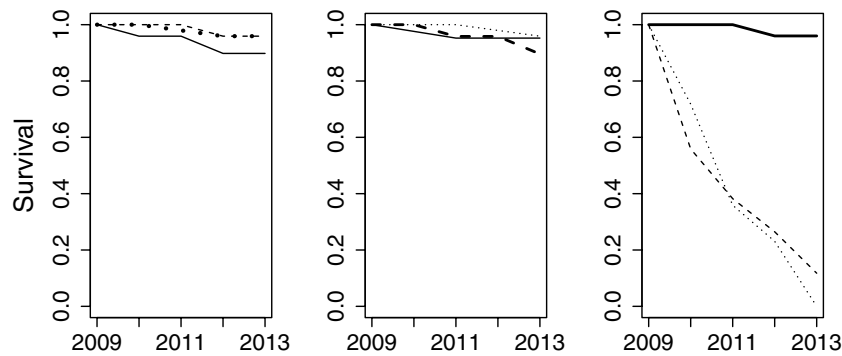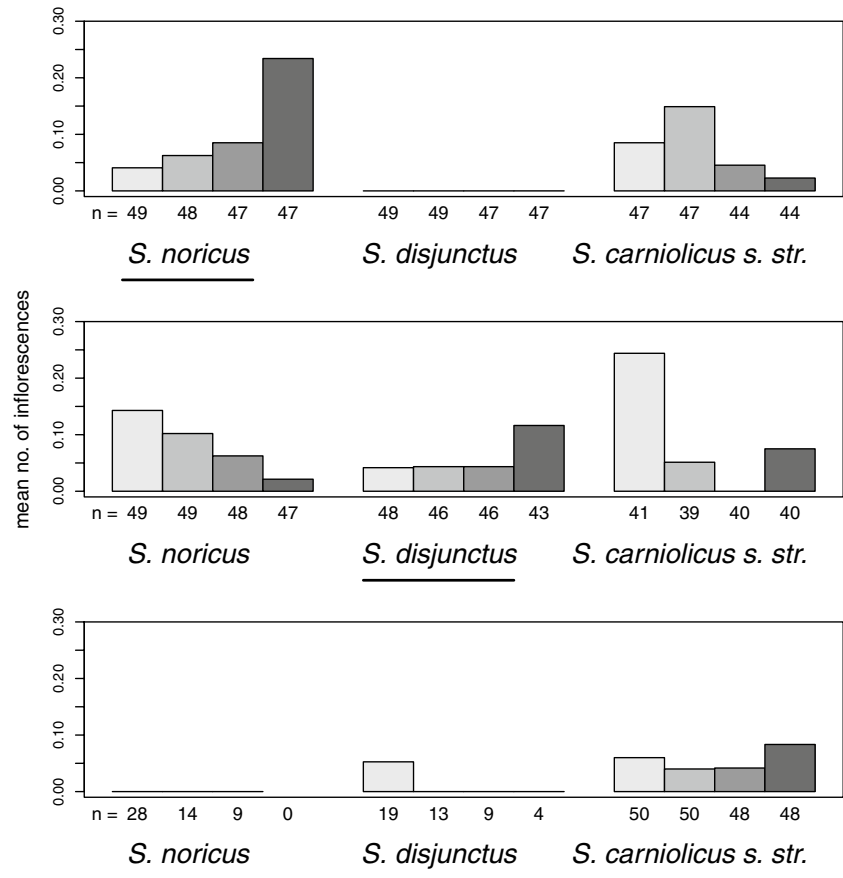

Fig. 5 Proportion of surviving adult individuals of species of the *Senecio carniolicus* agg. reciprocally transplanted to resident sites of diploid *Senecio noricus* (left), tetraploid *S. disjunctus* (centre), and hexaploid *S. carniolicus* s. str. (right). Dotted, dashed, and solid lines represent transplanted *S. noricus, S. disjunctus*, and *S. carniolicus s. str.*, respectively. Underlined names indicate the species to whose resident site individuals were transplanted

and measured the performance of the recently delineated diploid *S. noricus*, tetraploid *S. disjunctus*, and hexaploid *S. carniolicus* s. str. (Flatscher et al. 2015). Differences in the overall performance as well as in individual vital rates (Tables 1, 2) provide direct experimental evidence that these species are ecologically differentiated. This is not surprising, as multi-faceted phenotypic, distributional, and/or environmental differences exist between diploid and autopolyploid

populations in almost all the studied systems (Ramsey and Ramsey 2014). Differences in performance demonstrating niche differentiation were particularly found for mixed-ploidy systems in mountain regions. Diploid *Anthoxanthum alpinum* was found to occur at higher elevations than tetraploid *A. odoratum* (Flegrová and Krahulec 1999). Similarly, diploid *Chamerion angustifolium* performs better at colder, more elevated sites than tetraploid individuals of the same

species (Martin and Husband 2013; Thompson et al. 2014). In contrast, in *Empetrum nigrum* (Elvebakk and Spjelkavik 1995), polyploids are more cold-adapted. Consequently, the hypothesis of polyploids being more tolerant against harsh environmental conditions (Levin 2002) cannot be confirmed for all or even the majority of species. Despite the often fundamental role of niche differentiation in the establishment of autopolyploids (Parisod et al. 2010), transplantation experiments revealed no differences between cytotypes in *Ranunculus adoneus* (Baack and Stanton 2005). In the absence of niche differentiation, the distinct distribution of tetraploids was explained by chance dispersal events.

Most transplantation studies found niche differences among cytotypes to be adaptive, implying that the resident lineage outperforms foreign ones, and identified niche shifts as the predominant process of niche differentiation between diploids and their polyploid relatives. For example, superiority of local ploidy over foreign ploidy levels was shown in experiments involving tetra-, penta-, and hexaploid *Allium oleraceum* (Duchoslav et al. 2017), di- and hexaploid *Aster amellus* (Raabová et al. 2008), di- and tetraploid *C. angustifolium* (Martin and Husband 2013), and di-, tetra-, and hexaploid members of the *Claytonia perfoliata* group (McIntyre and Strauss 2017). Evidence for a broader niche and, hence, the general superiority of a single cytotype is less strong. A well-known example was provided by Buggs and Pannell (2007): diploid *Mercurialis annua* showed a consistently higher performance than its polyploid congener—not vice versa as might be hypothesised based on the frequently larger ranges of polyploids (see citation in Weiss-Schneeweiss et al. 2013). In *M. annua*, diploids are currently displacing polyploids mainly driven by asymmetrical pollen swamping (Buggs and Pannell 2006). Ramsey (2011) recorded a fitness advantage of hexaploid *Achillea borealis* over tetraploids in parental tetraploid habitats, but reciprocal fitness was not examined. No "home-range" advantage was also detected by Dona and Galen (2007) for *C. angustifolium*. Individuals of unknown ploidy transplanted within their resident plot had lower survival than those from other plots with no trend concerning distance or elevation.

For the *S. carniolicus* agg., we found a clear home-range advantage for two out of the three study species. *Senecio noricus* and *S. carniolicus* s. str. showed a higher overall fitness and higher vital rates of the transplanted adults than other species at their resident site (Tables 1, 2). In contrast, the overall performance of *S. disjunctus* was lower than that of *S. carniolicus* s. str. at its resident site (Table 2). Mechanisms and processes driving this pattern cannot be inferred directly from this study. However, it is likely that the asymmetric gene flow from the tetraploid *S. disjunctus* to the hexaploid *S. carniolicus* s. str. due to back-crosses of pentaploid hybrids with their hexaploid parents contributes to the competitive weakness of the former (Hülber et al. 2015),

as traits relevant for the adaptation to *S. disjunctus* habitats might be introgressed into *S. carniolicus*. However, at the resident site of *S. disjunctus*, both the vital rates of adult plants as well as the overall fitness considering only vegetative traits were maximally as high as those of *S. noricus*. This competitive disadvantage of *S. disjunctus* compared to *S. noricus* might be explained by a more negative effect of transplantation as *S. disjunctus* often grows in coarse-grained scree. This substrate does not allow digging out *S. disjunctus* plants with compact root balls, causing lower performance even if back-transplanted to the resident site.

Most transplantation studies focused on the early life-history stages (Baack and Stanton 2005; Buggs and Pannell 2007; Ramsey 2011; Martin and Husband 2013; Duchoslav et al. 2017), while much less empirical evidence is available for adults (Flegrová and Krahulec 1999; Raabová et al. 2008) or the whole life history (McIntyre and Strauss 2017). Our investigation of the *S. carniolicus* group clearly demonstrates that it is important to cover a broad range of life-history stages (e.g., from germination to reproduction), because the pattern of performance differences, from which niche differentiation is inferred, might change during the ontogenetic development. Our data suggest superior performance of polyploids during early life-history stages. Specifically, hexaploid *S. carniolicus* s. str. outperformed its lower ploid relatives by having higher germination rates in the first year of observation and stronger subsequent growth at all the sites (Table 2). Seedling establishment (i.e., survival through the observation period; Fig. 1) of *S. carniolicus* s. str., however, was lower than that of *S. noricus* and *S. disjunctus* at their resident sites (Table 2, Fig. 1), which implies a higher mortality rate of *S. carniolicus* s. str. seedlings. *S. carniolicus* s. str. is obviously able to establish in foreign habitats, but will likely die out gradually—a pattern already observed in other heteroploid species (Duchoslav et al. 2017). In contrast to seeds and seedlings, a home-range advantage was detected for transplanted adults of all study species. Resident species showed the strongest growth (Fig. 3, Table 2) and the highest reproduction (Fig. 4, Table 2). Thus, the adaptation of niche-differentiated members of heteroploid species or species groups to their local habitats might be much stronger at later life-history stages, while the performance of seeds and seedlings might be mainly determined by species- or ploidy-intrinsic, non-adaptive characteristics (such as larger seeds, see below). Performance differences indicating the lack of adaptation in single vital rates were also observed in other studies (e.g., higher germination of the foreign cytotypes of *C. angustifolium* in Martin and Husband 2013).

The by far most selective conditions for the survival of early and late life-history stages of foreign species were obviously experienced at the south-exposed, moderately steep resident site of *S. carniolicus* s. str. While adult

survival rates and seedling establishment of *S. carniolicus* s. str. were in the range of the other species at their resident sites, performance of foreign species was marginal (Figs. 1, 5). This was unexpected as we would have assumed the resident site of *S. noricus*, a flat kryoturbated terrace with a continuous cover of small stones situated close to a ridge, where strong winds prevent the growth of closed *Carex curvula* swards, to be the most challenging growing site, followed by the north-exposed resident site of *S. disjunctus*, which is cold, mossy, and instable likely due to occurrence of permafrost-stabilised screes. Anecdotal field observations from the early vegetation period (Sonnleitner and Schönswetter, unpublished field observations) render differences in snow cover, one of the major drivers of small-scale distribution of alpine plants (Körner 2003), unlikely to be responsible for the observed fitness differences. Whereas the resident site of *S. noricus* remains without snow cover for most of the winter, those of *S. carniolicus* s. str. and *S. disjunctus* experience similar snow cover duration; differences in exposition are apparently compensated by the lee situation of the south-exposed site of *S. carniolicus* s. str. Therefore, we suggest that the factor contributing most to the selective growing conditions is top soil desiccation in summer, which was also found to be a major impediment for survival in a transplantation experiment conducted with tetra- and hexaploid *A. borealis* (Ramsey 2011). The weakly continental climate of the investigated area (Gams 1931; Wakonigg 1978), which receives rain shelter from the North (e.g., Niedere Tauern chain) as well as from the South (Julijske Alpe/Alpi Giulie), is likely enforced by southern exposition, relatively open vegetation, and a dark raw humus layer (Turner 1958).

The increased drought tolerance of the early life-history stages of *S. carniolicus* s. str. is probably a ploidy effect, similarly as the species' taller growth (Flatscher et al. 2015) and larger seeds (M. Sonnleitner, pers. obs.). Larger seeds appear to develop into bigger seedlings: the average distance between the tips of the cotyledons (value before the slash) and the average length of the radicula at the time of the unfolding of the cotyledons (value after the slash) are 4.8 mm/3.8 mm ($n = 51$) in *S. noricus*, 5.0 mm/4.7 mm ($n = 28$) in *S. disjunctus*, and 6.0 mm/8.2 mm ($n = 36$) in *S. carniolicus* s. str. The bigger seedlings of the latter may have more resources in terms of water and nutrients to reach constantly humid soil layers; in addition, they might reach these layers earlier due to faster growth (but see Bretagnolle et al. 1995) than seedlings of the two lower ploid species, potentially conferring a crucial selective advantage during the early establishment. Still, it is important to note that at its resident site only half as many seedlings of *S. carniolicus* s. str. survived as did seedlings of *S. disjunctus* and *S. noricus* at their resident sites (Fig. 1). This low probability of establishment might contribute to the lower population densities of *S. carniolicus* s. str. as compared to *S. disjunctus* and

especially *S. noricus* in their respective resident sites (Sonnleitner and Schönswetter, unpublished field observations).

Reproduction exhibited positive and negative temporal trends in resident and foreign sites, respectively (Fig. 4), indicating a strong selective role of the environment. Strong evidence for adaptation in reproductive traits in contrast to vegetative growth has been frequently reported in plants (Bischoff et al. 2006), amongst other in a reciprocal transplantation study comparing vegetative traits of local and foreign cytotypes (Duchoslav et al. 2017). Differential reproductive success may, indeed, explain the lack of occurrence of *S. disjunctus* in the resident site of *S. noricus* and vice versa in spite of comparable survival rates of adult transplants of about 94% (Fig. 5), although survival usually has a larger influence on population growth rates than fertility in long-lived plants (Franco and Silvertown 2004). Even if growing sites of the two species are topographically separated—*S. noricus* usually inhabits flat ridges, whereas *S. disjunctus* is almost entirely restricted to north-exposed slopes—reproductive failure in the habitat of the respective other species may be an important mechanism for preventing gene flow between these reproductively strongly, but incompletely separated (based on controlled crossings; Sonnleitner et al. 2013) species. What prevents the plants from flowering is unclear; potential factors might include wind-induced mechanical stress for *S. disjunctus* at the resident side of *S. noricus* and competition by surrounding vegetation—including tall mosses such as *Hylocomium splendens* and *Rhytidiadelphus triquetrus*—for *S. noricus* at the site of *S. disjunctus*. Dedicated analyses of phytohormones and other metabolites may, in the future, shed light on this hitherto neglected aspect of polyploid research. However, interpretations of the reproductive performance within our study need to be made with caution because of the strong direct effect of transplantation at least in the first year of the experiment (see "Materials and methods" section).

In a strict sense, conclusions drawn from our results should be limited to the study area, because ploidy effects cannot be statistically distinguished from population effects. Based on extensive field studies and genetic analyses, the chosen populations can be regarded as spatially (Sonnleitner et al. 2016), genetically (Winkler et al. 2017), and ecologically (Sonnleitner et al. 2010, 2016) representative for the study species as such. This is also confirmed by the initial results from transplantation experiments of adult plants from three other mountain ranges into the resident site of the same species in the study area: comparisons with the resident individuals did not reveal differences in any of the vital rates (supplementary material). Thus, we are confident that our results can be generalised to other populations within the eastern Alps.

In conclusion, our study illustrates that there may be no clear-cut answer to the question if the resident or the higher

ploid species generally shows superior performance in polyploid complexes. Instead, our results clearly show that performance is life-history stage-dependent, and initially important species- or ploidy-intrinsic characteristics may be later outweighed by extrinsic factors, i.e., habitat selection. For instance, high-polyploid *S. carniolicus* s. str. outperformed lower ploid *S. noricus* and *S. disjunctus* with respect to seed germination and seedling growth, but this was later counteracted by higher mortality, rendering the resident species the best establisher in the long term, a pattern also reflected in adult growth and reproduction.

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