A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis

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Polyploidization, the doubling of genomic content, is an important mechanism of adaptation and speciation in plants (Ohno 1970, Levin 1983, Lumaret 1988, De Bodt *et al.* 2005, Soltis *et al.* 2008, Wood 2009). It is estimated that up to 70% of angiosperm species have polyploidy in their evolutionary history (Masterson 1994). Polyploids originate, often repeatedly, within diploid populations (Ramsey & Schemske 1998, Siena *et al.* 2008, Koopman *et al.*, 2008, Burnier *et al.*, 2009). As a consequence, the establishment of polyploid lineages requires that polyploids avoid minority cytotype exclusion (Levin 1975) and withstand competition with diploid cytotypes, which are initially present in (much) larger densities (Weeks 1993, Felber 1991, Baack 2005). A way to avoid competitive exclusion by diploid ancestors is spatial segregation due to niche differentiation, which has hence been evoked as a primary mechanism facilitating polyploid establishment (Husband & Schemske 2000, Levin 2003). A number of observations apparently corroborate this 'niche shift hypothesis' with polyploids often reported to occupy abiotically harsh environments at or beyond the ecological tolerance of their diploid ancestors (Kearney 2005, Brochmann *et al.*2004, Hijmans *et al.* 2007). For those cases were polyploidization is accompanied by apomixis this phenomenon has been termed "geographical parthenogenesis", first described by Vandel (1928). While these observations are suggestive of niche differentiation, experimental studies on the issue nevertheless delivered equivocal results (Baack & Stanton 2005, Buggs & Pannel 2007, Raabova *et al.* 2008).

The study of niche differentiation has recently made important progress triggered by the large-scale availability of climatic data (e.g. www.worldclim.org) and the development of new statistical tools (Warren *et al.* 2008, Broennimann *et al.* 2012, Blonder *et al.* 2014). These data and methods allow for improved measurement of (climatic) niche overlap or niche differences between taxa based on information about their distribution in geographical space (Petitpierre *et al.* 2012, Guisan *et al.* 2014). Originally developed in the context of invasion biology, the approach can be transferred to other issues and, indeed,

based on such an analysis, the idea of niche shifts as a generic prerequisite of polyploid establishment has recently been challenged (Glennon *et al.* 2014). However, using this methodological approach for assessing niche differentiation of di- and polyploids suffers from potential shortcomings, too. First, plant taxa may not only differ with respect to their climatic requirements, but also with respect to non-climatic niche dimensions like soil properties, nutrient availability or disturbance frequency (Grime 1979). These differences might be negligible at the macro-ecological scales relevant to plant invasions on different continents (e.g. Petitpierre *et al.* 2012), but might become (more) important when ranges of taxa are overlapping or are at least close to each other like it is the case with many pairs of di and polyploids. Further, the climatic data commonly used for these types of analysis are spatially interpolated from point measures, their resolution is rather coarse, and their accuracy partly questionable (Randin *et al.* 2009, Bedia *et al.* 2013, Franklin 2013). The climatic conditions indicated by these variables might hence not necessarily be representative for the microenvironment plants experience, particularly in landscapes with a pronounced relief like in mountainous terrain (Scherrer & Körner 2010, Faye *et al.* 2014). Moreover, measuring climatic conditions at too coarse a resolution may confound differences in climatic niche space of species with macroclimatic differences of their respective ranges.

One possible way to overcome problems with the adequate measurement of climatic and non-climatic niche dimensions is the use of environmental indicators derived from the vegetation accompanying the cytotypes to compare (Felber-Girard *et al.* 1996, Johnson *et al.* 2003). Its feasibility for comparing the niches of taxa across their full ranges has considerably increased with the growing availability of large vegetation plot databases during the recent years (e.g. Lenoir *et al.* 2012). From these databases presence and absence information for a species of interest can be derived at a fine spatial grain but over a large spatial extent which, in combination with vegetation-derived indicators, allows for applying the newly developed statistical tools to compare niches measured at a much finer scales and characterized by other than purely climatic dimensions. However, one potential obstacle to the application of this approach for comparing the niches of polyploids and their diploid ancestors is that the vegetation plots collected in these databases were commonly sampled for other purposes and usually do not contain sufficient information to distinguish (cryptic) cytotypes within one species.

In this study, we focus on one particular model system to develop an in-depth study of di-/polyploid niche differentiation which is based on recent methodological progress but tries to overcome the aforementioned problems by (1) consistent sampling and cytotype determination across (nearly) the whole spatial range of the species complex and (2) combining and comparing an analysis based on GIS-derived large-scale climatic data with an analysis based on microclimatic and non-climatic indicators derived from a large set of vegetation plots . In addition, we do not only focus on possible differentiation of cytotypes in terms of niche optima, but also in terms of niche breadth, niche expansion, and niche unfilling (Guisan *et al.* 2014). Moreover, we try to elucidate the role of competition for the

differentiation of realized niches by separately comparing sympatric and allopatric di- and tetraploid cytotypes. Our model system is *Ranunculus kuepferi* (Greuter & Burdet), a high-mountain buttercup native mainly to the European Alps. The species has survived the Last Glacial Maximum in refugia at the southwestern margins of the Alps and polyploids have emerged from diploid progenitors in this area (Burnier *et al.*, 2009). Subsequently, the tetraploid cytotypes have colonized a large part of the Alps while the diploids have largely remained restricted to their glacial refugia (Cosendai & Hörandl, 2010). To analyse niche relationships in this model system, we use the approach of Broennimann *et al.* (2012) and ask whether (1) diploids and tetraploids differ in the positon of their niche optima and/or in their niche breadths across their respective whole ranges; (2) whether these differences vary when comparing sympatric and allopatric cytotypes; (3) whether results based on GIS-derived macro-environmental data are consistent with results based on indicator-derived micro-environmental data.

1. Methods

1.1. Model organism

The white-flowering, perennial herb *R. kuepferi* has lineal-lanceolate leaves and small achenes, which are predominantly dispersed by wind (Müller-Schneider, 1986). The species usually occurs in various types of alpine grassland vegetation and pastures at elevations between 1300 – 2800 m. *R. kuepferi* has been the subject of several phylogenetic studies, which have revealed multiple origins of tetraploids at the southwestern fringes of the European Alps. Subsequently, these tetraploids have colonized a wide range of the Alps (Burnier *et al.*, 2009), while diploids retained a restricted range in their former glacial refugia in the south-west, probably because of higher fertility (Cosendai & Hörandl, 2010). Tetraploids and diploids moreover differ in their reproductive systems: while diploid populations are outcrossers, tetraploids mainly reproduce by apomixis, i.e. by asexual production of seeds, although they are, in principle, also capable of sexual seed production (facultative apomixis, Asker & Jerling 1992; Cosendai & Hörandl 2010). Some populations in the geographical contact zone are mixed and in a few populations also triploid, pentaploid and hexaploid individuals occur (Cosendai and Hoörandl, 2010). Triploids are probably the result of backcrossing events of diploids and tetraploids and have a mixed mode of reproduction (Cosendai and Hörandl, 2010). Some isolated small populations of the species occur in the Apennines and on the island of Corsica.

1.2. Study area

The study area comprises the Alpine part of *R. kuepferi's* geographical range (Fig. 1). Within this area we searched for records of populations in the literature (Cosendai & Hörandl 2010) as well as in herbaria (private herbarium of Philippe Küpfer, herbaria NEU (University of Neuchatel; Switzerland), GOET (University of Göttingen; Germany), W (Natural history Museum Vienna; Austria), GZU (Karl Franzes University of Graz; Austria), WU (University of Vienna, [former] Institute for Botany; Austria), Z (University of Zürich; Switzerland), KL (Herbarium of Carinthia; Austria), in the Global Biodiversity Information Facility (GBIF, www.gbif.org, last access 2014-05-13), and in the free internet, e.g. in "flickr" (www.flickr.com, last access 2014-05-13). Overall, we selected 102 sampling sites from these sources which comprise all known diploid populations and a large set of tetraploids covering the whole _Alpine range of the species (Fig. 1).

1.3. Sampling design

During the growing seasons of 2013 and 2014 we sampled all these populations. In essence, we searched for the species at the locations given either by verbal description or by geographical coordinates in the above mentioned sources. Once we had detected the population we randomly selected a plot of 100 x 100 m. Besides a number of parameters not relevant to this study we randomly selected four groups of three *R. kuepferi* individuals within this 100 x 100 and positioned a frame of 2 x 2 m around these groups. Subsequently, we recorded all vascular plants growing within this frame and collected the 12 individuals by digging them out. The individuals were then transported to the common garden of the University of Göttingen and cultivated for use in further experiments. Level of ploidy (and reproductive strategy) were determined by Christoph Schinkel and Elvira Hörandl.

1.4. Environmental data

Environmental conditions at the 102 sampling sites were characterized by two different sets of data. The first one, which we here call macro-environmental data, was derived from WorldClim (www.worldclim.org). WorldClim provides monthly averages of mean, minimum and maximum temperature as well as monthly precipitation sums for the period of 1950 – 2000, together with 19 bioclimatic variables which are derived from the monthly values at a spatial resolution of 30 arc-seconds (approximately 1km at the equator). To better represent water availability during the vegetation period we moreover calculated an ombrothermic index from these climatic data as Io = 10(YPP/YPT), where YPP is the summed precipitation of the months with an average temperature > 0°C and YPT is the sum of the average temperature of those months (Rivas-Martinez 1996 cited in Attorre *et al.* 2007). To make climate data resolution match the size of our sampling plots we statistically downscaled them to a resolution of 100 x 100 m (for detailed d*ownscaling* description see Appendix). From the array of climatic data we then selected four which (1) were not too closely correlated (Pearson $r < 0.75$) among each other to avoid collinearity issues (Dormann *et al.* 2012) and (2) represent the most important climatic drivers of plant growth: temperature

(maximum temperature of warmest month and annual temperature range) and water availability (precipitation of driest month and ombrothermic index). On top of these climatic variables we added slope inclination and information on substrate conditions. The former was calculated from a Digital Elevation Model of 100 m cell size and the latter using information from the European Soil Database (ESDB, http://eusoils.jrc.ec.europa.eu). With the data from the ESDB we calculated the percentage area of Soil Typological Units (STU) having a calcareous dominant parent material for every Soil Mapping Unit (SMU). For the Austrian part of the Alps, a fine-scaled map of substrate units was available (Bayer, K. & Pavlik W. 2009) which we used to compute the area of calcareous substrates within grid cells of 5' longitude × 3' latitude in order to get information compatible to that derived from the European Soil Database.

The second dataset, we call it ‚microenvironmental' data henceforth, was derived from the species lists of the four 2 x 2 m plots and their Landolt indicator values (Landolt 1977, 2010): Landolt indicator values represent an ordinal classification of plants according to the position of their realized ecological niche along different environmental gradients. They are similar to Ellenberg indicator values (Ellenberg 1992) but are specifically tuned to represent the niches of species in the European Alps. Although such indicator values are based on expert judgement and refer to the species' niche optimum only, they have been proven reliable and useful indicators of local-scale environmental conditions in many studies (Diekmann 2003). Based on the pooled species lists of the four 2 x 2 m plots we calculated, for each of the 102 populations, a simple, unweighted mean of the climatic indicator values for temperature (T), the average and the variability of soil moisture during the growing season (F and W, respectively), as well as for soil pH (R) and soil nitrogen content (N).

1.5. Statistical analysis

For comparing the niches of di- and tetraploid populations we used an approach introduced by Broennimann *et al.* (2012, Broennimann *et al.* 2014). The method uses kernel smoothers to calculate, first, the densities of species occurrences along environmental gradients and, second, the densities of environmental conditions in the respective study area(s). Species occurrence densities are then scaled by densities of environmental conditions to derive a description of the (realized) species niche which is independent of sampling effort (number of occurrences) and accounts for environmental variability in the study area (Guisan *et al.* 2014). As a corollary, however, the method not only needs information about the sampling sites of species but also on the availability of environmental conditions across the whole area. In addition to our sampling plots we hence used a set of vegetation relevés from the European Alps to characterize the density of environmental conditions across the Alpine range of *R. kuepferi*. The relevés were taken from the Alps vegetation database (Lenoir *et al.* 2012) and contain 8239 samples with a plot size < 125 m² that were done at elevations between 1000 and 3400 m.a.s.l. and that have a maximum of 5 % of tree or shrub cover. All

these samples are georeferenced and offer a complete list of vascular plants species. Hence both full macro-environmental and full micro-environmental densities could be calculated for them in the same way as for our own *R. kuepferi* sampling plots.

The method of Broennimann *et al.* (2012) translates a multivariable environmental space into a two-dimensional one by means of a principal component analysis (PCA). PCA space is then gridded and smoothed densities of species occurrences and background conditions per grid cell are calculated by a kernel density function. Finally, the species' density function is divided by the 'background' area's density function to standardize the estimated niche and make it comparable among different species which might have been sampled with different effort and in different areas. In our case, the background area was the same for the di- and tetraploid cytotypes to compare, but sampling effort (= number of sites) was higher for the latter.

Our subsequent comparison of the thus calculated two-dimensional niches of di and tetraploid cytotypes combined several approaches. First, we used the niche equivalency and similarity tests proposed by Warren *et al.* (2008) and implemented in the ecospat-package in R (Broennimann *et al.* 2014). Both tests rely on the niche overlap metric *D* (Schoener 1970) which ranges from 0 (no overlap) to 1 (complete overlap). For the niche equivalency test occurrences of both species to compare are pooled and randomly re-split into two datasets, with the respective numbers of occurrences of the two species kept constant. Splits are repeated 100 times and the position of the observed *D* within the 100 reshuffled *D*s is determined: niches are considered non-equivalent (null hypothesis rejected) if the observed *D* falls within the lower .05 quantile of the reshuffled *D*s. This is a strict test of niche conservativism (Glennon *et al.* 2014). By contrast, the niche similarity test examines whether the environmental niche of one species is more dissimilar or more similar to the niche of the other one than would be expected by chance. For this purpose, a number of points equal to the occurrences of one species is selected at random from the background area, the niche of this random sample is calculated and then compared to the observed niche of the other species by means of *D*. The distribution of 100 such random *D*s is then compared to the observed *D* for the two species: if the observed *D* is within the upper or lower .025 quantile of the resampled *D*s species x is said to be more similar or more dissimilar, respectively, to species y than expected by chance. The test is repeated in both directions, i.e. by resampling first the occurrences of x and then those of y.

While *D* offers a metric of niche conservativism vs. divergence it contains little information about how the niches of the two species differ. In particular, it does not differentiate between changes in niche optima and breadths (Glennon *et al.* 2014) and it does not indicate the main environmental gradients responsible for potential differentiation. For comparing niches in terms of optima and breadths we bootstrapped niche calculations by re-sampling occurrence points of both species 100 times. For each re-sample, we selected 100 cells of the gridded PCA space at random, but weighted by the species' occurrence density, and calculated the niche optimum as the median and the niche breadth as the distances

between the .025 and .975 quantiles along the two PCA axes, respectively. We then subtracted, for each pair of re-samples, the thus calculated medians and distances for species x from those of species y. If the central 95% of these differences did not include 0 we considered the niche optima and niche breadths to be different, respectively. Moreover, we calculated niche unfilling and expansion, i.e. the proportions of the niche of species x which is not occupied by species y and vice versa (cf. Petitpierre *et al.* 2012, Guisan *et al.* 2014). Finally, we plotted the direct overlay between the two niches in PCA-space for visual inspection of the environmental drivers mainly responsible for detected niche changes.

3.6 Set of comparisons

Apart from using the total set of sampled populations to compare the niches of di- and tetraploid cytotyes of *R. kuepferi* as a whole we further conducted a set of comparisons among differently defined (sub)sets of our 102 populations in order to explore an eventual role of competition in shaping the realized niches of tetraploids. In particular, we (1) restricted our comparison to the populations in the south-western part of the Alps, i.e. to the sympatric range of the two cytotypes; (2) by contrast, compared diploid to tetraploid populations sampled outside the south-western Alps only, i.e. compared niches of allopatric populations. We expected that, if competition with diploids is important for the niches of tetraploids, niche differentiation should be particularly pronounced in the sympatric range, while overlap may increase where diploids are absent, i.e. among allopatric populations. As a consequence, the niches of allopatric di- and tetraploids may also differ and we hence finally (3) compared the tetraploid populations in the south-western with the tetraploid populations in the rest of the Alps. Mixed populations, i.e. those that contained both di- and tetraploid individuals were excluded from all these comparisons.

Moreover, we conducted all four comparisons based on both the macro- and the micro-environmental variable sets. In this case we expected that the micro-environmental data might deliver a more precise definition of the species' niche and in particular, that niches of the widespread tetraploids might appear broader when using coarse-scale descriptors of climatic trends as compared to site-scale indicator values. As a corollary, differences in niche breadth suggested by macro-environmental variables may actually vanish when using micro-environmental predictors.

2. Results

Of the 102 populations sampled 23 were purely diploid and 60 were purely tetraploid. The remaining 19 populations, all from the contact zone in the south-western Alps, either contained di-, tri- and tetraploids (8 populations), or di- and triploids (2), or tri-, tetra- and pentaploids (9).

Based on the complete set of the 83 purely di- and tetraploid populations the macro- and micro-environmental analyses revealed largely consistent results: Schoener's *D* values indicate that the niches of the two cytotypes are not equivalent and are not more similar

than expected by chance (Table 1). Niche divergence is mainly based on a shift in the niche optimum, which is statistically significant on the first or on both PCA axes for the macro- and micro-environmental variables, respectively. By contrast, niche breadths do not differ significantly among the two cytotypes. Only with respect to niche unfilling/expansion do the two variable sets deliver contradictory results: while the macro-scale variables suggest that tetraploids have expanded their niches considerably with only a small part of the diploid niche becoming unfilled, the micro-scale data suggest that unfilling was more pronounced than expansion (Fig. 2).

When restricting the comparison to allopatric populations, the results remain largely stable: both datasets suggest shifts in the niche optima without changes in niche breadth, but the macro-environmental variables indicate predominant expansion and the micro-environmental variables predominant unfilling of the diploid niches by tretraploids (Table 1, Fig. 2).

By contrast, when comparing cytotypes in the sympatric area, differences among macro- and micro-environmental analyses become much more pronounced. With macro-scale data, the results are similar to those achieved with the complete set or for allopatric populations: there is a significant shift of the niche optimum, but no change in niche breadth. Niche unfilling, however, appears now more important than expansion. With the micro-environmental data, *D*-values are considerably lower and niches differ in both position of the optimum and breadth. In particular, the niches of tetraploids are much narrower with this restriction being mainly driven by a large part of the diploid niche space being not occupied by tetraploids, i.e. considerable niche unfilling (Table 1, Fig. 2).

Finally, comparison of allopatric tetraploids deliver complementary results with both variable sets: tetraploids outside the south-western Alps have shifted their niche optima as compared to tetraploid populations that co-occur with diploids; and they have significantly broader niches mainly due to a considerable niche expansion (Table 1, Fig. 2). These trends are consistent among both variable sets, but appear more pronounced with micro-environmental data.

With respect to the main environmental axes of niche divergence, macro-environmental variables indicate that tetraploids have shifted their niche optima towards soils less rich in carbon and, more importantly, towards both cooler and moister habitats. Micro-environmental results are similar with respect to a trend towards are lower pH, but suggest that temperature is the more influential climatic variable (Fig. 2, c,g). In the sympatric range, macro-environmental predictors indicate an approximately equal contribution of all variables to the shifting niche optimum. By contrast, with micro-environmental data optima in the sympatric range seem to differ mainly along gradients of nitrogen availability and annual soil moisture variability, with tetraploids restricted to sites poorer in nutrients and less stable in water supply. With respect to climatic variables, the optima hardly shifted, i.e. tetraploids appear excluded from both the coldest and warmest and the driest and moistest sites of diploids. Complementarily, when comparing allopatric ranges of tetraploids, the results suggest that tetraploids have expanded their niches with respect to more or less all

environmental gradients and in particular, to cooler and moister conditions as well as towards both more acid and more nutrient rich soils.

3. Discussion

Our results demonstrate that the environmental niche of tetraploid *R. kuepferi* differs from the niche of its diploid ancestor. When focussing on the entire geographical ranges of both cytotypes, this difference appears mainly driven by a shift of the niche optimum without a significant change in niche breadth. Analysing the subset of sympatric and allopatric populations separately indicates, however, that an initial narrowing of the tetraploids' realized niche was followed by a re-expansion associated with the eastward spread of the tetraploids. These findings suggest that within the south-western Alps tetraploids were, and still are restricted to a part of their potential niche by competitively superior diploids. Only after emigrating out of the sympatric area tetraploids were able to broaden their realized niches, partly by re-filling of the diploid niche and partly by niche expansion into cooler, moister and more acid conditions.

Taken together, these findings indicate that avoidance of competition by niche differentiation has been important for the establishment of polyploid lineages in *R. kuepferi*. In general, the ecological differences detected among the two cytotypes are largely congruent with the pattern the so-called "frozen niche variation" model (FNV, Vrijenhoek (1984, 1994, 2009) would predict. The FNV model is a hypothesis proposed to explain the phenomenon of geographical parthenogenesis and suggests that different apomictic lineages produced by e.g. autopolyploidy, represent certain "frozen" portions of the genotypic variation and thus parts of the diploids' niche. Those lineages that overlap with the niche optimum of the sexual population may become eliminated by competition from the diploids. Lineages occupying marginal niche positions can, however, establish because diploid fitness is reduced while the lack of gene flow associated with apomixis prevents the invasion of maladapted genes and hence stabilizes the "marginal" adaptation. As a corollary, the niche optimum of apomictics will shift towards marginal, more extreme habitats as compared to their diploid ancestors – as is the case for *R. kuepferi*. The change in reproductive system may have additionally directly facilitated the establishment of tetraploid *R. kuepferi* at and beyond the diploids' cooler range margin in particular: apomixis implies a shortening of the reproductive pathway and faster seed development as well as reproductive assurance if pollinators and mating partners are rare (Horandl 2006) – all of these factors appear highly beneficial in cool high-mountain environments with short vegetation periods and often severe limitation of pollinator activity due to adverse weather conditions (Kevan & Baker 1983, Arroyo *et al.* 1985, McCall & Primack 1992, Totland 1994, Bergman *et al.* 1996).

As recently highlighted (Glennon *et al.* 2014) niche differentiation has at least two dimensions, however, namely position of optima and breadth. Our separate analysis of sym- and allopatric populations strongly suggests that in *R. kuepferi*, initial differentiation was

associated with a severe reduction of niche breadth even more than with a shift in niche optimum. Importantly, a standard analysis based on the full ranges of both cytotypes would have probably reached the opposite conclusion (as niche optima differ on the first PCA-axis in these case, and niche breadths are identical). Moreover, the gradients along which cytotypes diverge differ among sym- and allopatric populations. These contradictory results highlight that the processes enabling sympatric polyploid establishment possible might not been adequately reflected in current geographical distribution patterns, or, put it another way, may become masked during subsequent spread and local adaptation of one (or both) cytotypes. In particular, our results indicate that the expansion of polyploid niches towards cooler conditions, which has repeatedly been observed in the context of genome duplication (e.g. Bierzychudek 1985, Hörandl 2006) does not necessarily represent a strategy to avoid competition as in *R. kuepferi* this expansion was apparently realized only after the cytotypes have become spatially segregated, i.e. only outside the sympatric area.

Independent of the subpopulations analysed, tetraploids have generally shifted their optima towards harsher and potentially more stressful environmental conditions – be it cooler, more acid or poorer in nutrients. The driver behind these "directional" shifts might have actually been an inferior competitive ability of tetraploids as compared to their diploid ancestors. The stress-gradient hypothesis (Bertness & Callaway 1994) states that competitive intensity will decrease under increasing environmental stress, and vice versa, a prediction that has largely been corroborated in alpine environments (e.g. Choler *et al.* 2001, Callaway *et al.* 2002, Dullinger *et al.* 2007). Actually, diploids apparently are superior to tetraploids both in terms of reproductive rates and growth (Schinkel *et al.*, in prep.) Even in the allopatric range, i.e. independent of direct competition with diploids, tetraploids might have hence been unable to expand at and beyond the benign range margins of diploids because they were unable to cope with the higher competitive intensity that generally prevails in communities assembled under warmer, less acid and less nutrient limited conditions.

If tetraploids are actually competitively inferior to their diploid ancestors why then have they been so tremendously more successful in re-colonizing the Alps after the last glacial maximum? Tolerance of cooler environments might have actually played a certain role as the diploids' range at the south-western margins has a slightly warmer climate than the more north-eastern parts of the Alps. However, we hypothesize that change in the reproductive system has been the most powerful driver of tetraploid spread. Ability to reproduce without mates or pollinators can be a key advantage in colonizing new areas ("Baker's Law", Baker 1967, Stebbins 1957) because a new population could establish from a single propagule. In addition, apomictics, which do not undergo recombination in the reproduction process, may maintain high levels of heterozygosity and hence reduce detrimental founder effects (Hörandl 2006). Accordingly, the large, though disjunct current range of tetraploids has been hypothesized to be driven by rare long-distance dispersal events of a few diasporas, e.g. by birds (Emadzade *et al.* 2011, Cosendai *et al.* 2013).

Finally, our analysis revealed partly inconsistent results at macro- and micro-environmental scales, in particular with respect to changes in niche breadth and to the net balance of expansion and unfilling. In all these cases, analyses with micro-scale data indicated a trend towards narrower niche space in tetraploids while on the macro-scale niche breadth did not differ or data even indicated a net niche expansion. These differences strongly suggest that while the broad-scale environmental variation of tetraploid populations is at least as or even more pronounced than that of diploid populations, the sites where individuals actually grow rather tend to shrink in environmental variability, a phenomenon largely coherent with a general trend of populations to become smaller from the south-western to the eastern Alps. As a corollary, basing analyses of niche differences on too large-scaled climatic data might lead to erroneous conclusions, particularly in the rugged relief of high mountain areas (Scherrer & Körner 2010, Faye *et al.* 2014). We hence strongly recommend to base any inferences from niche comparison analyses for mountain plants on appropriate, fine-scaled environmental data.

Acknowledgments

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Table 1 Results from the niche equivalency and the two niche similarity tests and from the comparison of changes of niche optima and niche breadth between di- and tetraploid populations of *R. kuepferi* (full, sympatric and allopatric range) and tetraploid populations within the sympatric and outside the sympatric area (allopatric apomicts range). The difference between niche expansion and unfilling of tetraploids (full, sympatric and allopatric range) and tetraploids outside the sympatric area is the subtraction of unfilling from expansion. Significant *p*-values are shown in bold. Niche broadening is symbolized by >, niche contraction by <.

	full range		**sympatric area**		**allopatric area**		**allopatric apomicts area**	
	macro scale	micro scale	macro scale	micro scale	macro scale	micro scale	macro scale	micro scale
Schoener's D	0,269	0,245	0,253	0,072	0,331	0,273	0,193	0,074
Equivalency	**0,020**	**0,020**	0,096	**0,020**	0,021	**0,020**	0,088	**0,025**
Similarity 1 → 2	0,436	0,264	0,256	0,296	0,368	0,245	0,333	0,299
Similarity 2 → 1	0,465	0,320	0,169	0,286	0,268	0,195	0,092	0,222
Niche optimum PC1	**0,000**	**0,000**	**0,000**	0,301	**0,000**	**0,010**	**0,000**	0,090
Niche optimum PC2	0,270	**0,000**	0,075	**0,011**	0,230	**0,000**	0,068	**0,000**
Niche breadth PC1	0.090 >	0.130 <	0.168 <	**0.000 <**	0.120 >	0.060 <	0.068 >	0.333 >
Niche breadth PC2	0.120 >	0.090 <	0.150 <	**0.043 <**	0.200 >	0.100 <	**0.000 >**	**0.000 >**
Expansion vs unfilling (Diff.)	0,231	-0,262	-0,066	-0,603	0,197	-0,333	0,575	0,284

Figure 1 Sampling sites (n = 102) in 2013 and 2014.

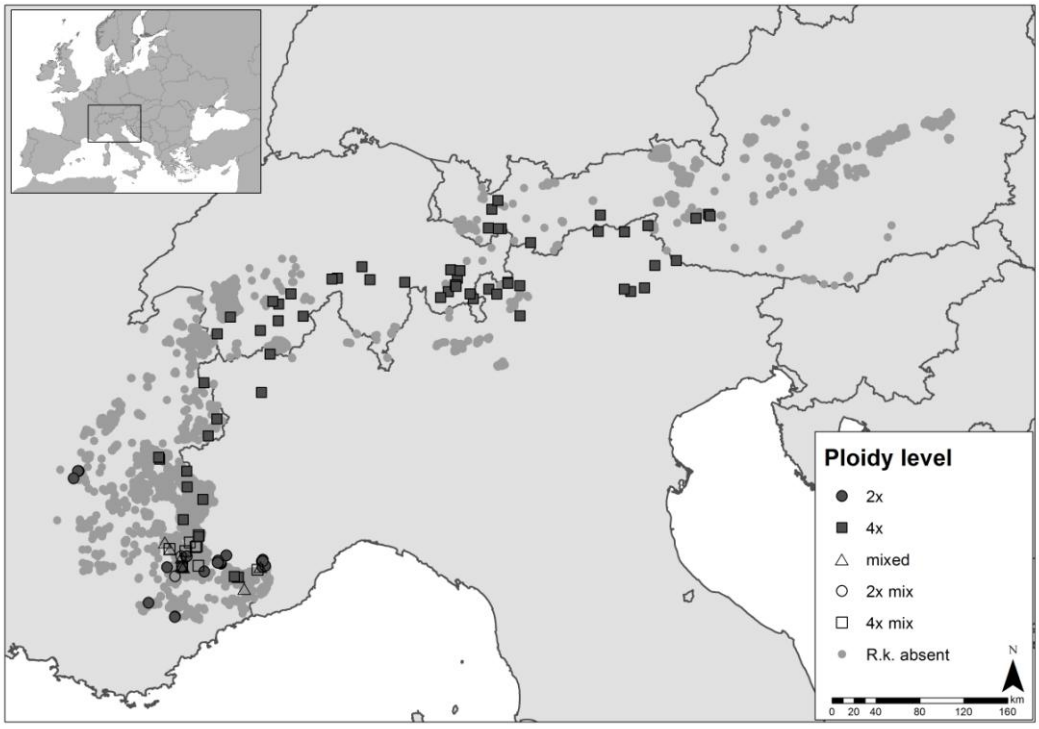
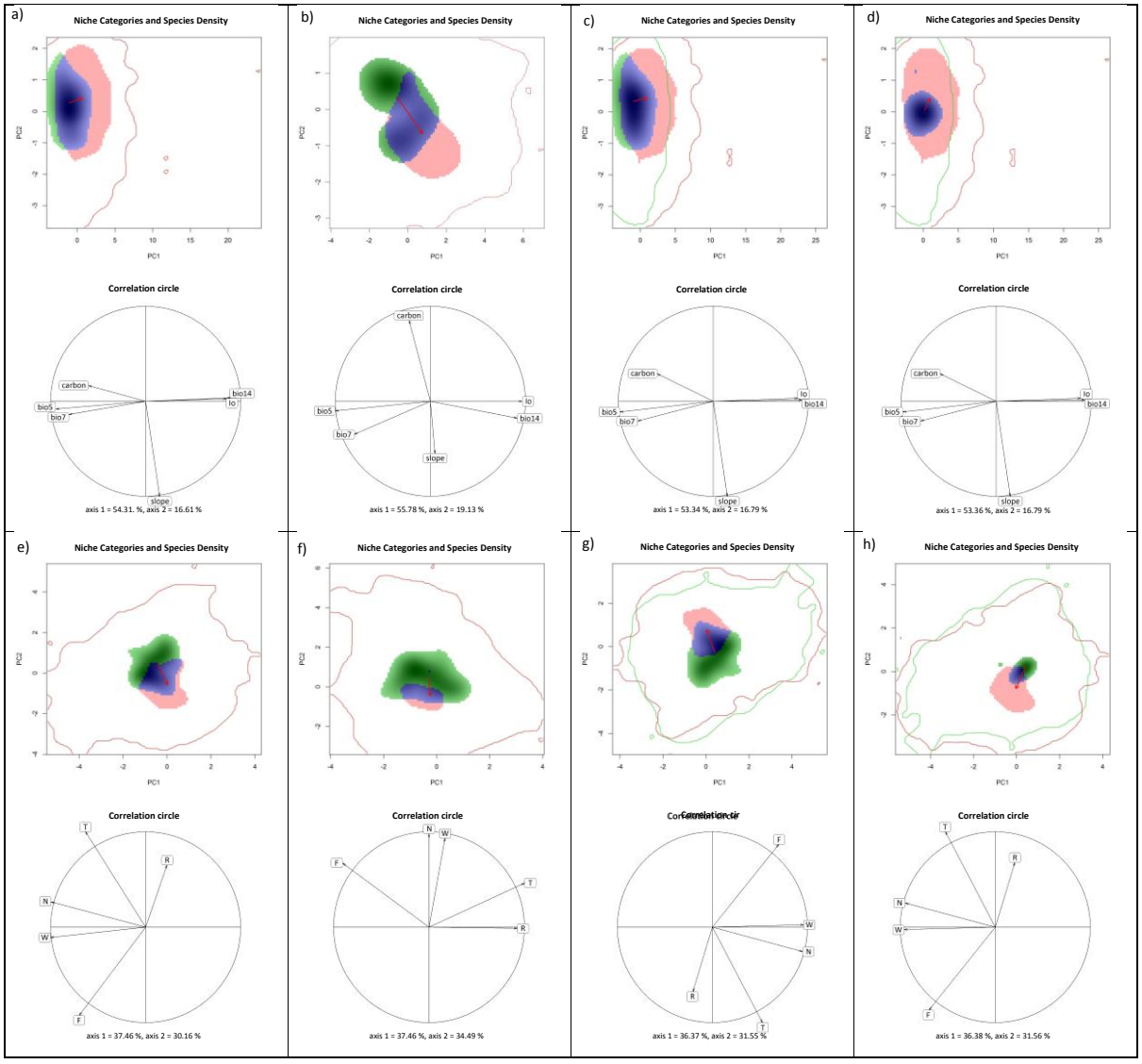


Figure 2 Niche change observed comparing di- and tetraploid *R. kuepferi* populations at macro-environmental (a-c) and micro-environmental (e-g) scales in their full (a, e), sympatric (b, f) and allopatric (c, g) range and comparing tetraploid *R. kuepferi* populations within the sympatric and outside the sympatric area (d, h) at macro-environmental (d) and micro-environmental (h) scale. Area of niche unfilling, stability and expansion of tetraploids (a-c, e-g) and tetraploids outside the

sympatric area (d, h) are shown in green, blue and red respectively. The red arrow links the centroid of the di- and tetraploids niche (a-c, e-g) and tetraploids niche in the sympatric and outside the sympatric area (d, h) respectively.

The correlation circle shows the loadings of individual environmental variables to the two PCA axes. bio5: maximum temperature of warmest month; bio7: annual temperature range; bio14: precipitation of driest month; Io: ombrothermic index; carbon: percentage of calcareous soils; slope: slope inclination; T: temperature, F: average soil moisture during the growing season; W: variability of soil moisture during the growing season; R: soil pH; N: soil nitrogen content. T,F,W,R,H are mean Landolt indicator values for the communities occupying the sampling plots.



Appendix

Climatic data:

The downscaling procedure can be summarized as follows: at the 1 km spatial resolution, we analysed the dependency of precipitation and temperature on elevation by means of linear regressions in circular moving windows of 15 and 25 km radius, respectively. We chose smaller moving windows for precipitation, because of the better fit in cross-validation exercises. By doing so, we extracted the hidden lapse rates and '0 m above sea level' temperature and precipitation intercepts inherent in the Worldclim maps. We stored lapse rates and intercepts to the centre cell of each window position and then spatially interpolated these regression parameters to a 100 m resolution by means of inverse distance-weighted interpolation. Finally, the interpolated regression parameters were applied for back conversion to climate maps using a 100 m digital elevation model, which was aggregated from the 90 m SRTM DEM[1] version 4.0 by means of the AGGREGATE command in ArcGrid. In summary, this procedure allowed us to first extract the hidden regression parameters of the Worldclim maps, and then to spatially scale them to the resolution of 100 m. The same approach to statistical downscaling of climatic parameters has already been used in several earlier studies[2,3,4,5,6].

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