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# Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae)

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Geographical parthenogenesis describes the enigmatic phenomenon that asexual organisms have larger distribution areas than their sexual relatives, especially in previously glaciated areas. Classical models suggest temporary advantages to asexuality in colonization scenarios because of uniparental reproduction and clonality. We analyzed population genetic structure and self-fertility of the plant species *Ranunculus kuepferi* on 59 populations from the whole distribution area (European Alps, Apennines and Corsica). Amplified fragment length polymorphisms (AFLPs) and five microsatellite loci revealed individual genotypes for all populations and mostly insignificant differences between diploid sexuals and tetraploid apomicts in all measures of genetic diversity. Low frequencies of private AFLP fragments/simple sequence repeat alleles, and character incompatibility analyses suggest that facultative recombination explains best the unexpectedly high genotypic diversity of apomicts. STRUCTURE analyses using AFLPs revealed a higher number of partitions and a stronger geographical subdivision for diploids than for tetraploids, which contradicts expectations of standard gene flow models, but indicates a reduction of genetic structure in asexuals. Apomictic populations exhibited high admixture near the sexual area, but appeared rather uniform in remote areas. Bagging experiments and analyses of pollen tube growth confirmed self-fertility for pollen-dependent apomicts, but self-sterility for diploid sexuals. Facultative apomixis combines advantages of both modes of reproduction: uniparental reproduction allows for rapid colonization of remote areas, whereas facultative sexuality and polyploidy maintains genetic diversity within apomictic populations. The density dependence of outcrossing limits range expansions of sexual populations.
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## INTRODUCTION

Geographical parthenogenesis (GP) is a term coined initially by Vandel (1928), describing the phenomenon that related sexual and asexual organisms have different distribution areas. Later, numerous studies on animals and plants confirmed that asexual organisms have larger geographical distribution areas, occur at higher elevations and at higher latitudes, and colonize more frequently previously glaciated or otherwise devastated areas than their sexual relatives (Bell, 1982; Bierzychudek, 1985; Van Dijk, 2003; Kearney, 2005; Hörandl, 2006, 2009). GP thus represents an exception from the predominance of sex in nature, which is by itself a paradox because of the high costs of sex (costs of male functions and of recombination; Bell, 1982; West *et al.*, 1999). The causality of GP is under dispute, and several, not necessarily exclusive hypotheses have been proposed (Bierzychudek, 1985; Peck *et al.*, 1998; Kearney, 2005; Hörandl, 2006, 2009; Song *et al.*, 2011).

Baker's law describes that uniparental reproduction is advantageous for colonization, as there is no need of a mating partner for founding a new population. The benefit of reproduction via single individuals is most efficient after long-distance dispersal (Baker, 1965, 1967). The negative consequence of such a colonization event would be a genetic bottleneck, because the founder individual(s) would carry only a subset of the genetic diversity of the source population (Hewitt, 2004). Thus, apomictic founder populations should be uniform with respect to genotypic diversity. Selection would favor the persistence of clones with a broad environmental tolerance, so-called 'general-purpose genotypes' and would eliminate other clonal lineages (Lynch, 1984; Vrijenhoek and Parker, 2009). According to this model, widespread clones would cover the distribution area.

The assumptions of Baker's law do not readily apply to pollen-dependent apomictic flowering plants. Apomixis, the asexual reproduction via seed, potentially allows for uniparental reproduction, because the unreduced egg cell develops parthenogenetically into an embryo (Supplementary information). Nevertheless, the great majority of apomictic species still needs pollination for fertilization of polar nuclei (pseudogamy; c. 90% of species; Mogie, 1992) for a normal development of the endospermic nutritive tissue within the seed. This mode of uniparental reproduction requires stigmatic and/or stylar self-compatibility (Supplementary information), as the pollen tube must penetrate the stigma and the style to fertilize the polar nuclei (Hörandl, 2008, 2010). In the case of self-incompatibility, seed set may fail because the asexual embryo lacks the nourishment of a

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functional endosperm (Supplementary information). This functional constraint was so far overlooked, as previous studies on Baker's law focused either on sexually selfing plants or on pollen-independent apomicts (Baker, 1965; Hao *et al.*, 2011). Under these considerations, pollen-dependent apomixis is expected to be advantageous only if (1) the sexual species is self-incompatible (SI) and thus depending on mating partners; (2) if the pollen-dependent apomict is self-compatible and thus can use self-pollen for the formation of seeds (Hörandl, 2010; Supplementary information).

Ecological models for GP rely on different adaptive potentials and resource competition between sexual populations and asexual clones. A mathematical model by Peck *et al.* (1998) suggests that asexuals could more easily adapt to environmental conditions in boundary areas, whereas immigration and introgression of maladapted sexual individuals would inhibit adaptation of sexual populations to local conditions. This model is independent from temporary climate shifts, but assumes equal founder abilities of sexuals and apomicts in remote areas. Thus, the model becomes less plausible at an early stage of colonization when long-distance dispersal is involved. Later on, sexuals may expand their range via stepwise short-distance dispersal; however, the faster-moving asexuals might have already built up an impermeable front (Mogie, 1992; Hewitt, 2004).

The Frozen Niche Variation Model, in contrast, predicts an origin of multiple clones from genetically diverse sexual progenitors; interclonal selection eliminates those clones that are overlapping with the niche optimum of sexual populations, and fixes arrays of clones in particular niches outside the optimum of the sexual species. Therefore, arrays of specialized clones partition more efficiently the total resource space than sexual populations (Vrijenhoek, 1979; Vrijenhoek and Parker, 2009).

Clonality, however, as a basic premise for all traditional models, is not necessarily the condition in asexual populations. Numerous population genetic studies have demonstrated that asexual populations harbor a considerable diversity of different genotypes (Loxdale and Lushai, 2003). Accumulation of mutations within clonal lineages, multiple origins from hybrid genotypes, or residual sexuality can create a considerable genotypic diversity within asexual lineages. In flowering plants, apomixis is usually facultative; in the case of apospory, apomictic and sexual developmental pathways start in parallel within the same ovule, but proceed during gametophyte development in the one or the other direction (Supplementary information). Both sexual and apomictic offspring may arise from differential development of ovules in the offspring of the same parental plant even within one generation. Thus, even rare sexual recombination events may have strong effects on genotype diversity within populations (Hörandl and Paun, 2007).

Polyploidy is frequently connected to asexuality and has been assumed to be a major causal factor for GP (Vandel, 1928; Bierzychudek, 1985). Polyploidy could provide to the species the advantage of genomic novelty, a higher genetic diversity and a broader ecological flexibility (Kearney, 2005). In flowering plants, this explanation for GP is not straightforward, as sexual polyploidy is common, but is in general not correlated to large distribution areas (Hörandl, 2006). Nevertheless, polyploids potentially can harbor a greater allelic diversity than diploid populations by combining gene pools of the diploid parents, which increases genetic diversity in polyploid asexual lineages (Cosendai *et al.*, 2011). Moreover, polyploidization is in flowering plants frequently connected to a breakdown of self-incompatibility systems and thus allows a shift from outcrossing to uniparental reproduction (De Nettancourt, 2001; Hörandl, 2010).

To get some empirical information on the premises of these hypotheses, we have studied population structure and pollination system of the alpine plant species *Ranunculus kuepferi* Greut. et Burd. The species has obligate sexual, diploid and apomictic tetraploid populations, with some rare triploid, pentaploid and hexaploid individuals. Tetraploids reproduce via facultative apomixis by developing sexual and apomictic initial cells (apospory) within the same ovule (Burnier *et al.*, 2009). A predominance of apomictically formed seed occurs in all alpine tetraploid populations (Cosendai and Hörandl, 2010). Triploids represent probably just backcrosses of diploids and tetraploids in the geographical contact zone and have a mixed mode of reproduction (Cosendai and Hörandl, 2010). The species exhibits a classical pattern of GP, with apomicts colonizing previously glaciated areas in the Alps, whereas sexuals are restricted in their distribution to ice-free refugial areas (Figure 1). The apomictic tetraploid cytotype originated multiple times via autopolyploidy and combined the gene pools of different sexual progenitor populations (Cosendai *et al.*, 2011). However, the lack of information on population genetic diversity and structure made it so far difficult to apply an explicit explanatory model for the GP pattern. Both sexual and apomictic plants are hermaphroditic and pollen-dependent for endosperm formation, but self-fertility of sexuals versus apomicts was so far unknown. Therefore, the determination of compatibility systems was essential to understand the ability for uniparental reproduction as a premise for the potential applicability of Baker's law.

We collected population genetic data throughout the range of the species and conducted experimental studies on breeding systems. Our study addresses the following questions: 1) Does population genetic structure in asexuals indicate the existence of widespread clones or of many different clones? 2) Is genetic diversity significantly different between sexual and apomictic populations? 3) Do breeding systems confirm the expectation of uniparental reproduction in apomicts and of biparental reproduction in sexuals? 4) Does population genetic structure support a hypothesis of combinatory effects of uniparental reproduction and facultative sexuality?

## MATERIALS AND METHODS

### Plant material

Materials were collected from 59 natural populations between 2004 and 2007 from the whole distribution area (Supplementary information; Figure 1). We sampled between 10 and 25 individuals per population, comprising totally 1009 individuals. This sampling added 29 populations and 630 individuals to an earlier study (Cosendai *et al.*, 2011) and allowed for a detailed analysis of population genetic diversity and geographical structure. Leaf material was dried in silica gel for molecular analysis. Determination of ploidy level of all individuals and sexual versus apomictic seed formation has been presented by Cosendai and Hörandl (2010). However, information on pollination and self-compatibility systems was so far missing. A part of the living plant collection was transferred to the University of Innsbruck (Austria) for experimental analysis of self-compatibility and of pollen tube growth.

### Molecular methods and analysis

DNA extraction and molecular methods were performed as in Cosendai *et al.* (2011). Six microsatellite loci (GeneBank number: Rk_11: JF308192; Rk_26: JF308190; Rk_27: JF308191; Rk_35: JF308193; Rk_37: JF308194; Rk_38: JF308195; see Cosendai *et al.* (2011) for details) were used to study a subset of 379 samples from 14 populations comprising 119 diploid, 15 triploid, 237 tetraploid and 4 pentaploid individuals. Raw data were sized and scored with GeneMarker 1.85 (SoftGenetics, State College, PA, USA) with parameter set at the size of selected microsatellite with a threshold of 100 RFU (relative fluorescence unit).

Amplified fragment length polymorphism (AFLP) fingerprint profiles were produced with a set of three primer combinations with four selective

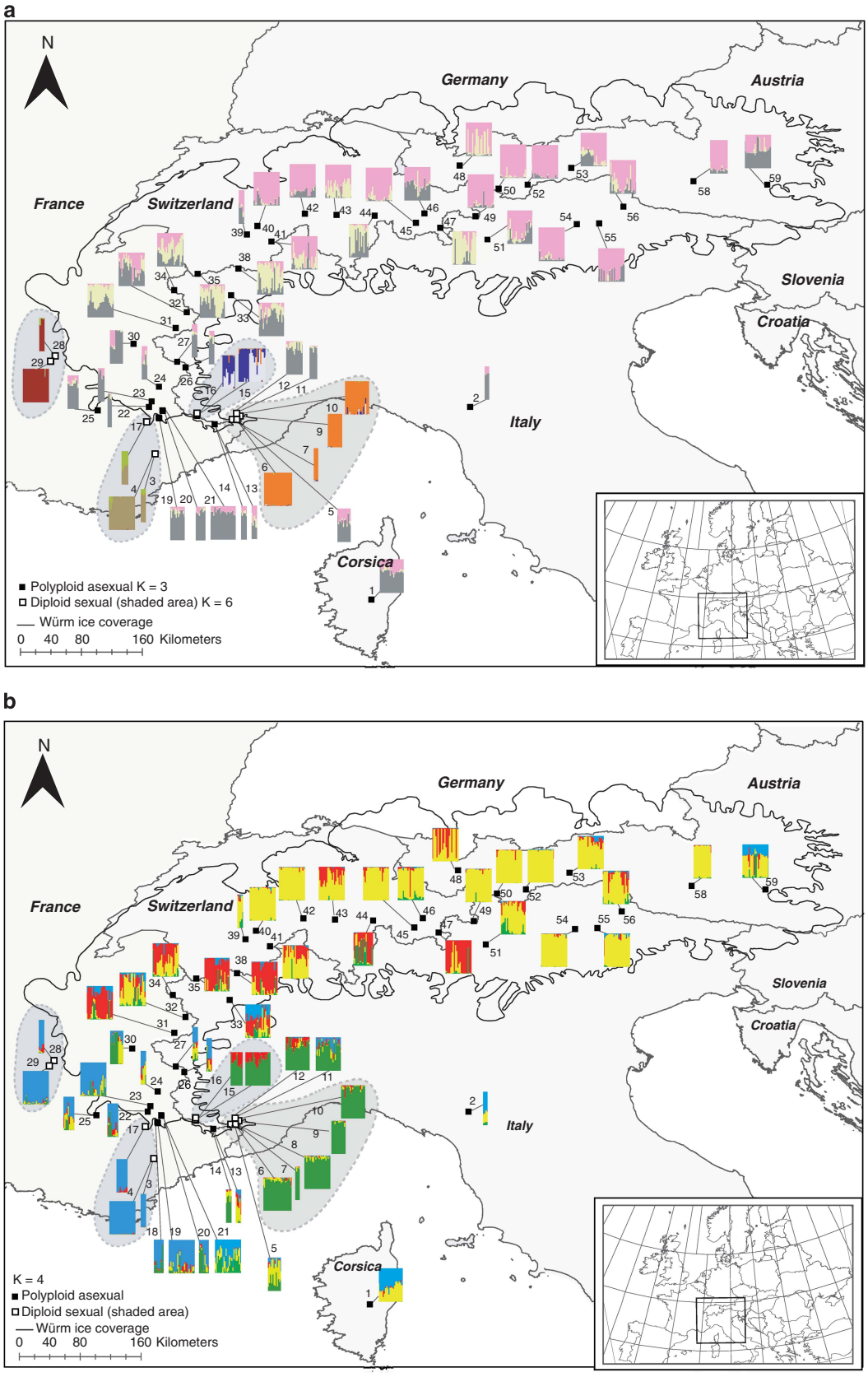


**Figure 1** Map of the distribution area of *R. kuepferi*; black squares, tetraploid populations; white squares, diploid populations; numbers beside the squares correspond to population numbers in Table 1. Colors represent the respective partitions identified by the STRUCTURE analysis. Individual posterior assignment probabilities (thin vertical lines) show the affiliation to the particular partitions. (**a**) STRUCTURE analysis with diploids and tetraploids analyzed separately and shown with different color schemes (see Materials and methods); (**b**) STRUCTURE analysis with all populations treated as diploids and shown with one color scheme.

**Table 1 Summary of private amplified fragment length polymorphism fragments (a) and simple sequence repeat alleles (b) of tetraploid populations (compared with diploids), number of genotypes and number genotypes/number of individuals (*G/N*)**

| *Population no.* | *Locality* | *No. of individuals* | *Allele 1* | *Allele 2* | *Allele 3* | *Allele 4* | *Allele 5* | *Allele 6* | *Total no. of private fragments* | *No. of actual genotypes* | G/N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *(a)* | | | | | | | | | | | |
| 1 | Corsica | 22 | | | | | | | 0 | 22 | 1.00 |
| 2 | Mt Cusna | 4 | | | | | | | 0 | 4 | 1.00 |
| 5 | Col de Tende | 12 | | | | | | | 0 | 12 | 1.00 |
| 11 | Colle della Perla_I_9596 | 7 | | | 1 | 1 | | | 2 | 7 | 1.00 |
| 12 | Colle della Perla II 9597 | 15 | | | | | | 1 | 1 | 15 | 1.00 |
| 13 | Notre Dame de la Fenestre | 5 | | | | 1 | 1 | 1 | 3 | 5 | 1.00 |
| 14 | Isola 2000 | 5 | | | | | | | 0 | 5 | 1.00 |
| 18–19 | Col des Champs | 13 | 1 | | | 1 | 1 | 1 | 4 | 13 | 1.00 |
| 21 | Col de la Cayolle | 32 | | | 1 | 1 | 1 | 1 | 4 | 32 | 1.00 |
| 22, 23 | Col d'Allos I | 10 | | | | | 1 | | 1 | 10 | 1.00 |
| 24 | Col de Vars | 5 | | | | | 1 | | 1 | 5 | 1.00 |
| 25 | Col de Raboux | 10 | | | 1 | | 1 | | 2 | 10 | 1.00 |
| 26 | Queyras LaCroix | 5 | | | | | 1 | | 1 | 5 | 1.00 |
| 27 | Queyras Montette | 5 | | | | | 1 | | 1 | 5 | 1.00 |
| 30 | Col du Lautaret | 12 | | 1 | | | | 1 | 2 | 12 | 1.00 |
| 31 | Mt Cenis | 24 | | | 1 | 1 | 1 | 1 | 4 | 24 | 1.00 |
| 32 | Col d'Iseran | 24 | | | | | 1 | 1 | 2 | 24 | 1.00 |
| 33 | Gran Paradiso | 23 | | | | 1 | 1 | 1 | 3 | 23 | 1.00 |
| 34 | Pt St Bernard[a] | 24 | 1 | 1 | | 1 | 1 | 1 | 5 | 24 | 1.00 |
| 35 | Gd St Bernard | 23 | | | | 1 | 1 | 1 | 3 | 23 | 1.00 |
| 38 | Cervinia[a] | 24 | | 1 | 1 | 1 | 1 | 1 | 5 | 24 | 1.00 |
| 39 | Jeizinen | 5 | | | | | 1 | 1 | 2 | 5 | 1.00 |
| 40 | Lötschental | 24 | | | | | 1 | 1 | 2 | 24 | 1.00 |
| 41 | Col du Simplon | 24 | | | | 1 | | 1 | 2 | 24 | 1.00 |
| 42 | Furka Pass | 24 | | | 1 | | 1 | 1 | 3 | 24 | 1.00 |
| 43 | Lukmanier Pass | 24 | | | 1 | 1 | | 1 | 3 | 24 | 1.00 |
| 44 | Rheinwald 9603 | 18 | | | | | | 1 | 1 | 18 | 1.00 |
| 45 | Julier Pass | 24 | | | | 1 | | 1 | 2 | 24 | 1.00 |
| 46 | Albula Pass | 24 | | | | | | | 0 | 24 | 1.00 |
| 47 | Bernina Pass | 23 | | | | 1 | 1 | 1 | 3 | 23 | 1.00 |
| 48 | Mt Kapall, St Anton | 24 | | | | | | 1 | 1 | 24 | 1.00 |
| 49 | Umbrail Pass | 24 | | | 1 | | | 1 | 2 | 24 | 1.00 |
| 50 | Kaunertal | 24 | | | | | | 1 | 1 | 24 | 1.00 |
| 51 | Tonale Pass | 23 | | 1 | 1 | 1 | | 1 | 4 | 23 | 1.00 |
| 52 | Timmelsjoch Pass[a] | 24 | | | 1 | | | | 1 | 23 | 0.96 |
| 53 | Tuxer Alps | 24 | | | 1 | 1 | | 1 | 3 | 24 | 1.00 |
| 54 | Rosengarten | 24 | | | | | | | 0 | 24 | 1.00 |
| 55 | Padon Pass | 24 | | | 1 | | | 1 | 2 | 24 | 1.00 |
| 56 | Mt Dürrenstein | 24 | | | | 1 | | 1 | 2 | 24 | 1.00 |
| 58 | Mt Sadnig | 16 | 1 | | | | | | 1 | 16 | 1.00 |
| 59 | Turracherhöhe[a] | 24 | | | | 1 | | | 1 | 22 | 0.92 |

| *Population no.* | *Locality* | *No. of individuals* | *Rk11_205* | *Rk27_187* | *Rk27_215* | *Rk27_221* | *Total no. of novel alleles* | *No. of actual genotypes* | G/N |
|---|---|---|---|---|---|---|---|---|---|
| *(b)* | | | | | | | | | |
| 1 | Corsica | 14 | 1 | 0 | 0 | 0 | 1 | 12 | 0.86 |
| 2 | Cusna | 4 | 1 | 0 | 0 | 0 | 1 | 4 | 1.00 |
| 12 | 9597 | 15 | 1 | 1 | 0 | 1 | 3 | 15 | 1.00 |
| 19 | Champs | 15 | 1 | 1 | 0 | 0 | 2 | 15 | 1.00 |
| 33 | Paradiso | 23 | 1 | 0 | 1 | 0 | 2 | 22 | 0.96 |
| 40 | Lötschenpass | 24 | 1 | 1 | 0 | 0 | 2 | 24 | 1.00 |
| 45 | Julier Pass | 24 | 1 | 1 | 0 | 0 | 2 | 23 | 0.96 |
| 48 | St Anton/Kapall | 24 | 1 | 1 | 0 | 0 | 2 | 24 | 1.00 |
| 53 | Tuxerjoch | 24 | 1 | 0 | 0 | 0 | 1 | 22 | 0.92 |
| 55 | Padon | 23 | 1 | 1 | 0 | 0 | 2 | 23 | 1.00 |
| 58 | Sadnig | 16 | 1 | 0 | 0 | 0 | 1 | 15 | 0.94 |
| 59 | Turracherhöhe | 24 | 1 | 0 | 0 | 0 | 1 | 22 | 0.92 |

[a]Populations used for character incompatibility.

nucleotides (fluorescent dyes in brackets): EcoRI-ACT/ MseI-CTCG (FAM), EcoRI-ACG/ MseI-CTCG (VIC), EcoRI-AGC/ MseI-CTGA (NED) following Cosendai *et al.* (2011). Raw data were sized and scored with GeneMarker 1.85 (SoftGenetics, State College, PA, USA). Settings of parameters with GeneMarker for scoring used a size range of 120–510 bp, and a threshold set at 50 RFU. The panel editor was used for filtering the irreproducible fragments, conserving only clear peaks. Non-reproducible bands identified by comparisons among replicated individuals were excluded from the further analysis. We did not score small fragments (50–120 bp) to avoid non-homologous fragments in this size class (Vekemans *et al.*, 2002). AFLP fingerprints with a total of 297 reproducible bands were obtained for a total of 1009 individuals over 59 populations with 163 diploids, 45 triploids, 749 tetraploids, 13 pentaploids, 3 hexaploids and 4 individuals with unknown ploidy level.

Presence of clones was estimated initially by scoring identical multilocus genotypes in AFLP as well as in simple sequence repeat (SSR) data (Martens *et al.*, 2009). The proportion of distinguishable genotypes (PG) was calculated by dividing number of genotypes/number of individuals per population. As multilocus genotype diversity reached the maximum of $G/N = 1$ (all

**Table 2 AMOVA of AFLPs (a) and SSRs (b)**

| *AFLPs* | *Source of variation* | *d.f.* | *Sum of squares* | *Variance components* | *% of variation* | *95% CI* | *FST total* |
|---|---|---|---|---|---|---|---|
| *(a)* | | | | | | | |
| All populations | Among populations | 71 | 14 545.564 | 13.09 | 36.82 | 0.34540 | 0.368*** |
| | Within populations | 937 | 21 048.938 | 22.46 | 63.18 | 0.38246 | |
| 2× only | Among populations | 10 | 2307.004 | 14.50 | 38.50 | 0.35246–0.41687 | 0.385*** |
| | Within populations | 152 | 3519.916 | 23.16 | 61.50 | | |
| 3× only | Among populations | 5 | 572.197 | 12.71 | 36.93 | 0.33554–0.40202 | 0.369*** |
| | Within populations | 39 | 846.425 | 21.70 | 63.07 | | |
| 4× only | Among populations | 42 | 8964.774 | 11.03 | 33.17 | 0.31275–0.3500 | 0.332*** |
| | Within populations | 706 | 15 691.125 | 22.23 | 66.83 | | |
| 2× versus 4× | Among populations | 53 | 12 615.932 | 12.83 | 36.42 | 0.34509–0.38274 | 0.364*** |
| | Within populations | 858 | 19 211.041 | 22.39 | 63.58 | | |

| *AMOVA SSRs* | *Fit* | *Fis* | *FST* | *Rit* | *Ris* | *RST* | *GST* |
|---|---|---|---|---|---|---|---|
| *(b)* | | | | | | | |
| All among population | 0.158** | 0.0218** | 0.1393** | 0.2282** | 0.1494** | 0.0926** | 0.178 |
| 2×, among populations | 0.1435** | 0.0146 | 0.1308** | 0.2461** | 0.1379** | 0.1254** | 0.114 |
| 3×, among populations | 0.1311* | −0.0462* | 0.1695 | 0.2968* | 0.3088* | −0.017 | 0.316 |
| 4×, among populations | 0.1748** | 0.0414** | 0.1392** | 0.2091** | 0.1464** | 0.0734** | 0.182 |

Abbreviations: AFLP, amplified fragment length polymorphism; All, all samples were used; AMOVA, analysis of molecular variance; CI, confidence index; d.f., degree of freedom; SSR, simple sequence repeat.
*Significant, $P \leqslant 0.5$.
**Highly significant, $P < 0.05$.
***Highly significant, $P \leqslant 0.005$.

individuals different) in all but two populations ($G/N > 0.90$), we refrained from further tests of identical multilocus genotypes (Arnaud-Haond *et al.*, 2007). We rather tried to get insights into the influence of mutations versus facultative recombination on genotypic diversity within a population. In a first step, we calculated the number of private SSR alleles and AFLP fragments of tetraploid populations compared with their diploid progenitor, respectively (Table 1). Further, we performed character compatibility analysis on AFLPs to get insights whether data structure within populations would support a model of recombination or mutation, respectively (Mes, 1998). This method is powerful for an indirect assessment of recombination in facultative apomictic plants and is applicable to dominant markers (Mes, 1998; Van der Hulst *et al.*, 2000; Paun *et al.*, 2006; Paule *et al.*, 2011). In a data set with a bi-allelic marker with presence or absence (1 or 0) at each locus, the presence of all four possible combinations of alleles at two different loci in four genotypes indicates incompatibility (that is, with four genotypes A, B, C, D, the matrix for two loci (I/II) with each two alleles 0 and 1 could be, for example, A: 0/0, B: 0/1, C: 1/0, D: 1/1). Incompatibility is an indirect signal of recombination as the data structure is reticulate (Mes, 1998; Van der Hulst *et al.*, 2000). In contrast, a hierarchical data structure (for example, A: 0/0, B: 0/0, C: 1/1, D: 1/1 at the loci I/II, respectively) fits to a model of mutations that are vertically transmitted and accumulated within a clonal lineage. In a multilocus matrix, the incompatibilities are summed over all pairwise comparisons of loci as matrix incompatibility count (MI). Character compatibility analysis calculates an initial count of MI within the population; the subsequent stepwise removal of genotypes with the highest contribution to MI (that is, to recombination) does not reduce MI in sexually outcrossing populations (because all individuals contribute to MI), but leads to a subsequent decline of MI counts in the case of apomixis. The MI curve declines rapidly if only few individuals contribute to recombination but slowly if several individuals are involved in recombination events. As MI reaches zero, all genotypes left represent just clonal diversity. We performed character compatibility on the AFLP data set as implemented in PICA 4.0 with the JACTAX.EXE option and the Jackknifing procedure for MI counts on four populations: two with the maximum number of private fragments, where an impact of mutations might be observed, and the two populations with $G/N < 1.0$ where clonality is expected (Table 1a).

Genetic diversity within and among populations was estimated on the AFLP data set by calculating FST values with AMOVA using Arlequin 3.5 (Excoffier and Lischer, 2010). Correlations of genetic distances (FST values) and geographic distances (calculated from ArcGIS 9.3.1, ESRI, New Redlands, CA, USA) were computed and a Mantel test was performed in XLSTAT Pro 2010 (Addinsoft, NY, USA).

Microsatellites were coded as an allele matrix and tested for linkage disequilibrium in diploid populations as described in Cosendai *et al.* (2011). Loci Rk_11 and Rk_26 had a significant LD ($P < 0.005$) to each other and to the other loci and were excluded from analysis. The allelic composition for each individual and diversity measures for ploidy levels have been presented by Cosendai *et al.* (2011). Here we present population-level statistics on genetic differentiation among populations: FST values (Wang, 2002) were calculated based on infinite allele model. GST was calculated as an equivalent to weighted averages of FSTs for all alleles, based on equal weight of each populations (Pons and Petit, 1996), and RSTs based on allele size estimated (Slatkin, 1995; Rousset, 1996), using a stepwise mutation model, both executed in SPAGeDI 1.3 (Hardy and Vekemans, 2002). This program allows the analysis of codominant data at any ploidy level and assumes polysomic inheritance as typical for autopolyploids. Autotetraploid origin of *R. kuepferi* has been assessed by Cosendai *et al.* (2011). We further calculated observed heterozygosity (number of heterozygotes/number of individuals) for each population to get insights into effects of mode of reproduction on allelic diversity, and tested values for ploidy levels via *T*-tests (Supplementary information).

To get information on the partitioning and distribution of gene pools, we performed STRUCTURE 2.3.2 analyses (Pritchard *et al.*, 2000) of the AFLP data using the recessive allele model implemented for analyses of polyploids and dominant data (Falush *et al.*, 2007). First, we analyzed diploids and tetraploids separately with the respective settings of ploidy levels to get insights into structuring of partitions within cytotypes (Figure 1a); individuals representing other, infrequent cytotypes (3×, 5× and 6×) were excluded from this analysis. In a third analysis, we focused on the geographical structure of partitions over the whole distribution area for all cytotypes, and thus we treated all samples as diploids (Figure 1b). For all three analyses, we applied admixture models with correlated allele frequencies without using *a priori* information on population origin. We performed $\Delta K$- plots after Evanno *et al.* (2005) ranging from 1–11 (for diploids), 1–43 (for tetraploids) and 1–56 (for the whole data set) to determine the optimal number of partitions (*K*'s) (Supplementary information). For each value of *K*, we ran 10 replicate chains of 500 000 MCMC iterations and discarded the first 10 000 burn-in iterations (detailed settings are given in Supplementary information). Barplots of the

**Table 3 Summary of allelic diversity and means of Ho of simple sequence repeat data**

| Population number | Ploidy level | Loci no. of alleles | | | | | Mean (alleles) | Mean (Ho)[a] |
|---|---|---|---|---|---|---|---|---|
| | | Rk_37 | Rk_35 | Rk_11 | Rk_26 | Rk_27 | | |
| 3 | 2× | 3 | 3 | 2 | 2 | 3 | 2.60 | 36.67 |
| 4 | 2× | 5 | 2 | 2 | 2 | 3 | 2.80 | 32.61 |
| 6 | 2× | 10 | 4 | 2 | 2 | 8 | 5.20 | 64.61 |
| 7 | 2× | 4 | 2 | 2 | 2 | 3 | 2.60 | 71.03 |
| 15 | 2× | 9 | 9 | 2 | 2 | 10 | 6.40 | 64.16 |
| 16 | 2× | 6 | 3 | 2 | 2 | 2 | 3.00 | 44.81 |
| 28 | 2× | 4 | 4 | 2 | 2 | 2 | 2.80 | 60.00 |
| 29 | 2× | 7 | 3 | 2 | 2 | 6 | 4.00 | 56.62 |
| 33 | 3×, 4× | 9 | 5 | 3 | 2 | 5 | 4.80 | 33.33 |
| 12 | 3×, 4×, 5× | 9 | 4 | 3 | 2 | 8 | 5.20 | 66.67 |
| 19 | 3×, 4×, 5× | 8 | 7 | 3 | 2 | 8 | 5.60 | 60.61 |
| 1 | 4× | 6 | 3 | 3 | 2 | 2 | 3.20 | 80.23 |
| 2 | 4× | 3 | 3 | 3 | 2 | 1 | 2.40 | 50.00 |
| 40 | 4× | 11 | 2 | 3 | 2 | 7 | 5.00 | 52.00 |
| 45 | 4× | 10 | 3 | 3 | 2 | 10 | 5.60 | 70.56 |
| 48 | 4× | 9 | 3 | 3 | 2 | 7 | 4.80 | 49.65 |
| 53 | 4× | 8 | 3 | 3 | 2 | 4 | 4.00 | 51.39 |
| 55 | 4× | 11 | 3 | 3 | 2 | 6 | 5.00 | 60.12 |
| 58 | 4× | 8 | 2 | 3 | 2 | 2 | 3.40 | 37.50 |
| 59 | 4× | 9 | 2 | 3 | 2 | 2 | 3.60 | 55.24 |
| Sum | | 149 | 70 | 52 | 40 | 99 | | |
| Mean | | 7.45 | 3.50 | 2.60 | 2.00 | 4.95 | | |

Abbreviation: Ho, observed heterozygosity.
[a]See details for observed heterozygosity in Supplementary material.

individual's posterior assignment probabilities were created by using CLUMPP 1.1 (Jakobsson and Rosenberg, 2007) and DISTRUCT 1.1 (Rosenberg, 2004) and mapped on the geographical distribution (Figure 1). All calculations were performed at the server Bioportal of the University of Oslo (Kumar *et al.*, 2009). To detect genetic clusters and identical genotypes, we produced a neighbor-joining tree based on the Jaccard index using FAMD 1.23 (Schlüter and Harris, 2006; Supplementary information). Bootstrap support of the neighbor-joining tree was computed with a random resampling of loci and replacing missing data with 1000 repeats, and a majority rule consensus tree was obtained in SumTrees (Sukumaran and Holder, 2009).

### Self-compatibility analysis

A total of 129 plants from 6 diploid and 14 tetraploid populations were bagged from bud to fruit stage with a cellophane bag or with translucent fine-mesh organza, which prevents both animal and wind pollination. The other cytotypes were not included because of the low number of individuals available. We applied three treatments during anthesis at the stage of papillate stigmas: 1) manual outcrossing: emasculated flowers were pollinated with fresh allopollen from at least three different individuals (we refrained from open pollination as the pollinator spectrum in the experimental garden may differ from the natural conditions in the alpine zone); 2) spontaneous selfing: no treatment, bagged only; and 3) manual selfing: self-pollen was applied to stigmas. For all treatments, the percentage of well-developed achenes was calculated from the total of achenes as a measure for reproductive success for each collective fruit as described previously (Hörandl, 2008). As a spatial separation of anthers and stigmas (herkogamy) can also inhibit self-pollination, we further examined pollen tube growth in the stigma and in the style. Flowers were hand-crossed ($n = 7$) or hand-selfed ($n = 9$) and fixed 3–5 days after pollination in FPA50 (50% ethanol, formalin, propionic acid; 90:5:5). In total, 105 carpels from tetraploid individuals and 37 carpels from sexual individuals were analyzed for pollen tube growth using the fluorescence standard method with aniline blue following Hedhly *et al.* (2003). Pistils were excised from the flowers, washed twice in distilled water (each times 1 h), soaked in 8 N NaOH solution at 60 °C for 15 min, rinsed twice more in distilled water and stained for at least 2 h with 0.1% aniline blue in Sörensen phosphate buffer, pH 8. Pistils were gently squashed and examined under a fluorescence microscope (Olympus BH2, Tokyo, Japan; excitation filter 405–435 nm). We identified five categories of pollen tube growth: 1) pollen tube missing; 2) growth stop on the stigma surface; 3) growth stop within the stigma; 4) growth stop within the style and 5) pollen tubes reach the base of the style and enter the ovary (Supplementary material).

Seed set after bagging experiments (percentages of well-developed achenes per collective fruit; Hörandl, 2008) is shown as boxplots. Data for seed set were not distributed normally. Therefore, the nonparametric Kruskal–Wallis test was used to test *a*) for differences among treatments within the cytotypes, and the nonparametric Mann–Whitney *U*-test for pairwise comparisons b) between treatments within cytotypes and c) between cytotypes. The statistic evaluation of pollen tube growth was accomplished by cross tables and Chi-square test (Pearson). In all tests, the critical level of significance was $\alpha = 0.05$. All analyses were performed with SPSS for Windows vs 12 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Genetic diversity within and among populations

All diploid, triploid, pentaploid and all except for two tetraploid populations were composed of individual multilocus AFLP genotypes (PG = 1.00). Only few tetraploid individuals shared identical genotypes (population number 52, one clone with three individuals, PG = 0.96, and number 59, two pairs with each two individuals; PG = 0.92; Table 1a). The tetraploid populations had altogether only six novel AFLP fragments compared with their diploid progenitors. Six of the tetraploid populations had no private fragments at all, whereas the others had a maximum of five private fragments within a population. The number of novel fragments remained in all cases far below the number of observed multilocus genotypes (Table 1a). In SSRs, shared multilocus genotypes appeared more frequently, which is probably due to the limited number of loci used; however, *G*/*N* values were also overall high (0.86–1.00). Only four private SSR alleles appeared in the tetraploid populations compared with the diploids, one of them shared among all tetraploid populations. The number of private SSR alleles reached a maximum of only three alleles per population.

Character compatibility analysis of AFLP data on the two populations with the maximum number of five private fragments (number 34, Petit St Bernard and 38, Cervinia) revealed similar results with a high initial MI (MI count = 11 847 and 9542, respectively) and a slow decline of MI after stepwise removal of individuals. With the minimum of four genotypes left, MI counts remained far above zero (74 and 209, respectively), indicating that genotypic diversity is still due to recombination (Supplementary information). The two populations with some identical multilocus genotypes (52 and 59) showed both a much lower MI (948 and 3212, respectively), but also a rather constant decline to MI = 2 and 4, respectively (Supplementary information). Strict clonality (MI = 0) is not observed in any population.

Measures of genetic differentiation were overall similar between sexuals and apomicts. FST values calculated for populations from AFLP data range from 0.332 and 0.385 for all groups (Table 2a). FST values of diploid sexuals differed from tetraploid apomicts only by 0.053. In summary, both sexuals and apomicts had genetic diversity distributed more within populations (ca. 65%) than among populations (ca. 35%) in the dominant AFLP data. In SSRs (Table 2a), FST

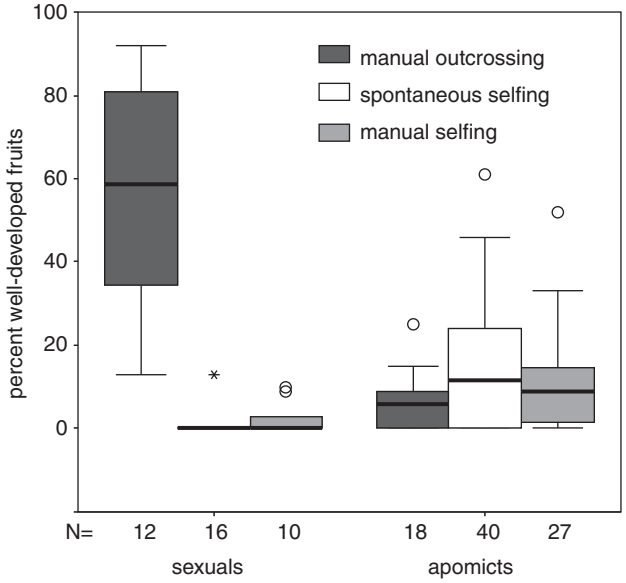


**Figure 2** Boxplots of the variation of percentages of well-developed achenes per collective fruit for diploid sexuals and tetraploid apomicts. The box shows the 25th and 75th percentile range and the median value, and maximum and minimum values within the normal range (capped bars); circles are outliers, asterisks (*) represent extreme values. *N*, number of flowers (collective fruits).

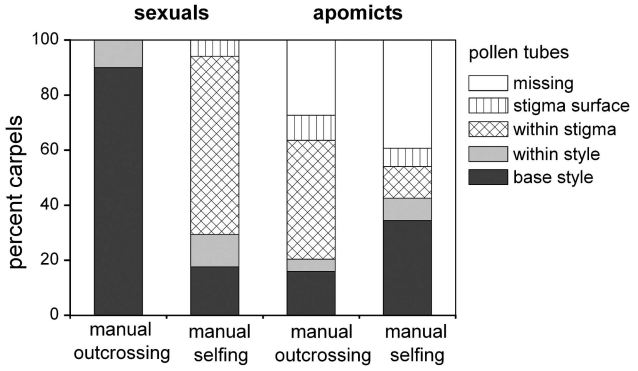


**Figure 3** Pollen tube growth in diploids and tetraploids after manual crossing and manual selfing. Values are percentages of carpels with respective pollen tube length; white, pollen tube missing; dotted, pollen tube growth stops at the stigma surface; cross-hatched, pollen tube growth stops within the stigma; gray, pollen tube growth stops in the style, and black, pollen tubes reach the base of the style and enter the ovary.

values ranged between 0.169 and 0.130, with a difference of only 0.08 between 2× sexuals and 4× apomicts. GSTs had the highest values in triploid backcrossed populations (0.316), in tetraploids 0.182 and the lowest value in diploids (0.114). Genetic differentiation based on allele size (RST) was highest in diploids (0.125), intermediate in tetraploids (0.073) and lowest in triploids ($-0.017$).

Levels of observed heterozygosity for three SSR loci were very similar between diploids, tetraploids and other ploidy levels with means of 53.37%, 56.13% and 53.54%, respectively (Table 3, Supplementary information), and were not significantly different from each other ($P=0.665$). Population means of allelic diversity for all five SSR loci (Table 3) ranged from 2.60 to 4.00 in diploids and had a grand mean of 3.75 alleles per population, whereas tetraploids had a range of 2.40–5.60 and a grand mean of 4.1 alleles per population; however, the number of alleles was not significantly different ($P=0.48$) between diploids and tetraploids. SSR loci differed from each other in their allelic diversity, as some loci presented up to 11 alleles (Rk_37), others only 2 (Rk_11 or Rk_26).

### Geographical population genetic structure

The STRUCTURE analysis of diploid populations revealed six groups as optimal partition (mean value of ln likelihood = −17 986). Three of the four geographical areas are each dominated by one partition (population groups 6–10, 3, 4 and 17, and 28–29; Figure 1a), while one geographical area comprises mostly two partitions (populations 15, 16). The sixth partition appears scattered with low frequencies. The degree of admixture remains low between the four geographical areas (Figure 1a). The separate analysis of tetraploid populations revealed three groups as optimal partition (mean value of ln likelihood = −681 988), with a predominance of one group in the populations of the eastern Alps (populations 40–46 and 48–59), but higher degrees of admixture in the southwestern parts (populations numbers 5, 13, 14, 18–27, 30 and 47). The STRUCTURE analysis with all individuals treated as diploids revealed four groups as optimal partition (mean value of ln likelihood = −136 196; Supplementary information). Sexual populations exhibit two dominant partitions with an east–west differentiation, whereas the other two partitions are infrequent; Figure 1b. The apomictic populations near the sexual area show admixture of all the diploid gene pools (numbers 5, 13, 14, 18–27, 30). In contrast, apomictic populations in the marginal areas have either genotypes admixed from other populations (31–35, 38, 41, 43, 44, 47, 48) or share one predominant partition (that is, yellow; for example 39, 40, 42, 45, 46, 49–58), which is rare in the examined diploid populations; Figure 1b. The neighbor-joining tree (Supplementary information) confirmed the diploid population groups as separate genetic clusters with bootstrap support >90%, but revealed no resolution for the majority of tetraploids. Only three spatially isolated tetraploid populations on Corsica, the Apennines and the easternmost Alps (numbers 1, 2, 59, respectively) do form well-supported genetic clusters in the neighbor-joining analysis (Supplementary information). The Mantel test identified a positive correlation between among-population diversity (FST value) and geographic distance, which is much stronger in diploids ($R^2=0.5899$; $y=0.002x+0.1968$; $P<0.0001$) than in tetraploids ($R^2=0.2272$; $y=0.0003x+0.2586$; $P<0.0001$).

### Breeding system

Manual outcrossing revealed seed set in all flowers of diploid sexuals, with a broad range of reproductive success (13–92% of well-developed achenes per collective fruit). After spontaneous selfing, no seed set was observed except for one flower (13%); after manual selfing, seed set was only 0–3%, with two outliers of 10% well-developed achenes; Figure 2. In contrast, tetraploid apomicts had good seed set after manual and spontaneous selfing (up to 52 and 61%, respectively), whereas manual outcrossing revealed lower reproductive success (0–25%) (Figure 2). Within cytotypes, the differences in seed set between the three treatments (manually crossed, spontaneously and manually selfed) were highly significant in diploid sexuals only, but not in tetraploid apomicts (Supplementary information). Diploids had significantly higher seed set between outcrossing versus both types of selfing, while manual and spontaneous selfing revealed no significant differences (Figure 2; Supplementary information). Tetraploids tended to a higher seed set after both types of selfing compared with outcrossing but the differences were not significant (Figure 2; Supplementary infor-

mation). Spontaneous selfing, which reflects best the situation of natural uniparental reproduction, revealed a significantly higher seed set in tetraploid apomicts than in diploid sexuals (Supplementary information).

The analysis of pollen tube growth confirms our hypothesis that the observed differences in the seed set are mostly explained by a differential degree of rejection of self-pollen tubes within the stigma and in the style (Figure 3). In diploids, manual outcrossing led in most carpels to pollen tube growth to the base of the style, whereas pollen tube growth mostly stopped within the stigma after selfing; only in 17% of carpels, tubes of self-pollen reached the base of the style. In tetraploid apomicts, manual selfing led to tube growth to the base of the style in about 35% of the carpels, manual outcrossing only in 17%. In general, in a high proportion of carpels, the pollen did not germinate at all in the apomicts. Within cytotypes, pollen tube performance significantly differed between selfing and outcrossing (diploids: $P<0.001$; tetraploids: $P=0.004$, Chi-square, Pearson). Differences in pollen tube growth between cytotypes and treatments were highly significant ($P<0.001$) except for diploid selfing and tetraploid outcrossing (not significant).

## DISCUSSION

### The relevance of genetic diversity

Population genetic data revealed no clonality in the tetraploid apomicts, but rather unique multilocus genotypes for almost all individuals, as in the sexual populations. This high genotypic diversity in *R. kuepferi* was also observed in earlier molecular studies based on smaller data sets (Burnier *et al.*, 2009; Cosendai *et al.*, 2011). Our study confirms that strict clonality, as expected from obligate asexuality, does hardly ever occur in natural asexual lineages (Loxdale and Lushai, 2003; Hörandl and Paun, 2007; Martens *et al.*, 2009). Genotypic diversity in apomictic populations can be due to multiple origins from diverse sexual ancestors, to accumulation of mutations, chromosomal rearrangements, or to facultative sexuality (Loxdale and Lushai, 2003; Hörandl and Paun, 2007; Vrijenhoek and Parker, 2009). In *R. kuepferi*, multiple origins from diploid sexual progenitors have combined alleles from all diploid populations in bi-allelic, tri-allelic and tetra-allelic SSR genotypes (details in Cosendai *et al.*, 2011). Polysomic inheritance in autopolyploids may even increase the number of allelic combinations and thus the genotypic diversity. The mutational dynamics is in *R. kuepferi* in both marker systems extremely low with a maximum of three SSR alleles or five AFLP fragments within a single population. Character compatibility analysis suggests that genotypic diversity is mostly due to facultative sexuality, which is supported by developmental studies. The occurrence of meiosis in parallel to aposporous embryo sac formation within the same ovule (Supplementary information) has been demonstrated by Burnier *et al.* (2009). Flow cytometric seed screening showed that partial sexuality appeared in about one-third of the seed samples analyzed, whereas the rest of the seed material was formed via apomixis (Cosendai and Hörandl, 2010).

Our study did not reveal any single widespread clone, and thus rejects the assumptions of the general-purpose-genotype model of a widespread single genotype (Baker, 1965; Lynch, 1984). In general, this model has gained low support in empirical studies on animals and plants, unless the geographical distribution is due to anthropogenic dispersal of asexuals (Vrijenhoek and Parker, 2009). The high genotypic diversity of apomicts in *R. kuepferi* fits better to the premises of the Frozen Niche Variation model, which assumes a high genotypic diversity. This model has gained broad support by numerous studies on animals and plants (Vrijenhoek and Parker, 2009). However, as apomicts in *R. kuepferi* do not exhibit clonal population structure, we cannot readily expect a 'freezing'of particular ecological niches by different clones.

Population genetic diversity in sexuals and apomicts has been shaped by different processes. In the sexuals, the historical fragmentation and geographical isolation of the four main sexual gene pools was probably a major cause for the reduction of genetic diversity within the sexual populations (Figure 1). Low genetic diversity within sexual populations is likely not due to frequent selfing or inbreeding as our observations on breeding systems do confirm predominant outcrossing for diploids. The FST values of AFLPs of diploid sexuals are above the average reported for outcrossers in dominant markers systems (0.27) and are nearer to values of mixed selfing-outcrossing systems (0.40; Nybom, 2004). The FST, RST and GST values in microsatellite data confirm a low genetic differentiation among populations (Nybom, 2004). Levels of observed heterozygosity in SSRs are in sexuals between mean values of mixed breeding systems (0.51) and outcrossers (0.63), but are clearly higher than mean values reported for selfers (0.05; Nybom, 2004). In contrast, the apomictic populations have a rather high genetic diversity due to polyploidy. Our microsatellite data even showed tri-allelic and tetra-allelic genotypes, which cannot be referred to novel alleles, but rather to polysomic inheritance of alleles in autopolyploids (Cosendai *et al.*, 2011).

### Geographical population structure of sexuals and apomicts

The separate STRUCTURE analysis of cytotypes revealed a higher number of partitions ($K=6$) and a stronger geographical subdivision for diploid sexuals than for polyploid apomicts ($K=3$). A lower substructure in such widespread polyploids is rather opposite to expectations for sexually reproducing plants, but is in *R. kuepferi* probably a result of facultative asexuality and the colonization history. Hence, a reduction of genetic diversity in asexuals happened rather on the level of gene pools than on the individual level. The loss of partitions could be due to bottleneck effects during colonization events by a few founder individuals. The combined analysis was less informative for cytotypes, but revealed just four optimal partitions for the species as a whole, with low likelihood for more groups (Supplementary information). All partitions of the tetraploids are derived from the diploid progenitors, which is in accordance with a hypothesis of autopolyploid origin (Cosendai *et al.*, 2011). The gene pools of the apomicts, especially those at the marginal areas, differentiated from the diploids by increasing frequencies of two partitions (marked as yellow and red in Figure 1b). Whether this shift is due to sampling effects during polyploidization and colonization, or relates to adaptive traits, needs further investigation.

In diploid sexuals, restricted gene flow among the four regional gene pools (Figure 1a) supports a hypothesis of geographical isolation in different glacial refugial areas. The positive correlation of geographical to genetic distance as shown by the Mantel test suggests isolation by distance among the regional clusters. In contrast, tetraploid asexual populations must have colonized rapidly the previously glaciated areas in the Alps after the retreat of glaciers, as geographical substructure and isolation by distance is rather weak. Populations near the southwestern center of origin exhibit high levels of admixture between populations (Figure 1b). Multiple origins of asexual tetraploids within the sexual area, that is, multiple as well as recent colonization of the same sites in adjacent areas may have enriched the gene pools of populations in these regions (Burnier *et al.*, 2009; Cosendai *et al.*, 2011). In contrast, the apomictic populations in the remote areas are more similar to each other (for example 39–56; Figure 1), with low levels of admixture (for example numbers 45, 46,

49–52, 54, 55, 58. We hypothesize that *R. kuepferi* was dispersed over the eastern and central Alps via founder events by single or a few apomictic diaspores. However, colonization was not conducted by clones, but rather by genetically similar individual genotypes. Colonization of remote sites could have happened via multiple events of long-distance dispersal from southwestern source areas. Long-distance dispersal, even transoceanic dispersal over thousands of kilometers, is frequent in flowering plants and has also been documented for many other species of *Ranunculus* (Emadzade *et al.*, 2011). The achenes of *R. kuepferi* have a cavity that aids wind dispersal (Müller-Schneider, 1986), but also dispersal via birds has been documented for species of *Ranunculus* (Emadzade *et al.*, 2011). As sexuals and apomicts have the same fruit morphology and thus an equal capacity for long-distance dispersal, the ability for establishment via uniparental reproduction is probably a crucial advantage for apomictic populations in remote areas.

The superior ability of asexuals to establish in remote areas also makes other theoretical models for GP less plausible. The mathematical model by Peck *et al.* (1998) suggests that immigration and introgression of maladapted sexual individuals would inhibit adaptation of sexual populations to local conditions. In *R. kuepferi*, it is questionable whether any obligate sexual population ever managed to establish in boundary regions, as diploids and obligate tetraploid sexuals are missing outside the southwestern Alps (Cosendai and Hörandl, 2010). In a postglacial re-colonization scenario, it might have been difficult for stepwise-migrating sexuals to expand their range, if the faster-moving apomictic populations have already built up an impermeable front (Mogie, 1992; Hewitt, 2004). In *R. kuepferi*, the geographical contact zone with triploid individuals (population numbers 11, 12, 17–19, 22, 23) indicates that expanding sexuals are being introgressed by backcrossing of tetraploid pollen donors with diploids. Although such introgression processes do not eliminate sexuality (Hörandl and Temsch, 2009), they may block stepwise range expansion of sexuals. A similar problem arises for the application of the theoretical consumer-resource model for cases of intermittent sexuality by Song *et al.* (2011), which suggests that frequencies of asexuality increase in marginal areas with decreasing resource richness and high mortality. However, different ploidy levels of sexual and apomictic plants may have a role for this model, and it is again questionable whether sexuals ever reached marginal areas. Empirical data on resource consumption by sexual and apomictic plants are missing. Strikingly, sexuality predominates in subnivale species under the extreme and resource-poor conditions of alpine glacier regions, whereas apomictic plants are more frequent in subalpine-alpine grassland habitats (Hörandl *et al.*, 2011).

### Self-compatibility

Our results confirm restricted self-fertility for sexuals and thus density dependence of biparental sexual reproduction. Bagging experiments, analysis of pollen tube growth and population genetic data confirm that diploids are largely SI with stigmatic or stylar SI. Diploids therefore do require mating partners for successful seed set. The lower seed set of tetraploids compared with diploids after outcrossing is in accordance with reproductive success observed in wild populations (Huber, 1988; Cosendai and Hörandl, 2010) and is quite typical for pseudogamous apomicts (Hörandl, 2010). As apomixis is in *R. kuepferi* mostly pollen dependent (Cosendai and Hörandl, 2010), disturbances of pollen tube growth may limit fertilization to form endosperm and reduce seed set. Nevertheless, the ability of seed set of apomicts in the situation of pollinator exclusion, compared with reproductive failure of diploid sexuals, is a crucial advantage for the establishment of populations in isolated founder situations. Preliminary results of experimentally transplanted founder individuals on natural sites confirmed regular seed set in apomicts, but not in sexuals (J Wagner and U Ladinig, unpublished data). For the explanation of the historical colonization scenario, actual observed frequencies of self-compatibility are probably less relevant. Only the apomictic founder plant must have been capable of self-fertilization, because any SI individual in isolation will fail to reproduce.

Self-incompatibility in diploids, but self-compatibility in their polyploid apomictic derivatives is a general trait in sexual/apomictic systems (reviewed by Hörandl, 2010). The genetic background of the breakdown of self-incompatibility in apomicts is not known, but could be a consequence of polyploidization (Hörandl, 2010). Alternatively, mentor effects by damaged or foreign pollen could have helped breaking the self-incompatibility system (De Nettancourt, 2001; Hörandl, 2010). As *R. kuepferi* has partly aborted pollen (Huber, 1988), self-compatibility could be also due to this form of pseudo-self-compatibility. The progeny of an autopolyploid self-compatibility individual can comprise both SI and self-compatibility genotypes, depending on the inheritance pattern of genetic control factors of SI (De Nettancourt, 2001). Therefore, after successful establishment, the tetraploid populations could well exhibit a mixed SI-self-compatibility system.

### A combinational model for GP

We draw the general conclusion that a combination of advantages of uniparental reproduction via apomixis and maintenance of genetic diversity via facultative recombination and polyploidy appears to be the best explanation for GP. The combination of uniparental reproduction, occasional sex and polyploidy is typical for apomictic flowering plants (Richards, 2003). Our case study on *R. kuepferi* thus represents a suitable model system for GP in angiosperms. The classical models for GP are probably not generally applicable and need reconsideration for facultative apomixis, as the premises of clonality do not apply. All genetic diversity measures between sexuals and apomicts are strikingly similar, suggesting that clonality is less relevant for GP than previously thought. In *R. kuepferi*, the density dependence of biparental reproduction appears to be the major disadvantage to obligate sexuality (Lewis, 1987; Hörandl, 2009). Other 'costs of sex' models (West *et al.*, 1999) are not easily applicable. Population genetic measures do not indicate a significant higher cost of recombination to sexuals compared with facultative apomicts. The cost of a male function in hermaphroditic systems remains basically the same in sexual and pseudogamous apomictic plants (Mogie *et al.*, 2007). As sexual outcrossers in *R. kuepferi* have a significant higher seed set than apomicts (Cosendai and Hörandl, 2010), a general fitness disadvantage of sexuality of a lower quantity of offspring does not apply. We have no information on long-term benefits to sexuality because of purging deleterious mutations (West *et al.*, 1999), as *R. kuepferi* represents probably an evolutionarily very young system. Nevertheless, *R. kuepferi* confirms the short-term success and an opportunistic behavior of asexual organisms in colonization scenarios (Van Dijk, 2003; Hörandl, 2009).

## DATA ARCHIVING

Data deposited in Genbank: accession numbers JF308190-JF308195 and in the Dryad repository: doi:10.5061/dryad.r37sj.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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