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Author's declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own.

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Table of Contents

Genetic analysis of fragmented populations of *Pinus cembra* L. in the Berchtesgaden National Park

Abstract – The conifer *Pinus cembra* L. (Swiss stone pine) is an endemic key stone species of the upper subalpine forest belt. It has been subject to various negative anthropogenic impacts in the past and is challenged by ongoing habitat fragmentation in the present. In addition, extreme climatic conditions in the species' habitat as well as individual traits make it highly susceptible to impacts of climate change, and a significant decrease of area habitable for the tree in future climates is expected. Especially small and fragmented populations at the margins of *Pinus cembra*'s distribution range have already shown signs of genetic depletion, restricted gene flow and inbreeding tendencies. Three Swiss stone pine populations embedded in the Berchtesgaden National Park in Bavaria, Germany, located at the northern species distribution boundary within the Calcareous Alps, are evaluated in the underlying study. Genetic and parentage analysis of juvenile and adult samples gathered from five sampling plots distributed over the three population areas are carried out using four chloroplast and twelve nuclear microsatellite markers. Results suggest, that the fragmentation of the three populations dates back a long time, and genetic exchange in the present is mostly restricted to the intra-populational level. Individual extents of genetic fragmentation and trends of inbreeding, genetic patterns and success of regeneration vary among the populations, therefore each of them is expected to display different degrees of success and viability in the future.

Keywords – *Pinus cembra*, Berchtesgaden National Park, chloroplast and nuclear microsatellite markers, population genetic fragmentation, genetic diversity, genetic differentiation, parentage analysis, gene flow, inbreeding, carbonate

BACKGROUND

Pinus cembra L. (Swiss stone pine or Arolla pine) is a keystone species of the treeline ecotone in the subalpine forest belt, restricted to an endemic distribution range in the Alps and the Carpathians (Rellstab et al. 2018, Neuschulz et al. 2017, Ulber et al. 2004). It has high biodiversity-endemic value and is quite important for the stabilization of hillslopes and therefore the prevention of soil erosion (karstification) and avalanches (Casalegno et al. 2010, Koch and Walentowski 2012, Walentowski et al. 2020). In the species' core range, the continentally influenced part of the Alps (Central Alps), it is distributed continuously, while at the margins of the distribution area in the Alps and at all sites in the Carpathian Mountains, populations are small and fragmented (Casalegno et al. 2010, Höhn et al. 2009, Farjon 2017, Huber et al. 2017, Politov et al. 2008). The species is considered a glacial relict, that has experienced a postglacial range expansion until it was slowly displaced upward by habitat competition with other plant species and climate warming during the Holocene and suffered a substantial range reduction (Höhn et al. 2009, Höhn et al. 2005, Huber et al. 2017). Swiss stone pine is mostly tolerant against the harsh climatic extremes in its regular habitat (Koch and Walentowski 2012). Adaptions to the climate at these elevations are a thick cuticula, long roots that ensure access to water during soil freezing in winter, as well as reduced water content and viscosity in the cell plasma of its needles during the winter months, which altogether results in frost resistance up to around -54 °C (Farjon 2017, Mayer and Erschbamer 2012)**.** Swiss stone pine grows in pure or mixed loose stands as a climax forest type with mostly *Larix decidua* Mill. (European Larch), but also *Pinus mugo* ssp. (Mountain Pine) at the upper and *Picea abies* (Norway Spruce) at the lower distribution ranges (Rohmeder 1941, Walentwoski et al. 2020, Dullinger et al. 2005, Langmaier and Hochbichler 2015, BfN (Bundesamt für Naturschutz) 2020, Höhn et al. 2009, Ewald 2007). Single or clustered individuals regularly act as pioneers on steep rocky outcrops or inhabitable placements like newly formed landslides or moraines above the treeline (Höhn et al. 2009, Casalegno et al. 2010, Ulber et al. 2004). Slight preferences for heightened mounds, closeness to dead wood and rocky structures have been visible among juvenile individuals, as those sites offer

better water- and temperature balances as well as protection from competitors and mechanical damage through snow (Welzmüller and Ewald 2016, Holtmeier 1986). These microhabitats are also lowering risks of infection, as the most relevant pathogens for *Pinus cembra*, *Gremmeniella abietina* Morelet and snow fungi (*Herpotrichia juniperi, Phacidium infestans*), seem to be mortal mostly for seedlings and saplings growing in pans with accumulating snow (Boden et al. 2010, Walentowski et al. 2020, Barbeito et al. 2013, Koch and Walentowski 2012, Holtmeier 1986). Caused by limited cold-tolerance, pathogenic insects (such as larvae of the midge *Cecidomvia pini,* the cone pyralid *Dioryctria abietella,* the cone geometrid *Eupithecia abietaria,* the weevil *Polydrusus atomarius* and larvae of the larch bud moth *Zeiraphera diniana* Guénée) do not seem to produce large rates of tree mortality (Dormont and Roques 2001, Ewald 2012). The limited colonization and damage of stone pine seeds by insects might also originate from the harvesting habits of the Spotted Nutcracker (*Nucifraga caryocatactes*). The bird empties most mature cones before larval development can be completed (Dormont and Roques 2001, Zong et al. 2010).

Pinus cembra's limited competitiveness due to its slow growth (1-2m in 50 years) implies that it is easily pressured by other species at the upper (*Pinus mugo* ssp.) and particularly at the lower (*Picea abies* L. (Karst)) boundaries of its distribution range (Koch and Walentowski 2012, Dullinger et al. 2005, Langmaier and Hochbichler 2015, Neuschulz et al. 2017, Ulber et al. 2004, Rohmeder 1941, Farjon 2017, Gugerli and Sperisen 2010, Höhn et al. 2005, Welzmüller and Ewald 2016). Negative human impact in the past (depletion due to extensive wood use and deforestation as well as karstification after excessive pasturing) led to the reduction of populations and lowering of upper distribution boundaries (Rohmeder 1941, Casalegno et al. 2010, Höhn et al. 2005, Farjon 2017, Klumpp and Stefsky 2004, Welzmüller and Ewald 2016, Walentowski et al. 2020). In the present, viability in extreme habitat conditions is aggravated by excessive cone harvesting for liquor preparation, habitat loss because of massive infrastructure required for mass tourism skiing and airborne pollution (Farjon 2017, Koch and Walentowski 2012, Klumpp and Stefsky 2004, BfN (Bundesamt für Naturschutz) 2020). Fragmentation and altering of the species' habitat in the past are still reducing chances of regeneration (Farjon 2017). In addition, Swiss stone pine is one of the species highly susceptible to impacts of climate change, since its survival in this narrow elevational belt of the Alps is strictly limited by temperature and steep environmental gradients occurring in the forests below and at the treeline ecotone (Casalegno et al. 2010, Mosca et al. 2016, Gruber et al. 2009b, Dullinger et al. 2020). The European Alps generally seem to tolerate an increase in temperature of 1-2K concerning plant species and ecosystems due to a certain inertia, but any further warming combined with anthropogenic impacts might have severe consequences (Theurillat and Guisan 2001). Long-term trends of mean annual temperatures within the Swiss Alps have already surpassed mean increment rates of the Northern Hemisphere twice (Rebetez and Reinhard 2008). Expected and partly already occurring changes are warmer and more humid conditions in winter alpine climate, and much warmer and drier conditions in summer, with the latter additionally leading to decreasing soil moisture (Cannone et al. 2008, Beniston 2006, Rebetez and Reinhard 2008, Lang and Stary 2017, Gobiet et al. 2014). Depending on individual species' traits, several responses to those trends have been documented. Warmer and drier conditions throughout the year, the infiltration of habitats by species from the montane areas and probably intensified competition with spruce, mountain pine and larch will force stone pine to shift upslope (Casalegno et al. 2010, Theurillat and Guisan 2001, Geburek and Myking 2018, Gruber et al. 2009a, Vittoz et al. 2008, Mosca et al. 2012, Heinze and Holzer 2013, Ewald 2012). The movement is facilitated by the increasing availability of areas for colonization with the ongoing abandonment of agro-pastoral landscapes throughout the last centuries, although such areas are often exhibiting quite specific grassland soils that may not be an immediately adequate substrate for high altitude conifers (Heinze and Holzer 2013, Casalegno et al. 2010, Dirnböck et al. 2003, Motta et al. 2006, Nicolussi et al. 1995). While such upward invasions and range expansions are partly already taking place for *Pinus cembra* and fellow coniferous tree species of these elevations (Casalegno et al. 2010, Farjon 2017, Motta et al. 2006, Motta and Nola 2001, Theurillat and Guisan 2001, Dirnböck et al. 2008), this secondary succession dynamic is happening rather slow and the prediction of migrating dynamics is complicated due to species-specific traits such as recruitment and dispersal limitation, slow growth rates and long generation times (Dullinger et al. 2004). Previously pastured areas situated on lower elevations than undisturbed conifer stands will be easier to invade (Vittoz et al. 2008) but will therefore suffer from changing soil composition and nutrient-water-cycles on the local scale (Dirnböck et al. 2008). For a successful upslope migration, seedling establishment and survival (often above the treeline) must be ensured

over several decades. Unfavorable moisture availability, snow cover duration or disturbances could hamper this process (Vittoz et al. 2008). Fragmentation trends within the endemic species' populations are likely to be enhanced by climate change as the subalpine belt's surface area is decreasing, areas higher up left for colonization will often be situated on steep slopes and may be impossible to reach for unfavorable orographic reasons (Theurillat and Guisan 2001, Ewald 2012, Heinze and Holzer 2013). Results of a study by Casalegno et al. (2010) revealed the limitation of areas suitable for colonization in the future very clearly. On the basis of two scenarios (A2a, B2a) concerning different rates of human population growth, levels of energy use and technical developments, implemented on a given baseline of climate change variables, loss of habitable areas for Swiss stone pine was calculated to be around 70% and 50% by 2080, respectively, and the remaining distribution area was moved upslope. Hence, already fragmented patches may become even more fragmented and might finally disappear on the local scale (Theurillat and Guisan 2001). Positive growth trends of Swiss stone pine (radial/height) caused by rising soil and air temperatures in summer and higher precipitation rates during the previous winter months have been observed numerous times (Vittoz et al. 2008, Gruber et al. 2009b, Motta and Nola 2001, Theurillat and Guisan 2001), while negative growth trends often seem connected to drought stress or late frost events (Vittoz et al. 2008, Boden et al. 2010, Oberhuber 2004). Growth and upslope shift trends could be further fueled by the access to greater carbon supplies to a certain extent, as both, atmospheric nitrogen and atmospheric CO_2 levels, have been increasing during the last century (Nicolussi et al. 1995).

The Spotted Nutcracker, mutualist scatterhoarder of cembran pine seeds, has verifiably been trying to sustain the durability of the collected seeds by caching them at places unfavorable for pine establishment (Neuschulz et al. 2015**,** Närmann et al. 2017). This non-random seed-caching behaviour could have detrimental effects on regeneration success of *Pinus cembra.* The sensible equilibrium between bird and tree could be hampered, when decreasing snow cover in the wake of climate change makes it easier for the bird to empty more of its caches, and therefore reduces chances of stone pine regeneration even further (Närmann et al. 2017). The tree's ability of seed dispersal as well as its biotic interactions might be regarded as key traits for the endurance of current and future threats of climate warming and landscape fragmentation, since these factors appear to control seedling establishment more than abiotic conditions across and beyond the species' upper distribution limits (Neuschulz et al. 2017, Zong et al. 2010, Vittoz and Engler 2007**,** Dullinger et al. 2003a). Upslope movement of stone pine establishment in the course of climate warming may depend on the nutcracker's seed caching behavior very strongly, which underlines the importance of this highly specialized plant-bird mutualism for local range expansions (Salzer 2011, Neuschulz et al. 2017).

Regeneration of Swiss stone pine seems to thrive best with enough light (radiation values of 4 to 8 MJ/m^2*d) and warmth, which requires a crown cover of $\leq 50\%$ and appropriate slope and exposition for earlier snowmelt in spring (Welzmüller and Ewald 2016). On the other hand, warmer temperatures could cause negative impacts on seedling establishment and sapling development. Persistent snow cover during winter can prevent deep soil freezing and attenuates effects of winter desiccation, abrasion or frost especially for that age group (Holtmeier 1986, Oberhuber 2004, Pfeifer et al. 2005). Progeny of *Pinus cembra* could be likely pressured further, since *Phacidium infestans* infections are facilitated by earlier snowmelt, and could strongly increase with climate change (Barbeito et al. 2013). The fact that the upward expansion of the species could proceed slower than projected climatic changes are taking place makes Swiss stone pine highly vulnerable and suggests extinction risks for the fragmented population patches at the margins of the species' distribution (Casalegno et al. 2010). Especially at the northern periphery of its distribution range within the Alps, where *Pinus cembra* appears scattered on some of the highest massifs of the Calcareous Alps as a remnant of cooler time periods, regional extinction risks are thought to be quite high under constant climate warming (Ewald 2012). In case of the occurrence of climate scenario B1 until the year of 2100, Ewald and Kölling (2009) estimated temperature amplitudes to move upslope for about 400 meters within the Bavarian Alps. If this estimation proves true, local stone pine forests will be significantly threatened. Hence, although Swiss stone pine stands are protected by several national and international protection regimes (e.g. BNatSchG § 30 in conjunction with Art. 23 BayNatSchG in Germany, habitat type 9420 within the Natura 2000 network in Europe) as well as regionally in conservation areas (e.g. Berchtesgaden National Park, nature reserve Allgäuer Hochalpen), survival and thriving future

development of the local populations seem uncertain in light of current and future threats (Koch and Walentowski 2012, BfN (Bundesamt für Naturschutz) 2020, Walentowski et al. 2020).

1. INTRODUCTION

Population fitness can not only be measured from an ecological point of view, but is also positively and significantly correlated with heterozygosity, quantitative genetic variation and population size. Due to inbreeding depression and genetic drift, fitness, as well as adaptability to changing environmental conditions in the future, are reduced in smaller and fragmented populations and their risk of extinction of environmental or demographic stochasticity is hence increased (Reed and Frankham 2003, Young et al. 1996, Salzer and Gugerli 2012). The distribution of *Pinus cembra*, a conifer native to the subalpine ecotone, is highly fragmented in the periphery of the Alps (Politov et al. 2008). The pine species is mostly outcrossing (Rellstab et al. 2018, Höhn et al. 2009), and is - given the combination of seed dispersal by the far-ranging Spotted Nutcracker and high mobility wind-mediated pollen dispersal over long distances - predisposed for extensive gene flow among and within populations (Ulber et al. 2004, Höhn et al. 2009, Politov et al. 2008). However, these variables do not seem to fully prevent populations with low effective sizes and low stand densities from inbreeding tendencies (Salzer 2011, Politov et al. 2008). The outcrossing rate, i.e. the proportion of male gametes received from any paternal plant unrelated to the maternal plant is one of the principal measures when assessing mating systems of plants (Politov et al. 2008). The opposed process, inbreeding, can occur through selfing by self-fertilization or by biparental inbreeding (= mating among relatives). It can be promoted when effective population sizes are low and restricted seed and pollen dispersal distances lead to a formation of family structure (Politov et al. 2008, Chybicki and Dzialuk 2014, Vranckx et al. 2011). Selfing can even be necessary to assure reproduction (Chybicki and Dzialuk 2014, Lewandowski and Burczyk 2000). Low pollen flow distances for instance have resulted in self-pollination rates up to 31% for *Pinus cembra* stands (Politov and Krutovsky 2004). Inbreeding manifests itself mainly in low seed quality (germination success) and high embryo abortion levels for this species (Politov et al. 2008, Salzer and Gugerli 2012, Höhn et al. 2005, Krutovskii et al. 1995). Coupled with adverse weather conditions, peripheral populations of *Pinus cembra* already suffering from inbreeding depression have been documented to have a significantly worse rate of parental seed production and reduced offspring performance compared to populations in the species' core distribution area (Salzer 2011, Salzer and Gugerli 2012). Such fragmented populations often cannot counterbalance with gene flow, and thus, show enhanced rates of genetic isolation resulting in reduced effective population sizes, increased genetic drift, lower allelic richness, lower genetic diversity and higher genetic differentiation (Geburek and Myking 2018, Vranckx et al. 2011**,** Tóth et al. 2019, Salzer and Gugerli 2012). These signs of spatial isolation patterns (Dzialuk et al. 2014, Salzer 2011, Gugerli et al. 2009) commonly increase from the center to the periphery of the species' distribution range (Eckert et al. 2008, Tóth et al. 2019). In addition, caching patterns of the nutcracker were observed to support the development of spatial genetic structures in the filial generation, especially in populations with low mature tree densities. Given all these findings, information about gene flow patterns within and among generations is important for making predictions about Swiss stone pine's chances of survival during changing environmental conditions, especially in small and fragmented populations, where isolation by distance is likely to be already mirrored in genetic patterns and regeneration rates are not always sufficient to secure long-term persistence of the populations (Salzer 2011, Welzmüller and Ewald 2016, Höhn et al. 2005).

Research has found significantly lower gene diversity and higher genetic differentiations in Northern Alpine populations compared to stands growing within the species' core distribution range in the Central Alps (Gugerli et al. 2009). The scattered stands that can be found in the Bavarian part of the Northern Calcareous Alps are genetically isolated relic populations, which survived because of local favorable climate far from the main distribution area in the Central Alps (Welzmüller and Ewald 2016). Situated in these margins of *Pinus cembra*'s Bavarian distribution range is the Berchtesgaden National Park. With local precipitation levels being relatively high, stone pine stands within the park are special from an ecological and geological point of view, since most of the Swiss stone pine populations that have been examined genetically until now stock on silicate substratum and it might be interesting to study fragmented cembran pine populations on carbonate bedrock. Analysis of stone

pine stands at Gesäuse National Park in Austria, which have similar growing conditions concerning fragmentation levels and geology, have revealed low regeneration rates (Langmaier and Hochbichler 2015). Stands of *Pinus cembra* in the area of Berchtesgaden have experienced a long history of significant anthropogenic influences until the decrease of pasturing during the last century and the foundation of the National Park in 1978 (Mayer 1970, Köstler 1950). Extreme local distribution boundaries, like observed differences in elevation, have led Köstler and Mayer (1970) to the interpretation that Swiss stone pine exists in a non-stationary state here and is, due to anthropologically or climatically induced timberline-dynamics, not situated in an ecological-biological equilibrium.

Genetic analysis limited to adult individuals might underestimate genetic consequences of habitat fragmentation, as extinction debts often only occur after a certain time lag (Vranckx et al. 2011). Hence, since numbers and genetic quality of progeny are crucial for population viability, the genetic condition of adult trees and offspring of the fragmented *Pinus cembra* stands within the Berchtesgaden National Park will be examined throughout this study to generate hypotheses about the future of these stands in view of present and arising threats. Main questions of this research therefore are: i) Is any genetic depletion visible from the parent to the filial generation? In detail, which relatedness patterns and genetic structures are apparent in the individual populations? Are there apparent inbreeding trends? ii) How advanced is the degree of genetic fragmentation within and between populations? Is there any gene flow visible among different populations? iii) Which inferences can be made concerning future performance of the individual populations?

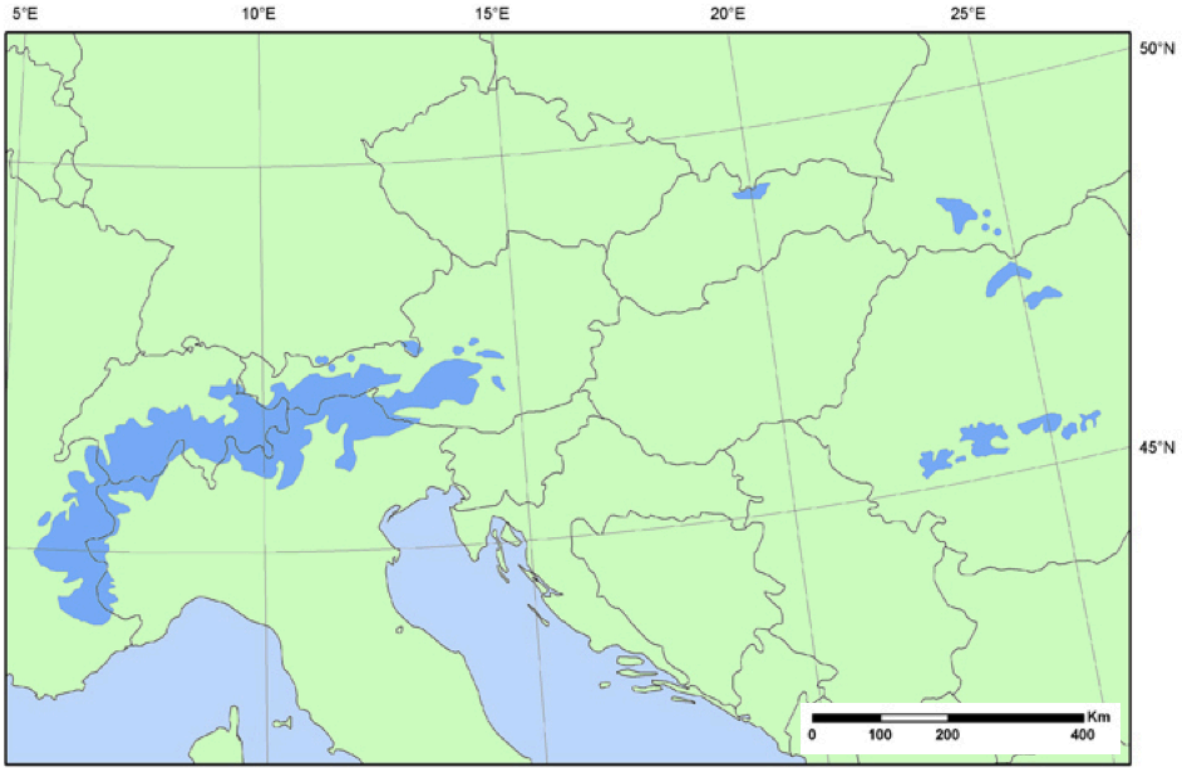
2. MATERIAL AND METHODS

Field work was carried out in the summer months of 2018. DNA processing and analysis took place from autumn 2018 until late spring 2019 in the BFW (Austrian Research Centre for Forests, Vienna) genome lab.

2.1 Study species

Pinus cembra, subsection *cembrae* (Stone Pines), section *Strobus* (White Pines), is a five-needled, evergreen, monoecious conifer (Rohmeder 1941, Politov and Krutovsky 2004, Chybicki and Dzialuk 2014). Its endemic distribution range includes the European Alps where it mainly inhabits the subalpine forest belt from 1500 to 2400 meters, and the Carpathian Mountains where its stands can be found from 1300 to 1700 meters above sea level (Ulber et al. 2004) (Figure 1). The pine species grows slowly and mostly does not reach more than 25 meters in height, mean sapling growth rates per year are 2,5 cm (Rohmeder 1941, Welzmüller and Ewald 2016). In return it has a long reproductive lifespan and reaches between 400 and 1000+ years when undisturbed (Ulber et al. 2004, Nicolussi et al. 2009, Walentowski et al. 2020, Farjon 2017, Koch and Walentowski 2012). In its natural high altitudinal distribution range, the tree reaches sexual maturity with 60 to 80 years (Rohmeder 1941). Wind-borne pollen dispersal often happens up and down the slopes and follows a heavy-tailed distribution, which means that long-distance dispersal is taking place to a small extent (Klumpp and Stefsky 2004, Chybicki and Dzialuk 2014). Chybicki and Dzialuk (2014) calculated an average pollen dispersal distance of ~1300m, but detailed knowledge about pollen dispersal of this species is still scarce. Other sources merely documented ~300m on average within marginal stands (Salzer 2011). In addition, Norway Spruce trees are believed to act as physical barriers to air movement between trees and therefore, self-pollination might be promoted in mixed stands where spruces grow next to stone pines (Salzer 2011). The closed seed cones of *Pinus cembra* take two years to ripen, flowering and seed production take place every two to three years, while mast years occur every five to ten years (Walentowski et al. 2020). Seed load depends on age and crown development of the individual tree, thus solitary trees of old age carry much more seeds (Mattes 1982).

Figure 1: Distribution of Swiss stone pine (*Pinus cembra*) throughout Europe (blue color). On the left the distribution belt covering the continental part of the Alps including the countries France, Swiss, Italy, Austria and small parts of Germany. On the right the Tatra mountains in Slovakia and Poland and the southern Carpathians in Ukraine and Romania. Source: EUFORGEN 2009, www.euforgen.org.



The pine species exists in mutualism with the Spotted (or European) Nutcracker (*Nucifraga caryocatactes*, *Corvidae*) which is the most significant dispersal vector of the seeds through synzoochory (Mattes 1982, Salzer 2011, Farjon 2017, Zong et al. 2010, Neuschulz et al. 2017, Neuschulz et al. 2015). It stores multiple seeds together in caches (~10.000 caches per bird and year) beneath the soil surface when the cones ripen from August to October (Mattes 1982). Thanks to the nutcracker's excellent spatial memory for natural landmarks, it later recovers ~80% of the stored seeds for consumption and to feed its offspring (Mattes 1982). The bird disperses Stone pine's seeds up to 20 kilometers in distance and 700 meters in altitude (maximum values) in its sublingual pouch, in which it transports on average 40 pine seeds (Mattes 1982, Zong et al. 2010, Tomback and Linhart 1990). It has even been witnessed crossing mountain valleys with filled pouches (Mattes 1982, Rolando and Carisio 1999). The caching behavior of the nutcracker often results in multi-trunk growth forms (tree clusters), which are much rarer in solely wind-dispersed conifers (Tomback et al. 1993, Tomback and Linhart 1990, Kratochwil and Schwabe 1994). Above ground tree parts may on the one hand profit from this special way of coexistence with enhanced biomechanical stability against snow- or windborne pressure (Kratochwil and Schwabe 1994, Tomback and Linhart 1990), on the other hand competition for root space, water or nutrients could arise (Tomback et al. 1993). Such clusters often lead to clumped population dispersion patterns (Tomback et al. 1993, Tomback and Linhart 1990). Strong spatial genetic structure has already been documented supporting the assumption that individuals within clusters are highly related (Salzer 2011).

2.2 Research area and sampling sites

The Berchtesgaden National Park typically consists of 75% core zone without human interference and 25% management zone with limited interference (Becker 2016). Swiss stone pine takes up around 10% of area covered with forest in the core zone with an altitudinal range between 1400 and 1914 meters above sea level (Becker 2016). Maximum altitudes for stone pine have been observed on southern and western slopes in the 1970s (Köstler and Mayer). Geologically, the National Park consists of Dachstein Kalk and Ramsau Dolomit, featuring small fractions of Werfener Schichten (Mayer 1959, Bayerisches Staatsministerium für

Landesentwicklung und Umweltfragen, 2001). So, as opposed to the main distribution area in the Central Alps (silicate bedrock), local Swiss stone pine stands stock on carbonate. Throughout the Bavarian Alps, carbonate Larch-Pine-Forests only exist as isolated relicts situated in high-subalpine elevations, with the biggest patches located in the Berchtesgaden National Park (Walentowski et al. 2020, Koch and Walentowski 2012) (Figure 2).

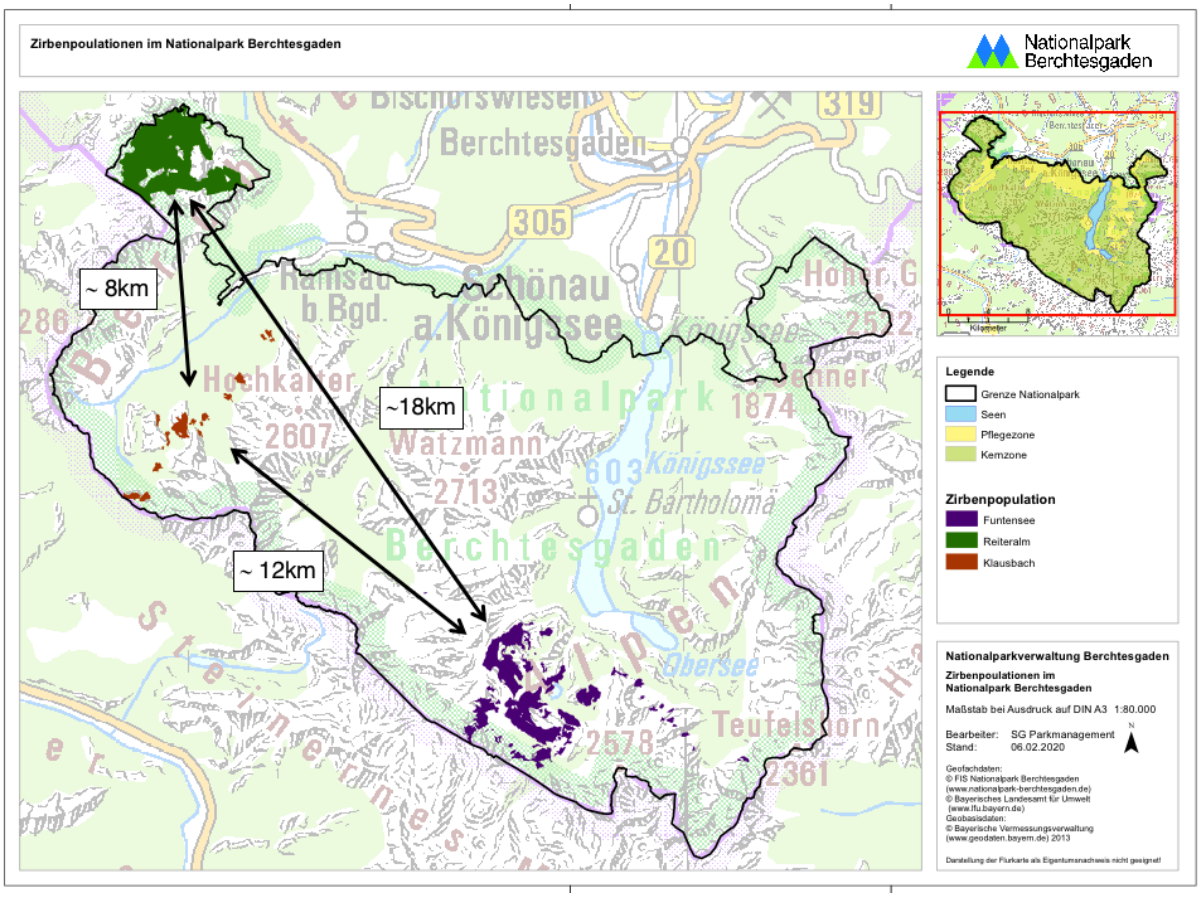
Figure 2: Population of *Pinus cembra* within the Berchtesgaden National Park at the margins of the Northern Calcareous Alps. On the left, distribution of *Pinus cembra* in blue, Calcareous Alps of Berchtesgaden circled in red. On the right, area of the National Park in green. Sources: EUFORGEN 2009, www.euforgen.org (left), Federal Agency for Nature Conservation (BfN), 2019, using data provided by the Bundesländer. Basic Spatial Data: © GeoBasis-DE / BKG 2018 (right).

Despite the carbonate bedrock, upper soil layers are quite acidic, displaying more or less heavy, dystrophic Tangel-Rendzina and Lehm-Rendzina, as well as Kalksteinbraunlehme (Koch and Walentowski 2012, Walentowski et al. 2020, Köstler and Mayer 1970). The National Park area is characterized by mild temperatures and high precipitation rates during the growing season, with maximum temperatures in July (Hartl-Meier et al. 2014). Yearly temperature amplitudes are around 18,6°C in the lower montane and 17,7°C in the subalpine elevation belt (Hartl-Meier et al. 2014). Like in most of the northern peripheral populations, precipitation rates are higher than at the core distribution zone of *Pinus cembra* in the Central Alps (Gugerli et al. 2009, Nicolussi et al. 2009), with annual precipitation values from 1500mm in the valleys to 2800mm at the highest grounds (Becker 2016). Trends to warmer (+ ~1.4°C) and drier (- ~30mm) growing seasons with increased radiation (+ ~55h sunshine duration in the high mountains) have been documented since 1990 (Hartl-Meier et al. 2014).
Anthropogenic impacts on forests have been extensive in the vicinity of Berchtesgaden (Köstler 1950). The first intrusions date back to the Bronze Age and peaked during the Middle-Ages, when high-altitude forests were harvested or destroyed for and by agro-pastural landuse, wood use for salines, mining and other wood-related industries (Holtmeier 1986, Köstler 1950). Wood of *Pinus cembra* was extensively harvested for the production of wooden goods or firewood for alpine pastures (Freiberg 1980, Köstler 1950). Even hardly accessible terrain like the Reiteralm has undergone several clearcuts, and as a consequence, the timberline was lowered for at least 100 meters (Mayer 1959, Köstler and Mayer 1970, Freiberg 1980). Remaining forest structures were persistently influenced by wood pastures (cattle, sheep, goats) (Holtmeier 1986, Köstler 1950, Freiberg 1980). When exposed to anthropogenic or natural deterioration, easily karstified soils experience a regressive development that can result in primary stages like Spaltenböden or alpine Protorendzina once the humus layer has been eroded due to missing protection by vegetation (Köstler and Mayer 1970). This predisposition for erosion increases with annual precipitation rates varying between 2000 and 2500mm. Hence, soils covering the research area were prone to major degradation. It often takes centuries or even millennia to reach new equilibria of soil- and vegetation development. Analysis of pollen has proven that it takes 3000 to 4000 years for the development of matured, dystrophic Tangelhumus under Larch-Pine-Forests or Mountain-Pine-Shrubs on Dachstein Kalk (Köstler and Mayer 1970). Irreversible damage cannot be ruled out: Single remnants of extensive stone pine stands which existed during the Postglacial period or even until around 1930 can be found at some areas in the National Park (e.g. Steinernes Meer, Reiteralm) several hundred meters above the current treeline, on terrain that is nearly completely without vegetation nowadays due to karstification dynamics (Mayer 1959, Mayer 1951, Walentowski et al. 2020). More or less simultaneously to anthropogenic land-use changes, unfavorable climatic trends have intensified the decline of treelines starting in the late Holocene (Nicolussi 2015, Mayer 1970), Therefore unequivocal conclusions about these trends are difficult to draw (Motta and Nola 2001, Nicolussi et al. 2005, Vittoz et al. 2008). The establishment rate of stone pine seedlings as well as radial growth

rates were favored by warmer climate after the end of the Little Ice Age and, in combination with the cessation of wood pastures, have since been documented to slowly rise (Mayer 1970, Kudernatsch et al. 2016, Freiberg 1980).

Today, *Pinus cembra* occurs in three populations throughout the National Park, all within the core zone. The biggest one is situated in the far northwest on the Reiteralm mountain range. It is also the densest one, with an accumulated area of 415,2 ha. The Reiteralm plateau is situated at the margins of the Berchtesgaden Calcareous Alps, but it is shielded by mountain peaks (e.g. Hochstaufen, Sonntagshorn) and therefore displaying inner climate dynamics (Köstler and Mayer 1970). The second biggest population's stands are growing roughly circular around the Funtensee in the south of the National Park with an accumulated area of 356 ha. It is more scattered than the stands on Reiteralm, with small patches reaching to the southeast. Two sampling plots were established within each of the two populations (RA1/RA2 and F1/F2). The third population in the park is scattered loosely along the south-eastern slopes of the Klausbach Valley, with a total area of 57,9 ha. Although regeneration is very hard to find at that smallest population, one sampling plot was established in one of those patches at the Sittersbach High Valley (ST). It seemed interesting in terms of genetic patterns: The oldest measured stone pine during inventory in the National Park (799 years of age in 2012) was found at this sampling plot. Also, the Klausbach Valley population is situated between the two bigger ones and could serve as steppingstone for the Spotted Nutcracker. The populations at the Reiteralm plateau and near the Funtensee are roughly 18 kilometers apart which could be a crucial distance, since the bird reportedly covers maximum distances of 20 kilometers with a full sublingual pouch (Mattes 1982) (Figure 3).

Figure 3: *Pinus cembra* – populations within the Berchtesgaden National Park and linear distances between them. In green the Reiteralm population, in brown the Klausbach Valley Population, in purple the population in the Funtensee area.



2.3 Sampling design

The sampling plots were chosen based on the results of the last forest inventory conducted in the National Park from 2010 to 2012. Based on those statistics about tree species distribution, areas at the Reiteralm plateau and around the Funtensee were selected, which displayed enough regeneration for sampling. Different plots were inspected at each area to find the ones that are most representative in terms of altitude, slope and exposition. For each chosen sampling plot, altitude, slope, exposition, bedrock, soil type and thickness of organic soil cover, degree of soil coverage, percentage of dead wood within each sampling plot and vegetation (applying a cover-abundance scale roughly based on Braun-Blanquet) were measured or gathered from earlier literature about the area. Climatic data from the weather stations closest to the sampling plots provided by the National Park was used to calculate mean temperature values per year.
Sampling plots were created circular (adopting the lasting magnetic centers of the inventories of 1995/1997 and 2010/2012). Each adult tree within a 25meter radius from the sampling plot center (sampling area 1963,5m^2), and every offspring individual within a 20meter radius from the center (1256,6m^2) was included. Needle tissue samples (15-25mg dry weight, approx. 20 needles) were taken from side shoots of every individual and stored dry and cool (~4°C). Additionally, height, DBH (= diameter at breast height), special attributes and coordinates for every adult tree and height, estimated age, special attributes and coordinates for each regeneration individual were measured. (Equipment: Trimble GeoExplorer 7 series GNSS Handheld with antenna, Haglöf Vertex IV Hypsometers, Haglöf Tree Caliper, metal searching tool Heliflux GA-72c). At sampling plots RA1 and RA2 on Reiteralm mountain range, respectively two solitary adult individuals in close distance to the sampling plots (but outside the 25 meter radius) were sampled as well, to see if due to their more accessible position to all sides any evidence that they were harvested more frequently by the Nutcracker was showing. To receive a general impression regarding the Spotted Nutcracker's activity level and with that possible differences among the population areas, sightings and traces (seed forgeries) of the bird were documented, as well as calls per hour while being at the sampling sites for several days.

2.4 DNA processing

Needles were cut into 1mm pieces, cleaned with acetone, vacuum-dried and lyophilized in liquid nitrogen, then grinded using a shaking mill (TissueLyser shaking mill, QIAGEN, Hilden, Germany) at 25Hz with two steel balls per tube. The homogenized material was stored at -60°C. DNA extraction was conducted using the Invisorb Spin Plant Mini Kit (STRATEC Molecular, Birkenfeld, Germany), applying the recommended protocol. Individual DNA concentrations were measured with a NanoDrop ND-100 Spectrophotometer (Thermo Fischer Scientific, Ulm, Germany). Individual DNA dilution was adjusted depending on which concentration had been measured before for each sample (supplementary information table 1). Organellar DNA markers have proved useful to research genetic diversity patterns and detect genetic structures within populations, as they reflect long long-term genealogies of species in a spatial context (Petit and Vendramin 2007). In *Pinus cembra* (as in most Gymnospermae), the chloroplast genome is transferred strictly through pollen and subsequently by seed, while the nuclear plant genome is inherited biparentally via seed (Lendvay et al. 2014, Höhn et al. 2009, Gugerli et al. 2009, Petit and Vendramin 2007). Hence, use of both chloroplast simple sequence repeats (cpSSRs) and nuclear simple sequence repeats (nSSRs) allows insights into differences between pollen and seed dispersal patterns and to effects of gene flow and drift on the population structure. Therefore, DNA amplification was performed with 4 chloroplast loci (cpSSRs) in combination with 12 nuclear microsatellite loci (nSSRs) (supplementary information table 2). 240µL millipore H_2O per 10µL for each chloroplast primer, and 90µL TE-buffer per 10µL for each nuclear microsatellite primer were used for dilution. Polymerase chain reactions (PCR) were based on a multiplex schema with different annealing temperatures depending on the primer group (see supplementary information table 2 and 3) and performed on a thermo cycler PTC-100^{TM} Programmable Thermal Controller (MJ Research, Inc.). PCR results were reproduced on a Sequencer (SCIEX CEQ 8000 Genetic Analysis System Beckman-Coulter, Vienna, Austria) with all samples including the CEQ DNA size standard kit 400 (Beckman-Coulter) and analyzed using the Frag 3 - program. It offers the appropriate size range for the products of the amplification process generated by the primers in use. Alleles were assessed and binned with the fragment analysis tool of Genome Lab Systems

(Genome Lab Analysis Systems, Beckman-Coulter). Follow-up visual inspections of the size fragment chromatograms ("peaks") helped to level primer dilutions accordingly for PCR reruns if necessary.

2.5 Data analysis

Genetic clustering within the codominant data was analyzed using STRUCTURE (Version 2.3.4, Pritchard et al. 2000). By using a Bayesian clustering approach and applying the Markov Chain Monte Carlo (MCMC) estimation, this program offers insights to genetic distribution patterns among the inserted samples (Porras-Hurtado et al. 2013). It identifies population structures amongst the data and assigns individuals to the most suitable group, depending on similar variation patterns (Porras-Hurtado et al. 2013). Based on the assumption that all loci are at Hardy-Weinberg and linkage equilibrium (Pritchard et al. 2010), the software produces individual analyses for an assumed ancestral population number (*K*) that is appropriate for the acquired samples (Porras-Hurtado et al. 2013). In the case of this study, *K* was analyzed for 1 to 10 population values using an admixture model and correlated allele frequencies, a running length of 100.000 repetitions with a burning period of 100.000 and ten runs per *K.* For easier visualization of likelihoods and detection of the *K* value that best fits the implemented data, the results were processed through STRUCTURE HARVESTER (Earl and von Holdt 2012), a web-based program that collates STRUCTURE results using the Evanno method (Earl and von Holdt 2012). CLUMPAK version 1.1 (Cluster Markov Packager Across *K*, Kopelman et al. 2015) software was used to sum up and graphically represent the results provided by STRUCTURE. For both nuclear microsatellite and chloroplast data, distance- and frequency-based analyses were executed using GenAlEx 6.5 (Peakall and Smouse 2006, Peakall and Smouse 2012): Allele frequencies and allelic patterns, diversity indices and differentiation, heterozygosity and polymorphism were calculated for each locus, as well as means with corresponding standard errors (SE) over loci for each population and over all populations. Shannon's (partition) diversity indices for all pairwise population combinations were also applied within the program, as well as an AMOVA (hierarchical analysis of molecular variance) and a PCoA (Principal Coordinates Analysis) over all populations. Inbreeding coefficients within the codominant data, e.g. Nei's and Hedrick's standardized G_{ST}, were estimated with 999 permutations and bootstraps with the same program. Additional intra- and inter-population analyses of the haploid chloroplast data were conducted using HAPLOTYPE (version 1.05, Eliades and Eliades 2009), therein haplotypic richness (H_R) using the rarefaction method (El Mousadik and Petit 1996), Nei's index of genetic diversity estimated without bias (Nei 1973), Nei's minimum genetic distance and genetic differentiation among populations with respective contributions of each of them to the total diversity (Finkeldey and Murillo 1999).
For parentage analysis of the nuclear microsatellite loci, Cervus (version 3.0, Kalinowski et al. 2007) and Colony (Version 2.0.6.5, Wang 2012) were used. All successfully genotyped adult individuals (N=105) were put into the programs as possible father or mother candidates, all regeneration individuals as offspring (N=183). CERVUS software utilizes a maximum likelihood method and two likelihood enhancements for assignment of parents to their offspring: The level of confidence in assignment is determined via simulation, and all likelihood ratios are inferred allowing for possibilities of mistyping, incompleteness or incorrectness in some genotypes of offspring and parents (Kalinowski et al. 2007). The likelihood ratio in the results provided by the program is described with the LOD score (natural logarithm of the likelihood ratio, Marshall et al. 1998), with positive LOD scores indicating higher likelihood of candidate parents to being true parents, and Delta representing a derivative of LOD scores or rather a difference between the LOD scores of the most likely parent candidates (Kalinowski et al. 2007). Allele frequency analysis, simulation of parentage analysis (maternity, paternity and parent pair with sexes unknown) and parentage analysis for maternity, paternity and parent pair (sexes unknown) were calculated using the following parameters: 10000 simulated mating events, two most likely parents sorted by joint LOD-score, a minimum number of six genotyped loci, 0,99 proportion of loci typed, a mistyping rate of 0,01 and a relaxed confidence interval set to 80% and 95% for strict confidence, respectively. Due to the small area sampled altogether compared to the full size of the populations in the National Park area, the proportion of sampled individuals was set to 0,2. Data was also checked for self-fertilization. In addition to parentage assignments, COLONY software also estimates clonemates (duplicates), inbreeding estimates and full- and half-sib relationships among the analyzed individuals and their multi-locus genotypes using one likelihood and two pairwise likelihood methods under the allowance of genotyping errors, assuming the markers to be in linkage

equilibrium. Data was processed using a model that assumes the presence of inbreeding by inferring a population level inbreeding coefficient (equivalent to Wright's F_{IS}) (Wang 2018). Parentage candidates are assumed unrelated to each other, which could prove critical throughout the analysis, as surely there are at least some kinsman-like relations evident between the adult sampled individuals (Tomback et al. 1993).

3. RESULTS

3.1 Population parameters

In total, 289 samples were taken. At the Sittersbach High Valley sampling plot, there were only six adult individuals included within the 25m radius, all six being in varying states of deterioration and offspring was nowhere to be found. Therefor data from this plot will have to be interpreted very carefully if even possible. Specific numbers of sampled adult trees and regeneration individuals per sampling plot are shown in table 4 and visualized in figures 4 – 8, supplementary information.

Underlying plant community of all three sampling sites is the carbonate Larch-Pine-Forest (*Vaccinio-Pinetum cembrae* or *Rhododendretosum hirsuti – Pinetum cembrae*), which, as a special feature, displays acidic as well as alkaline indicator plants, e.g. *Rhododendron ferrugineum* (acidic) and *Rhododendron hirsutum* (alkaline) (Mayer and Erschbamer 2012, Koch and Walentowski 2012, Walentowski et al. 2020, Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen 2001: map 6). Due to high relief energy, plant communities are unevenly distributed and often occur in small unities within the Calcareous Alps of Berchtesgaden (Mayer 1959). All three sampling sites show different variants of their underlying plant association. The Sittersbach High Valley sampling plot displays a *Pinus mugo* ssp. coverage of 50-75% and plenty of *Vaccinium myrtillus/vitis-idaea*, *Erica carnea* and *Lycopodium annotium*, while *Rhododendron* species are not so numerous. Sampling plot RA2 at Reiteralm sampling site, situated on a hilltop and much drier than RA1, features 50-75% *Pinus mugo ssp.* and high amounts of *Vaccinium ssp., Rhododendron ssp.*, and *Lycopodium annotium*, while RA1 displays less *Pinus mugo ssp.* (~25%) and some species of tall herb fringe communities (*Parnassia palustris, Adenostyles alliariae*). The two plots at the Funtensee sampling site both show patches of *Erico-Rhododendretum hirsuti* (F1 /F2) and *Seslerio-Cericetum sempervirentis* with *Alnus viridis* and *Vaccinium myrtillus/vitis-idaea* (F1) and less *Pinus mugo ssp.* coverage (<5%) (Freiberg 1980, personal observations). Impacts of wood use for construction and fire wood on high pastures are still visible at the Funtensee population, where single *Pinus cembra* stumps were found within the sampling plots as remnants of single stem harvest in earlier times (Figure 9b).

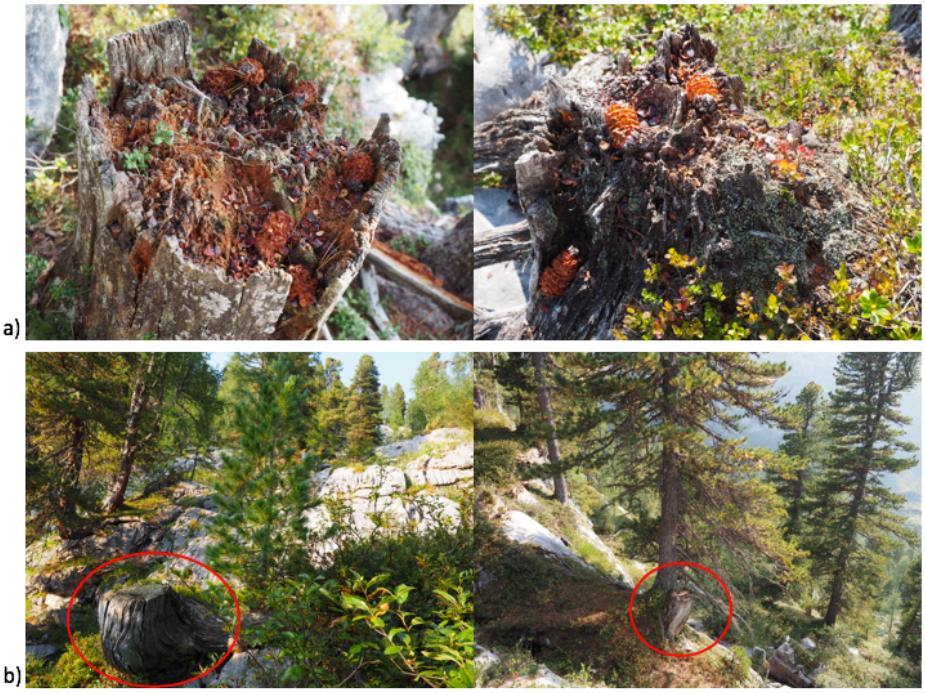


Figure 9: a) Seed forgeries of the Spotted Nutcracker at sampling plots 1 and 2 at the Funtensee population.
b) Single *Pinus cembra* stumps at sampling plots 1 and 2 at the Funtensee population (circled in red).
Fotographs: Hannah Hochsattel.

Apparent activity of the Spotted Nutcracker (i.e. frequency of calls, sightings during flight or while the bird was sitting on trees, emptied cones or seed forgeries) was lower at the Sittersbach High Valley sampling area as compared to Funtensee or Reiteralm (Figure 9a). More descriptive data of each sampling area and plot can be gathered from table 5 (supplementary information).

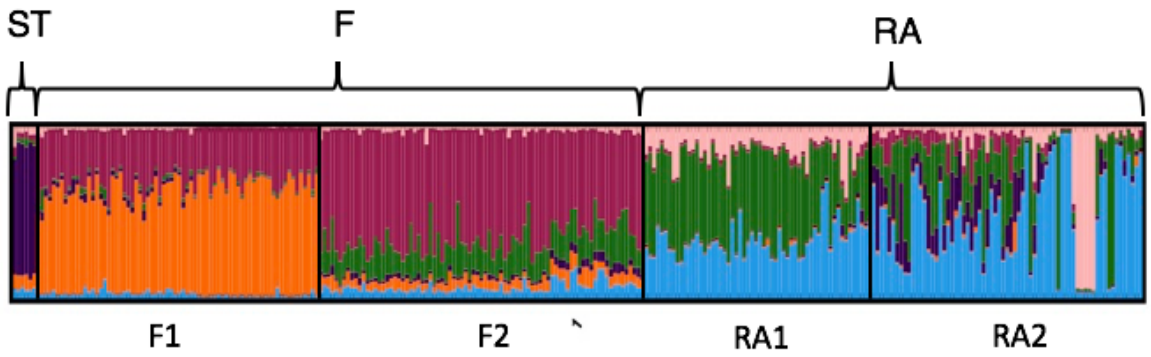
3.2 Fragment analysis

288 out of 289 individual samples taken were successfully genotyped for cpSSRs and nSSRs.

3.2.1 Codominant loci (nSSRs)

STRUCTURE and STRUCURE-HARVESTER results (e.g. mean likelihood L(*K*) and variance per *K* value, Figure 10 supplementary information) identified *K* = 6 as the most probable number of ancestral genetic clusters. The three populations are clearly distinguishable by the allele frequency distributions (Figure 11 and figures 12 and 13, supplementary information).

Figure 11: CLUMPAK single bar chart showing allele frequency distribution in *K* = 6 genetic clusters. The differences between the sampling areas (ST = Sittersbach, F = Funtensee, RA = Reiteralm) and even the sampling plots (except for RA1 and RA2) are easily recognizable.



With allele frequency subsets mostly found in the Sittersbach High Valley population placed in between subsets dominating the Reiteralm and the Funtensee sampling area, the distribution of the genetic clusters mirrors the geographic distribution of populations in the National Park (Figure 14, supplementary information).
Adult generation and following generation of each sampling plot ($\sum$ = 9) were put into the analysis programs as individual populations for the easier interpretation of the results, hence, in the following result section the three populations Reiteralm, Funtensee and Klausbach Valley will be referred to as sampling areas to avoid confusion. 93 alleles were detected in total among all 12 nSSR loci, with values ranging from 2 to 16 per locus (data not shown). Numbers of different and effective alleles were higher for filial compared to adult samples for all sampling plots (Table 6, supplementary information). Mean observed heterozygosity (H_O) values per population were mostly higher than expected heterozygosity (H_E). Both observed and expected heterozygosity values for adult and juvenile samples were higher than 0,5, and thus indicated high levels of genetic diversity. Private alleles (A_P) were found at low levels, with the highest mean number of 0,25 at F1_V and none found at F2_V, RA2_V and RA1_B and no obvious differences between adult and juvenile samples. Polymorphic loci (P) were calculated for all juvenile populations with 100% each, also for adult populations with the exceptions of ST and RA_1, which both showed a value of 91,67% (all values shown in table 6, supplementary information). Mean fixation indices did not differ much from 0 altogether which indicates that there are only little deviations from Hardy-Weinberg equilibrium (Table 7). Sums of mean F_{IS} values across loci did not indicate homozygotic excesses neither for juveniles nor for adult samples (-0,027 and -0,091, respectively), but showed higher heterozygosity levels for adult samples.

Table 7: Mean of F-statistics and estimates of effective migrants over 12 nuclear microsatellite loci for sampled regeneration, sampled adults and both combined. F_{IS}, inbreeding coefficient within individuals; F_{IT}, degree of inbreeding over populations; F_{ST}, (average F_{ST} over loci) inbreeding coefficient within in subpopulations, relative to total = genetic differentiation among populations; N_M, number of effective migrants; SE, standard error.

	F_{IT}	SE	F_{IS}	SE	F_{ST}	SE	N_M	SE
Regeneration	0,001	0,046	-0,027	0,046	0,027	0,006	21,45	8,682
Adults	-0,034	0,044	-0,091	0,046	0,052	0,008	6,624	1,422
Combined	-0,016	0,043	-0,062	0,044	0,043	0,007	7,679	1,506

Inbreeding depression (F_{IT}) seems to be more pronounced within the juvenile (0,001) than within the adult samples (-0,034). Values of genetic differentiation (F_{ST}) were generally low, insignificant for juveniles (0,027) but surprisingly higher for adults (0,052). Alternative mean F_{ST} estimates for individual populations, based on the average F_{ST} over loci showed highest differentiation rates for all four sampling groups of Reiteralm (data not shown). Mean numbers of effective migrants (N_M, gene flow parameter) were higher for the juvenile sampling groups (21,45) than for the adult groups (6,624), which means gene flow is still evident and even higher in the juvenile populations. Concerning genetic diversity among the 9 populations inferred, the PcOA revealed dense clustering of all populations into a single group when using a distance matrix. Using a covariance matrix, values were distributed less dense, but still quite evenly in one single cluster. The first two axes explained 19,07% of the variation in a distance matrix and 16,32% in the covariance matrix, respectively (data not shown). Results of the AMOVA showed much higher percentages of molecular variance within populations (93%) than among populations within sampling areas (2%) or among the three sampling areas (5%) (Figure 15, table 9, supplementary information). Similar results were gathered through Shannon's partitioned Diversity Index: Percentage of diversity was much higher within populations (92%) than among populations within sampling areas (4%) or among sampling areas (4%) (table 10, supplementary information). Scaled diversity according to Shannon's Index calculations is visible in Figure 16, supplementary information.

3.2.2 Chloroplast loci (cpSSRs)

In total, 22 alleles were counted among all four cpSSR loci, with values ranging between 4 and 7 per locus (data not shown). Mean resulting numbers of different and effective alleles were with few exceptions higher for juvenile than for adult samples of all sampling plots (Table 11, supplementary information). Mean unbiased diversity values over all loci (uh) ranged between 0,311 (RA2_B) and 0,658 F2_B). All sampling groups were polymorphic (P = 100%). Total numbers of intra-population analysis revealed private haplotype numbers ranging between 0 and three for all populations, except for the juvenile sampling group of RA1 (A_P = 17, data not shown), mirrored in the mean numbers of juvenile private haplotypes (A_P) and juvenile haplotypic richness (R_h), which are higher than the equivalent values of the adult samples (Table 12). Mean values of genetic diversity (H_e) as well as total values of gene diversity within each population (H_s, table 13) and total gene diversity (H_t) of juvenile sampling groups slightly exceeded numbers for adult sampling groups.

Table 12: Mean numbers calculated for regeneration, adult samples and both combined. N, sample size in each population; A, number of haplotypes detected in each population; A_P, number of private haplotypes; N_e, effective number of haplotypes; R_h, haplotypic richness; H_e, genetic diversity; D^2_sh, mean genetic distance between individuals.

	N	A	A_P	N_e	R_h	H_e	D^2_sh
Regeneration	45,750	17,000	6,750	9,013	14,793	0,904	4,801
Adults	21,000	9,800	3,000	5,707	3,352	0,834	8,137
Combined	32,000	13,000	3,222	7,176	3,556	0,865	6,654

Table 13: Total numbers calculated for regeneration, adult samples and both combined. H_s, gene diversity within each population; D_st, diversity due to differentiation; H_t, total gene diversity; F_st, proportion of the total genetic differentiation.

	H_s	D_st	H_t	F_st
Regeneration	0,884	0,039	0,923	0,042
Adults	0,791	0,055	0,846	0,065
Combined	0,850	0,056	0,906	0,062

However, rates of the total genetic differentiation (F_st) and diversity due to differentiation were lower within all combined juvenile sampling groups (D_st) (Figure 17, supplementary information). Combined for each sampling area, Reiteralm displayed the highest Fst values (data not shown). The Principal Coordinate Analysis (PCoA) revealed clustering of one of the juvenile sampling groups on Reiteralm (RA1_V) mostly separate from all other samples, explained by 54,01% of variation via distance matrix and 44,47% variance via covariance matrix on the first two axes (data not shown). AMOVA calculated higher molecular variance within populations (82%), than among populations within sampling areas (17%), or among sampling areas (1%) (Figure 18 and table 14, supplementary information).

3.3 Parentage analysis

Parentage analysis with CERVUS identified 18 candidate mothers, 15 candidate fathers and 2 parent pairs at high stringency (Table 15, supplementary information). The two parent pairs displayed identical parental candidates, therefore they can be considered as being selfed. All detected offspring-parent-relationships were found within sampling plots with no overlapping. Relaxed confidence interval results revealed 61 candidate mothers, 58 candidate fathers and 3 parent pairs with unknown sexes (data not shown).
Parentage analyses within COLONY provided roughly similar results as compared to analysis by CERVUS: 29 candidate mothers and 9 candidate fathers. However, inferred parentage assignments occurred with single overlaps between the sampling areas Reiteralm and Funtensee, which could suggest evidence of genetic exchange between those populations (Table 16, supplementary information). The program also identified 48 cases of full sibship and 383 cases of half sibship among all juvenile samples (Figure 19, supplementary information). However, these high numbers should be interpreted with caution, since the program's power is impaired to distinguish relationships when sample sizes are small and marker information is limited (Wang 2018). Calculated inbreeding estimates were quite high for the selfing parameter of the FL_PLS method (0,0820) as shown in table 17 (supplementary information).
The four solitary adult trees sampled outside of the sampling plots at RA1 and RA2 did not display increased assignments during parentage analysis.

4. DISCUSSION

4.1 Comparison between cpSSRs and nSSRs

Fragment analysis results of the 4 chloroplast loci used in this study were in line with similar research carried out in the Alps, which also reported high levels of intra-populational variability and low differentiation values among populations (Höhn et al. 2005, Krutovskii et al. 1995, Höhn et al. 2009). The value of 17% inter-population variation gained from our results is close to the average in paternally inherited markers for conifers (16,5%) (Lendvay et al. 2014). This genetic pattern, seemingly evident also in the Carpathian distribution patches (Höhn et al. 2009), can be explained by the low numbers of generations that have passed since the species' expansion in the postglacial period due to long generation times (Gugerli et al. 2009). The occurrence of private haplotypes as well as the low F_st values (0,062 for all populations combined) indicate that the degree of isolation between the three populations in the National Park is not significantly high. This interpretation is corroborated by Gugerli

et al. (2009) and Lendvay et al. (2014), who mentioned the general capacity of conifers for substantial gene flow among populations through pollen as a possible explanation for low values of inter-population variation. Furthermore, high genetic diversity despite enhanced fragmentation and small population sizes within stone pine populations has been documented for chloroplast DNA before (Dzialuk et al. 2014, Höhn et al. 2009). Analysis results of the 12 nSSRs on this very local scale showed lower genetic variation among populations (2%) than the 11,6% average for biparentally inherited markers normally reported for conifers, but similarly low levels among and high levels within populations have been reported before for analyses with codominant loci in *Pinus cembra* (Lendvay et al. 2014, Tóth et al. 2019). The low overall F_{ST} value (total of 0,044 combined for adult and juvenile samples) and the mean number of 7,68 effective migrants across loci imply the existence of at least some nuclear genetic exchange between populations.
The haploid chloroplast genes behave as single haploid loci that do not undergo recombination and have lower effective population sizes compared to diploid genes (Lendvay et al. 2014). Therefore, genetic differentiation is expected to be higher for chloroplast than for nuclear genes, while genetic diversity rates should theoretically be lower in the chloroplast genome (Lendvay et al. 2014). Results of the underlying study however showed higher genetic differentiation as well as higher total gene diversity for chloroplast genotypes. The same phenomenon has been reported by Lendvay et al. (2014) and Powell et al. (1995). It might suggest a development directed to restricted gene flow. Another explanation could be dispersal distances of pollen exceeding those of the seeds (Gugerli et al. 2009), but with the Nutcracker as active dispersal vector, this seems highly unlikely. However, highly directional transport by the bird may obscure patterns.

4.2 Comparison between adult and juvenile genotypes

Especially the analysis results of the 12 nuclear microsatellite markers showed some differences between juvenile and adult genetic performance of the samples. Allelic richness, expressed in the number of effective alleles as well as in the rate of polymorphism was higher for juveniles, while genetic differentiation rates were overall lower than within adult samples. Values of observed heterozygosity did not show significant signs of decrease or increase. Since genetic diversity did not change significantly from parent to filial generation, the analyzed populations do not seem to have experienced input by genetically distant immigrating sources (Salzer 2011). Inbreeding tendencies (F_{IS}) were slightly more pronounced within juvenile samples. Apparent inbreeding (mostly by selfing) was also found by both parentage analysis programs. The high number of significant mother-offspring assignments found during parentage analysis in spite of the proportionally small area sampled in total, supports the assumption that the Spotted Nutcracker caches seeds close to the maternal source trees if seeds are abundantly available (Salzer 2011). The doctoral thesis by Salzer (2011) reported that seeds put into one distinct cache by an individual bird seem to be collected mostly from one single tree (with exceptions), indicating that seeds are cached within a spatially restricted area by the nutcracker, while numbers of pollen donors for one tree are presumably higher than one. The resulting relatedness rate of trees in spatial proximity could explain enhanced inbreeding among related individuals in the populations examined in the present study.
Regarding differences between the three populations in the National Park, the Funtensee population seems to be the most vital. Chloroplast analysis reveals the highest value of total genetic heterozygosity for the juvenile samples of this area, and the lowest proportion of genetic differentiation of offspring among all sampling plots.
The population on the Reiteralm plateau stands out in several regards. It displayed the lowest results concerning numbers of private alleles and rates of polymorphic loci, as well as the highest genetic differentiation within the nSSR analysis. Chloroplast microsatellites also showed the highest F_st values out of the three populations (values of all sampling plots per population combined), and the lowest mean diversity value was detected for one of the sampling plots there. The juvenile sampling group of RA1 showed by far the highest number of private haplotypes (17) as compared to all other sampling groups (0-3), which was mirrored in the more or less distinct cluster of RA1_V in the principal coordinate analysis (PcOA). According to those findings, genetic isolation of the Reiteralm population seems to be more pronounced, and tendencies of genetic drift from one generation to the next are visible. Less obtrusive results for sampling plot RA2 might be based on better pollen dispersal due to suitable topographic features (hilltop) (Chybicki and Dzialuk 2014). In addition, RA1 displays lower adult tree density in and closely around the sampling plot (extrapolated 66,21 trees per hectare) as compared to RA2

(152,79 trees/ha). Lower mate availability might facilitate the occurrence of genetic drift and non-random mating, just as the probability for self-fertilization might increase with decreasing numbers of neighbouring individuals (Salzer 2011, Lewandowski and Burczyk 2000). A negative association between pollen availability and population size was already indicated by Salzer and Gugerli (2012) for small compared to core populations in the Swiss Alps, whereas Chybicki and Dzialuk (2014) considered long-distance pollen dispersal due to low stand density favoring gene flow between non-related individuals more likely.

The sampling plot at Sittersbach High Valley must be regarded separately from the plots in the Funtensee and Reiteralm area since there was no regeneration and adult trees occurred at very low abundance only. Dead wood proportion within the sampling plot took up to 25%. Köstler and Mayer already reported high amounts of dead wood compared to low numbers of living individuals, a preponderance of strong diameters, trends to obsolescence and visible recent karstification dynamics in 1970, indicating low vitality of the local stands. It seems like this situation is still lasting to date, at least at elevations close to the timberline (personal observations). In addition, calls of the Spotted Nutcracker were less frequent and no seed forgeries or emptied cones were found at or close to the site (personal observations). According to (Mattes 1982), numbers of breeding pairs and, thus, dispersal activity might be lower in areas with low tree density as a result of limited food availability. Worse parental seed production rates, low germination success and high embryo abortion levels are probable consequences of inbreeding depression within peripheral populations of *Pinus cembra* (Salzer 2011, Salzer and Gugerli 2012, Politov et al. 2008, Höhn et al. 2005, Krutovskii et al. 1995). Hence, genetic erosion tendencies are strongly implied by the current state of the Klausbach Valley population. Displaying the smallest accumulated area and the highest degree of geographic fragmentation among the three populations embedded in the National Park, it seems likely that this population has experienced the highest degree of genetic fragmentation and, thus, suffers from decreased population fitness. Chloroplast analysis of the existing small number of samples shows the highest degree of differentiation (0,194) and the lowest value of total gene diversity (0,017) among all sampling groups. According to Dzialuk et al. (2014) and Mosca et al. (2016), this perfectly mirrors what is to be expected from fragmented populations with affected genetic processes and inhibited gene flow. However, it is difficult to prove this assumption without filial samples or a higher number of sampled adult trees. Otherwise, an accumulation of poor seed years combined with unfavorable climatic conditions (north-west exposure, medium amounts of precipitation combined with higher mean annual temperature compared to Reiteralm and Funtensee, lowest elevation among all sampling plots) and strong competition by *Pinus mugo ssp.* (50-75% abundancy within the sampling plot) might have contributed to the current state of deterioration of the *Pinus cembra* stands at Sittersbach High Valley. The population is distributed in patches along the whole Klausbach Valley, so local observations might not be valid for all of those patches. However, inventory data of 2010-2012 indicates a similar situation throughout the whole population, given that regeneration was once only found at one of the inventory plots at very low quantity (Bernd Becker, project leader of the forest inventory conducted in the National Park 2010-2012, personal communication, July 2018).

5. CONCLUSION

Due to the limited sampling numbers used for analysis in this study, conclusions must be taken with caution.

Overall, no trend of homozygote excess was visible in local *Pinus cembra* stands from the parent to the filial generation, but inbreeding trends are evident to varying degrees in the populations (i). Apparently, there was no input from a genetically distant source in recent times. Gene flow, indicated by higher results for gene flow parameters of the juvenile samples and overall higher genetic differentiation rates within the sampled adults, seems to occur on the intra-populational scale mostly (ii). Results are indicating that the three *Pinus cembra* populations growing in the National Park have been separated for a long time, and that individual population sizes and limited connectedness among them have led to the recent extent of genetic fragmentation in each of the populations. They display different genetic patterns and levels of fitness, expressed also by inconsistent success of regeneration (iii): Among all sampling plots, the Funtensee population on average shows the highest numbers of regeneration. Genetic analysis suggested vital rates of genetic heterozygosity and differentiation. The Reiteralm population shows more pronounced patterns of genetic isolation and tendencies of genetic drift

from adult to juvenile generation. However, success of local regeneration seems to be secured. The smallest and most fragmented population along the Klausbach Valley does not exhibit signs of successful regeneration and displays the lowest adult tree density. Genetic analysis of the small rate of sampled individuals located there implies inhibited gene flow. These stands might be pressured to the limit of their viability with the increase of climatic extremes, since they are already showing a strongly skewed age distribution and very poor fitness performance, especially at the upper distribution boundaries in the Valley. However, due to the species' long generation times and regularly occurring mast years, it is likely that even small amounts of unrecovered seeds, that successfully establish themselves and survive the first crucial years, could maintain Swiss stone pine's viability on the long term (Neuschulz et al. 2015, Närmann et al. 2017). The combination of germination with favorable biotic and abiotic conditions may result in successful waves of establishment, as it has been reported before (Welzmüller and Ewald 2016, Köstler and Mayer 1970). The long generation turnover may, next to *Pinus cembra*'s advantageous combination of wind-borne pollen and subsequent avian seed dispersal, also contribute to diminish the effects of spatial isolation and may prevent further genetic isolation (Höhn et al. 2009, Geburek and Myking 2018, Höhn et al. 2005, Salzer 2011, Kramer et al. 2008). In this regard, the important role of the Spotted Nutcracker as primary seed dispersal vector is unequivocal (Salzer 2011, Farjon et al. 2017, Boden et al. 2010). Especially for such isolated populations with small effective population sizes, the nutcracker's contribution to the stone pine's dispersal is assumed to have great influence on genetic structures of the populations (Salzer 2011). Although findings suggest, that the bird's profit of the mutualism is bigger than the tree's, the amount of seeds that establish themselves in fitting microhabitats seems to be high enough to ensure regeneration (Närmann et al. 2017). Unemptied seed caches may accelerate *Pinus cembra*'s establishment beyond its current distribution range and up to higher elevations, which has already been reported (Salzer 2011, Heinze and Holzer 2013). The movement of seeds by nutcrackers over long distances may enhance gene flow also among patchily distributed populations like the one in the Klausbach Valley, and lead to higher and more widespread levels of genetic multiplicity, random spatial genetic structures and a more effective preservation of the gene pool (Klumpp and Stefsky 2004, Salzer 2011). However, Gregory et al. (2009) reported impacts of climate change between the years of 1980 and 2005 to have caused shrinking distribution ranges of *Nucifraga caryocatactes.* The bird was one out of ten species whose habitat decreased the most during this period of time. This could potentially suggest a further decrease of dispersal and, thus, regeneration rates of Swiss stone pine.

During a study concerning mountain forest growth response to climate change carried out in the Calcareous Alps of the Berchtesgaden area in 2014 (Hartl-Meier et al.), the authors found the mountain forests within the National Park to be quite resistant against the impacts of climate change up to that point. However, species that exist in fragmented and small populations in high-elevation habitats and display low fecundity rates or late age of sexual maturity will be prone to greater adaptational lags (Aitken et al. 2008). Hence, the actual potential of the local *Pinus cembra* stands to adapt to the ongoing changes may first manifest itself several generations later. Since active conservation management is not intended by the fundamental objectives of core zones in National Parks, survival of the three local Swiss stone pine populations will depend on their ability to persist in the present distribution ranges and their upslope regeneration potential on the long run (Casalegno et al. 2010). The colonization of new areas available due to the abandonment of pastures may partly counterbalance the grounds lost to climate change at lower elevations, given that increasing numbers of stone pine seedlings have successfully established themselves on alpine grasslands since the 1980s (Kudernatsch et al. 2016, Mosca et al. 2016). Local extent and manner of the individual populations' reactions to enhanced climate warming with all its side effects will not emerge clearly until the course of actual occurrence.

Snapshots of current genetic population patterns do not necessarily indicate a population's ability to retain genetic fitness in the long run. Little gene flow (~1 individual/generation) can be sufficient to reduce consequences of genetic drift (Young et al. 1996). Hence, further observations by monitoring over long time spans and repeated sampling would be necessary to draw wider conclusions. Especially protected areas with undisturbed life cycles, like the Berchtesgaden National Park, can provide valuable insights into *Pinus cembra*'s genetical and ecological resilience to upcoming threats (Aitken et al. 2008), and the knowledge gained here could provide useful information for other populations of Swiss stone pine growing under similar conditions and degrees of fragmentation.

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SUPPLEMENTARY INFORMATION

Table 1: Ratio of DNA-dilution with H_2O, depending on DNA concentration contained in each sample.

DNA - concentration	Ratio	Dilution
< 50ng μL^{-1}	1 : 1	25µL H_2O + 25 µL DNA
> 50ng μL^{-1} and < 200ng μL^{-1}	1 : 10	25µL H_2O + 2,5µL DNA
> 200ng μL^{-1}	1 : 50	25µL H_2O + 0,5µL DNA

Table 2: Different primers used for amplification: Group 1 to 4 for nuclear microsatellite loci, group 5 for chloroplast loci. Details concerning location of primers, primer names, repeats, colourants for dyeing, different annealing temperatures, expected lengths of fragments, loci, original primer sources and colours of size fragment chromatograms.

	Location	Primername	Repeat	Colourant	Annealing temperature	Expected length	Locus	Source	Colour
Group 1	nSSR	Pc-HJM	$[TC]_{10}$	Cy5	58	168-188	KF692109	Newly designed by Lendvay et al. 2014	blue
	nSSR	Pc-BUG	$[GA]_{13}$	Cy5		101-133	KF692108		blue
	nSSR	Pc-YAU	$[TCA]_7$	IRD700		144-162	KF692110		green
Group 2	nSSR	Pc-28Z	$[CA]_9G[AC]_5$	Cy5	58	288-324	KF692111		blue
	nSSR	Pc-CQG	$[AC]_8[AT]_5$	IRD700		169-179	KF692107		green
	nSSR	Pc-SWK	$[AT]_{10}$	Dy751		236-318	KF692112		black
Group 3	nSSR	Pc-1b	$[GT]_{19}$	IRD700	55	166-202	EU629205	Salzer et al. 2009	green
	nSSR	Pc-7	$[CT]_6[AT]_4[GT]_{25}$	Cy5		342-384	EU629210		blue
	nSSR	Pc-18	$[TG]_{12}$	Dy751		152-156	EU629204		black
Group 4	nSSR	Pc-22	$[CA]_7TA[CA]_{12}$ $[TA]_2$	IRD700	55	345-375	EU629203		green
	nSSR	Pc-23	$[TG]_6CG[TG]_2$	Cy5		221-257	EU629206		blue
	nSSR	Pc-35	$[GTT]_7$	Dy751		162-165	EU629209		black
Group 5	cpSSR	Pt63718	$[T]_9$	IRD700	58	90-95	Pinus taeda	Vendramin et al. 1996	green
	cpSSR	Pt15169	$[C]_8[T]_8A[T]_8$	Cy5		113-118	Pinus taeda		blue
	cpSSR	Pt36480	$[T]_{11}$	Cy5		143-147	Pinus taeda		blue
	cpSSR	Pt26081	$[T]_{14}$	Dy751		109-112	Pinus taeda		black

Table 3: Thermal cycling programs for polymerase chain reaction (PCR).

Primer groups	Step 1: Initial incubation	Step 2: Melt /Denaturation	Step 3: Annealing	Step 4: Extend	Step 5:	Step 6: Final extension	Step 7: Final step
1, 2, 5	Hold at 95°C for 15 minutes	30 cycles at 94°C for 30 seconds	Hold at **58°C** for 90 seconds	Hold at 72°C for 60 seconds	Go to step 2), 34 cycles	Hold at 60°C for 30 minutes	Hold at 4°C for infinity
3, 4	Hold at 95°C for 15 minutes	30 cycles at 94°C for 30 seconds	Hold at **55°C** for 90 seconds	Hold at 72°C for 60 seconds	Go to step 2), 34 cycles	Hold at 60°C for 30 minutes	Hold at 4°C for infinity

Table 4: Numbers of sampled individuals (adult trees and regeneration) per sampling plot, max. DBH (diameter at breast height), max. height of adult trees, extrapolated number of adult trees/regeneration individuals per hectare. Sampling radius for adult trees: 25m → 1963,5m^2 sampling area per plot. Sampling radius for regeneration: 20m → 1256,6m^2 sampling area per plot.

Sampling area	Sampling Plot	Age Group	Code	No. of Individuals sampled	Max. DBH [cm]	Max. Height [m]	Extrapolated No. of Individuals /[ha]
Sittersbach High Valley	ST	Adult	ST_B	6	105 (ST_B05)	13,6 (ST_B05)	30,56
Funtensee	F1	Adult	F1_B	33	105 (F1_B09)	18,6 (F1_B12)	168,07
	F1	Regeneration	F1_Vj	40	-	-	318,32
	F2	Adult	F2_B	24	78 (F2_B20)	16,4 (F2_B23)	122,23
	F2	Regeneration	F2_Vj	59	-	-	469,52
Reiteralm	RA1	Adult	RA1_B	13	81 (RA1_B13)	20,2 (RA1_B13)	66,21
	RA1	Regeneration	RA1_Vj	45	-	-	358,11
	RA2	Adult	RA2_B	30	94 (RA2_B25)	15,8 (RA2_B10)	152,79
	RA2	Regeneration	RA2_Vj	39	-	-	310,36
$\sum$				289			

Figure 4: Sampling plot RA1 with all sampled adult trees and offspring individuals. Adult trees RA1_B13 and RA1_B10 are the two solitary tree samples outside of the 25meter sampling radius.

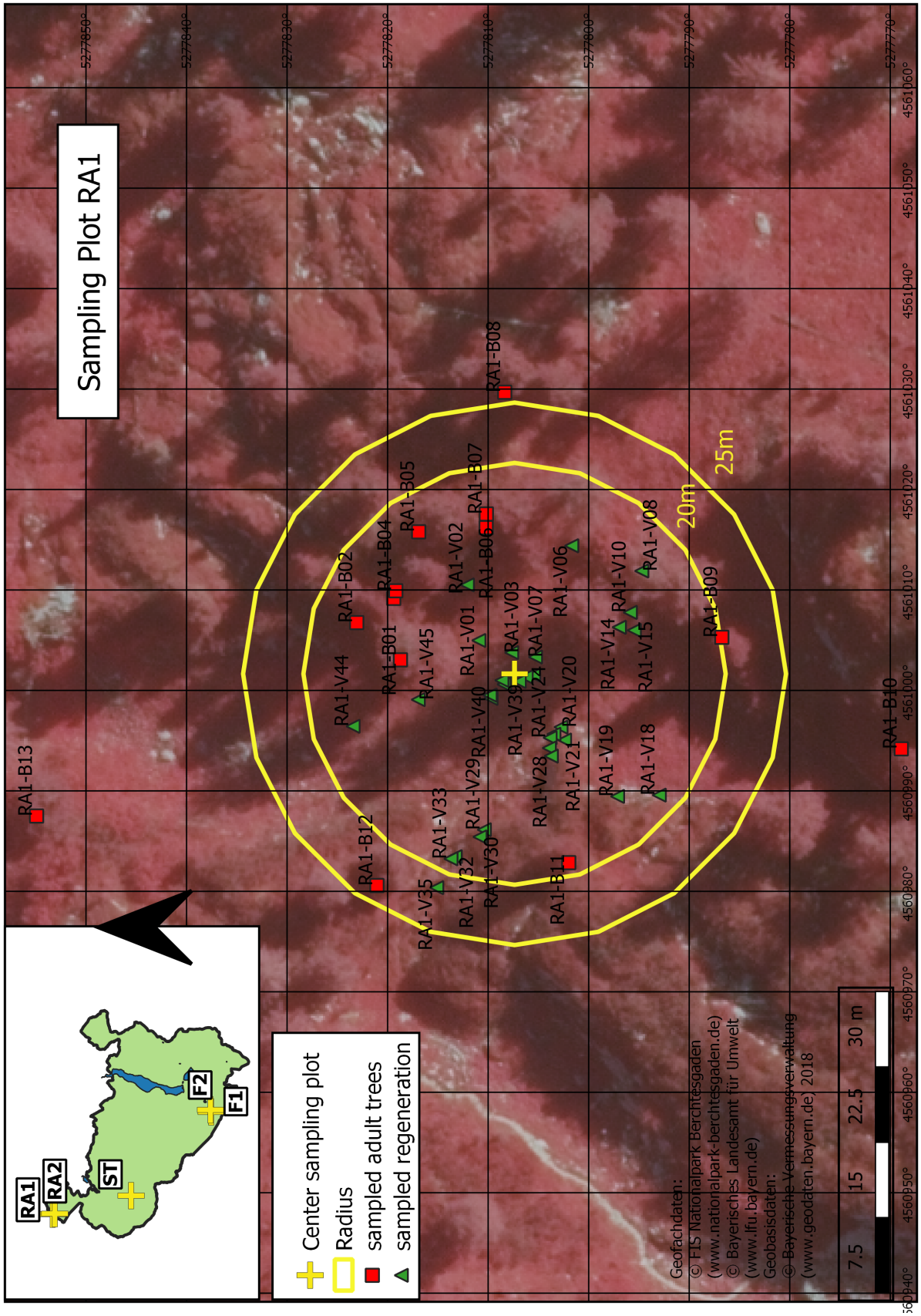


Figure 5: Sampling plot RA2 with all sampled adult trees and offspring individuals. Adult trees RA2_B30 and RA2_B31 are the two solitary tree samples outside of the 25meter sampling radius.

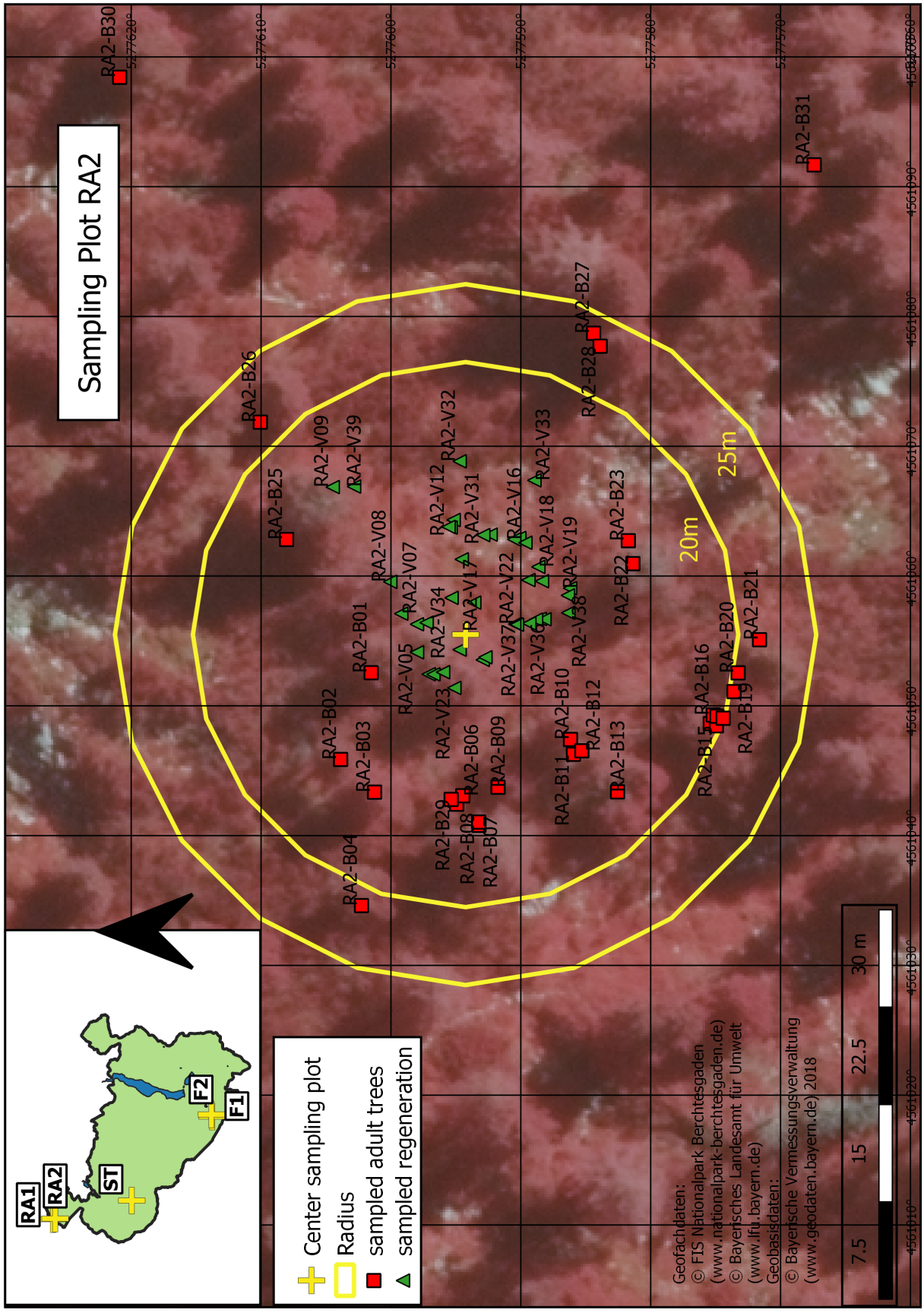


Figure 6: Sampling plot ST with all sampled adult trees and offspring individuals.

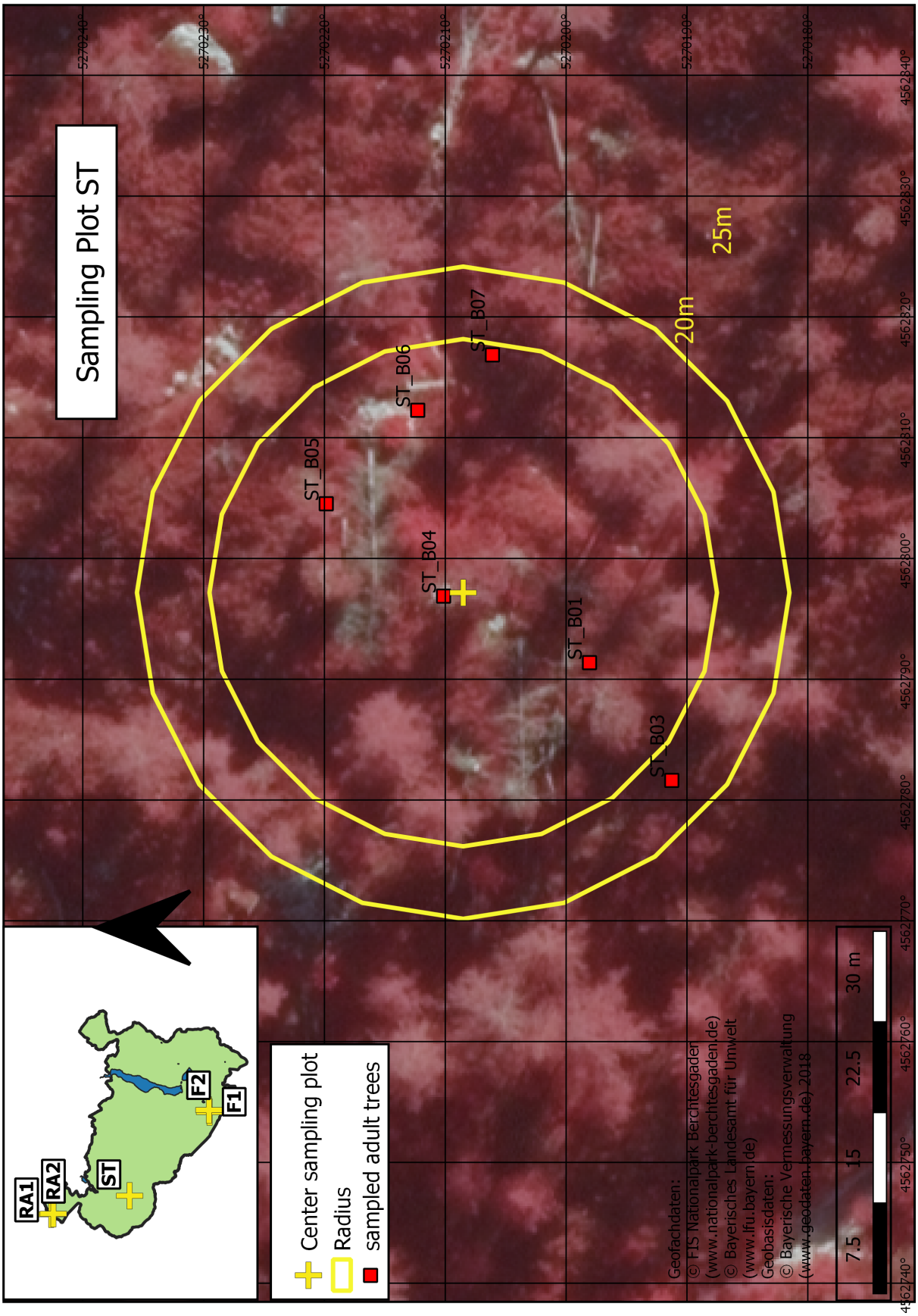


Figure 7: Sampling plot F2 with all sampled adult trees and offspring individuals.

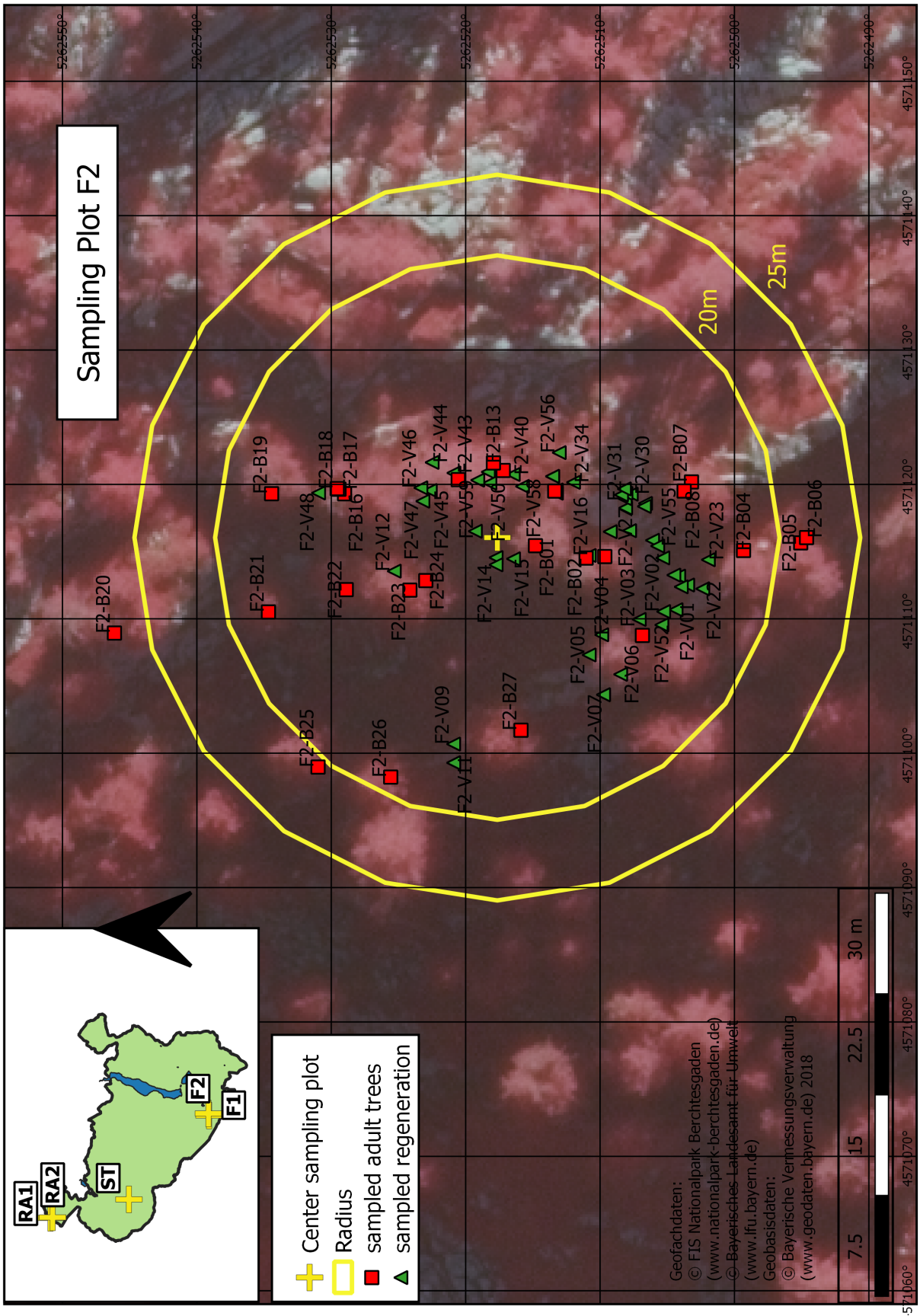


Figure 8: Sampling plot F1 with all sampled adult trees and offspring individuals.

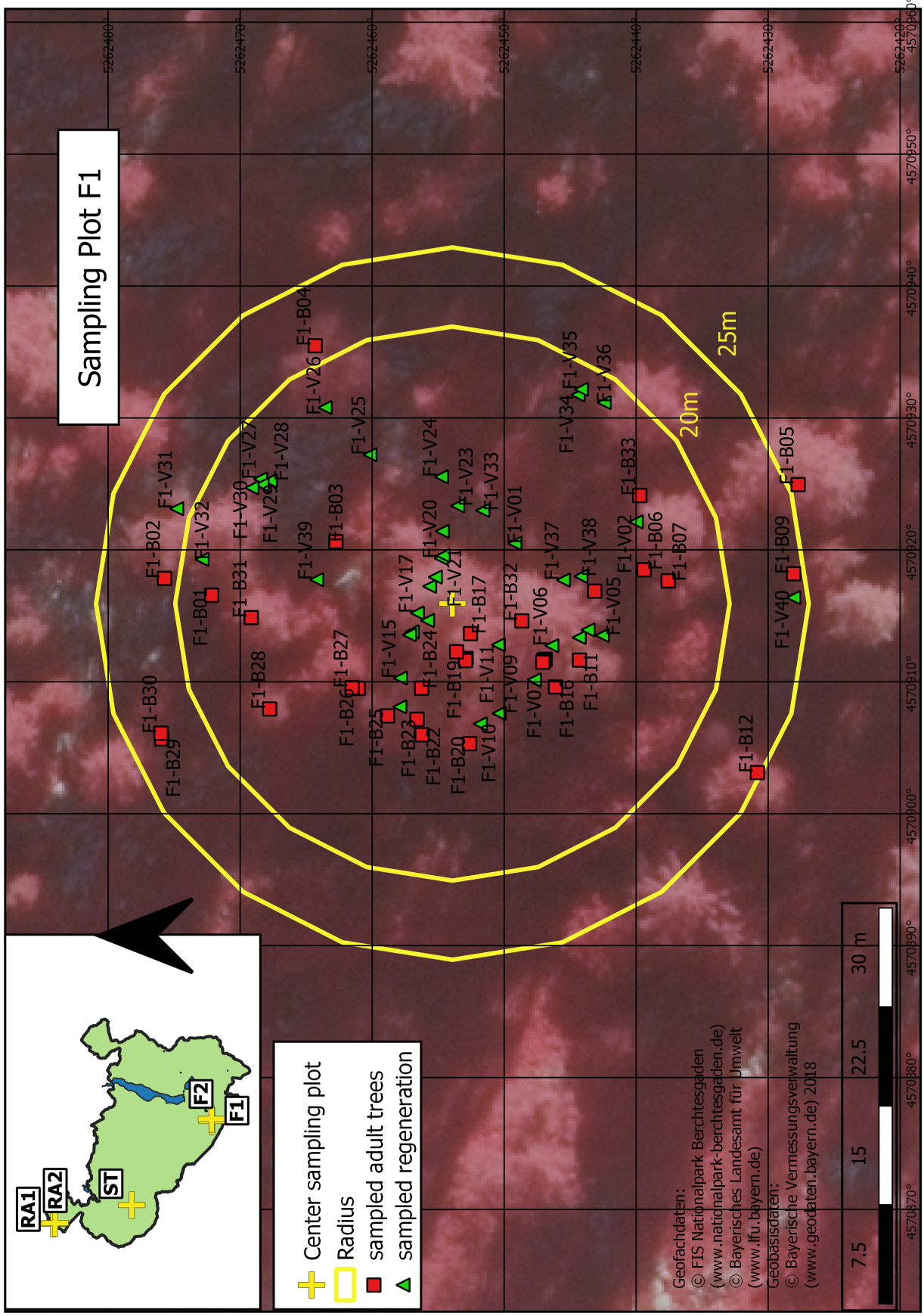


Table 5: Parameters of the different populations/sampling plots. Sources: Personal observations/measurements; Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen (2001); Freiberg 1980; Koch and Walentowski 2012; Mayer and Erschbamer 2012; Climatic data provided by the Berchtesgaden National Park.

	Reiteralm	Funtensee	Sittersbach High Valley
Elevation (measured at the center of each sampling plot)	RA1: ~ 1637m RA2: ~ 1697m	F1: ~1752m F2: ~1864m	ST: ~1557m
Slope angle	RA1: ~ 19° RA2: mostly ~0°, southern edge ~11°	F1: ~ 30° F2: ~ 36°	~9°
Exposure	RA1: (North-)East, middle slope RA2: Hilltop (south)	South (-West) F1: Middle slope F2: Upper slope	North-west Middle slope
Mean annual temperature	2-3°C	1-2°C	~ 5 °C
Mean annual precipitation	~1958mm	2000-2500mm	~ 2100mm
Bedrock	Dachsteinkalk	Dachsteinkalk, small areas of pink-colored Jurassic bedrock	Dachsteinkalk, Hangschutt, (Fleckenmergel)
Soil type	Dystrophic Tangelrendzina, bedrock and patchy Syrosems	Dystrophic Tangelrendzinen, few Kalkverwitterungslehme, Polster- and Protorendzina, bare bedrock	Dystrophic Tangelrendzina, bedrock and patchy Syrosems
Thickness of organic soil cover	Medium-heavy (~30cm)	Heavy, 30-50 cm	Medium-heavy (~30cm)
Degree of soil coverage [%]	100%	80%, otherwise bare rock	100%
Vegetation	RA1/RA2: *Vaccinio-Pinetum cembrae / Rhododendretosum hirsuti – Pinetum cembrae* RA1: patches of all herb fringe communities	F1/F2: *Vaccinio-Pinetum cembrae / Rhododendretosum hirsuti – Pinetum cembrae, Erico-Rhododendretum hirsuti* F1: *Seslerio-Cericetum sempervirentis*	*Vaccinio-Pinetum cembrae, Erico-Rhododendretum hirsuti*
Dead wood on sampling plots [%]	RA1: < 1% RA2: < 5%	F1: < 5% F2: < 5%	5 – 25%
Damaged offspring	RA1: ~ 18% (mostly pathogens, some mechanical damage) RA2: ~15% (some mechanical damage, some browsing/rubbing damage)	F1: ~ 10% (mostly pathogens) F2: ~ 14% (mostly mechanical damage, some pathogens)	-
Activity of the Spotted/European nutcracker (Nucifraga *caryocatactes*)	Calls: Frequent throughout the whole area (max. silent period: 15mins) Sightings during flight: Yes Sightings sitting on trees: Yes Seed forgeries: Yes Emptied cones: Yes	Calls: Frequent throughout the whole area (max. silent period: 15mins) Sightings during flight: Yes Sightings sitting on trees: Yes Seed forgeries: Yes Emptied cones: Yes (see Figure 4a)	Calls: Yes, approx. 1/hour Sightings during flight: Yes Sightings of sitting on trees: Yes Seed forgeries: No Emptied cones: No

Figure 10: Plot of mean likelihood L(K) and variance per *K* value with *K* = 6 showing highest likelihood and lowest variance interval (circled in red).

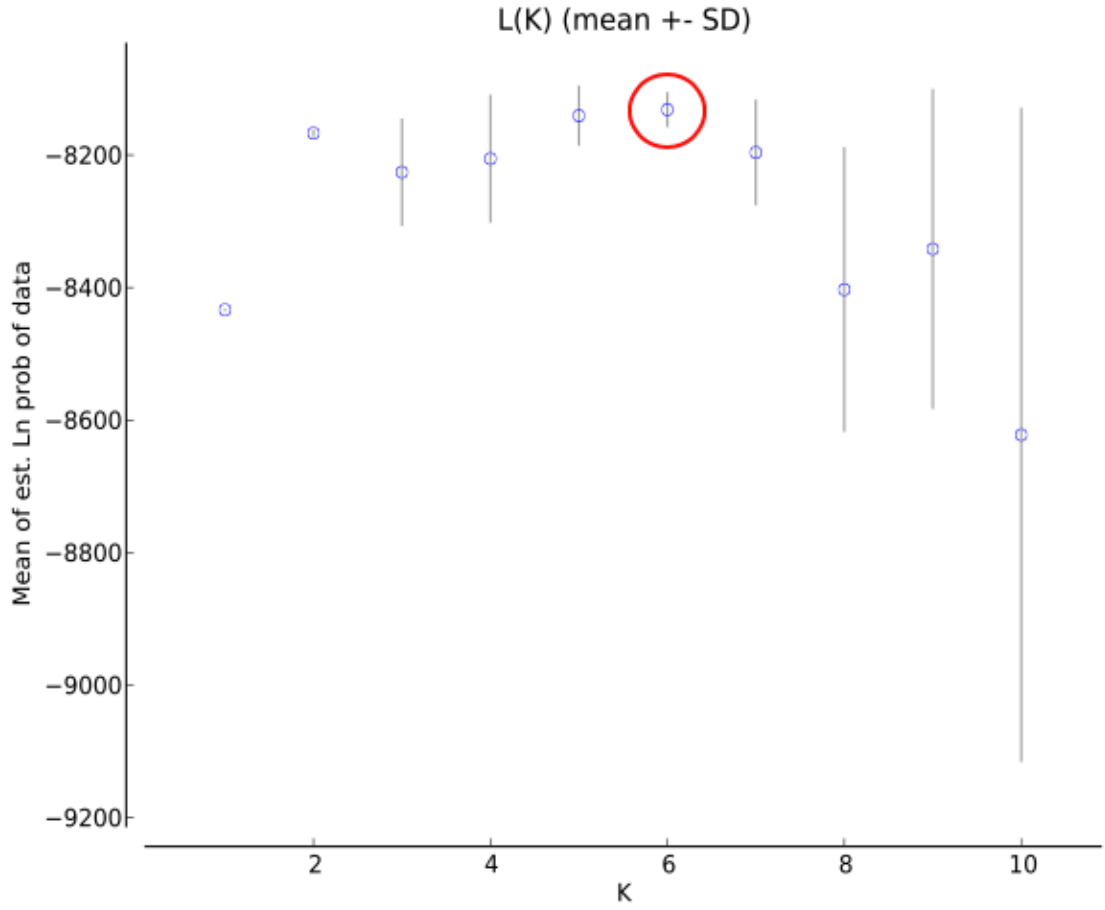


Figure 12: STRUCTURE summary plot of estimates of *Q* (= estimated membership coefficients for each individual, in each cluster) showing allele frequency distribution for *K* = 6 ancestral genetic clusters. The differences between the sampling areas (ST = Sittersbach, F = Funtensee, RA = Reiteralm) and even the sampling plots (except for RA1 and RA2) are easily recognizable.

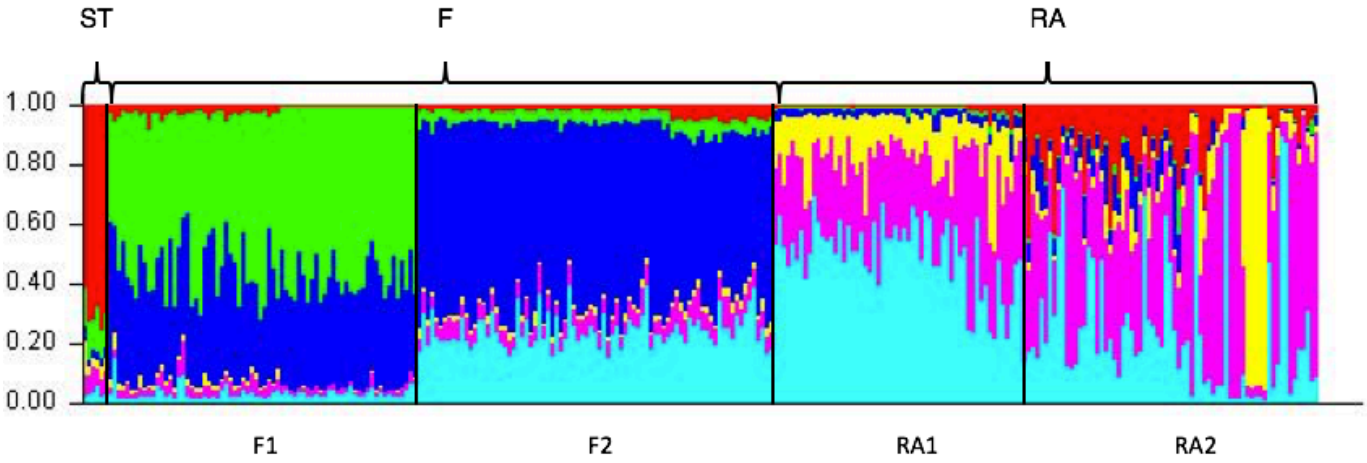


Figure 13: STRUCTURE multiple lines bar plot of *Q* for *K* = 6. Each vertical line represents one sampled individual. The lines are partitioned into *K* = 6 coloured segments that represent that individual's estimated membership fraction in each of the 6 inferred ancestral clusters. Sampling plots and adults/regeneration are subdivided by black lines and labelled underneath.

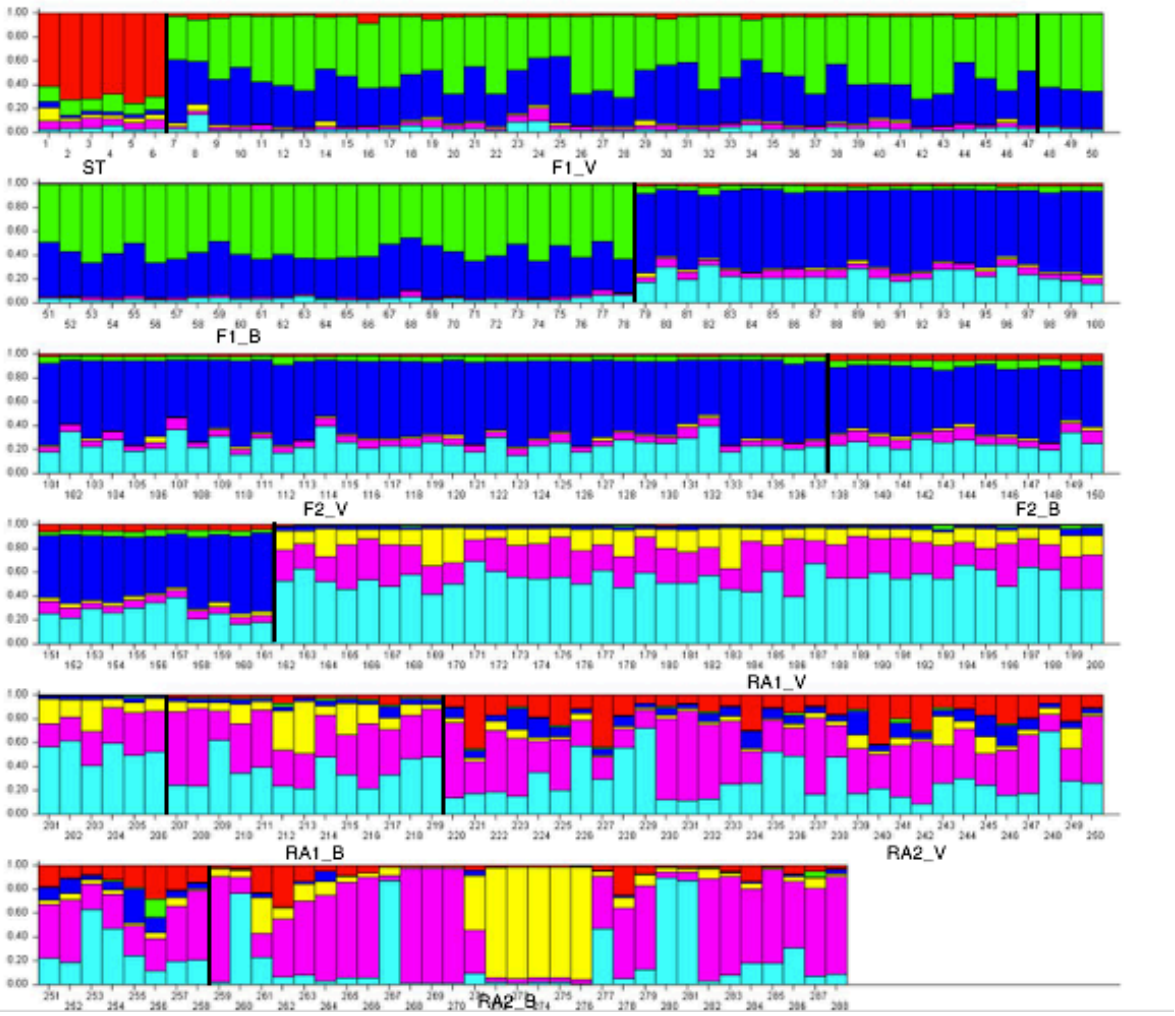
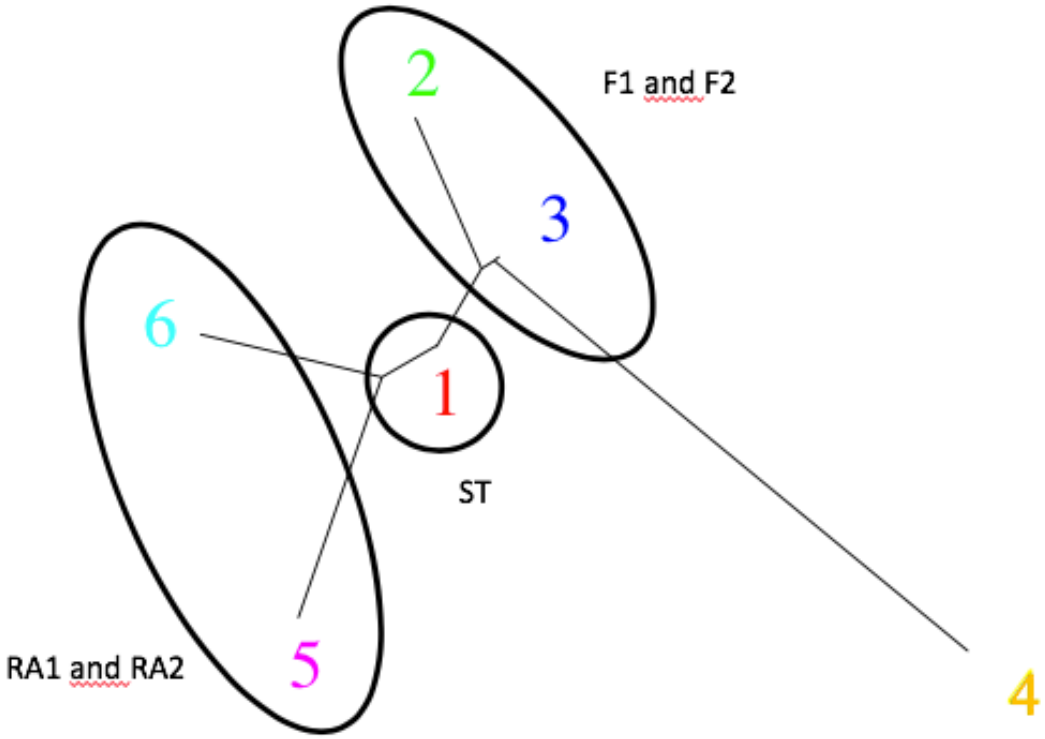


Figure 14: Representation of genetic distances of the 6 genetic clusters gathered from STRUCTURE shown in a tree plot. Circles indicate grouping of populations: cluster 1 dominating the Sittersbach High Valley samples, clusters 2 and 3 being found mostly in the samples gathered at the Funtensee area (plots F1 and F2) and clusters 5 and 6 mostly evident in the Reiteralm samples (RA1 and RA2).



a)

Adult Generation													
Sampling Area	Sampling Plot	N	N_A	SE	N_E	SE	H_O	SE	H_E	SE	P [%]	A_P	SE
Funtensee	F1_B	32	4,833	0,815	2,793	0,436	0,622	0,087	0,548	0,065	100,00%	0,083	0,083
	F2_B	24	5,167	0,952	2,97	0,431	0,656	0,083	0,581	0,06	100,00%	0,167	0,167
Reiteralm	RA1_B	13	4,25	0,789	2,744	0,484	0,521	0,079	0,525	0,068	91,67%	0	0
	RA2_B	30	4,833	0,842	2,652	0,417	0,531	0,082	0,537	0,057	100,00%	0,167	0,112
Sittersbach	ST_B	6	3,667	0,607	2,865	0,453	0,708	0,109	0,55	0,069	91,67%	0,167	0,112
		105											

b)

Juvenile Generation													
Sampling Area	Sampling Plot	N	N_A	SE	N_E	SE	H_O	SE	H_E	SE	P [%]	A_P	SE
Funtensee	F1_V	40	5,5	1,011	2,9	0,374	0,64	0,085	0,579	0,06	100,00%	0,25	0,131
	F2_V	59	6	1,087	3,015	0,46	0,622	0,079	0,588	0,057	100,00%	0	0
Reiteralm	RA1_V	45	4,917	0,883	2,935	0,461	0,587	0,077	0,571	0,059	100,00%	0,083	0,083
	RA2_V	39	5,667	0,948	3,107	0,547	0,563	0,077	0,58	0,061	100,00%	0	0
		183											

Table 6: Means and standard errors of standard measures of genetic diversity for the a) adult and b) juvenile age group of every sampling plot over 12 nuclear microsatellite markers. N, number of genotyped individuals; N_A, number of different alleles; N_E, number of effective alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P, percentage of Polymorphic Loci; A_P, number of private alleles unique to a single population; SE, standard error.

Table 8: Sums of F- and G-statistics over all populations and loci calculated for regeneration, adults and both combined. F_{ST}, genetic differentiation among populations; F_{IS}, inbreeding coefficient within individuals; G_{ST}, analog of F_{ST}, adjusted for bias; G_{IS}, inbreeding coefficient within individuals, adjusted for bias; P, Probability;

	F_{ST} (Tot)	P (F_{ST})	F_{IS} (Tot)	P (F_{IS})	G_{ST} (Tot)	P (G_{ST})	G_{IS} (Tot)	P (G_{IS})
Regeneration	0,027	0,001	-0,041	1	0,019	0,001	-0,029	1
Adults	0,053	0,002	-0,108	0,999	0,027	0,002	-0,072	0,999
combined	0,044	0,001	-0,077	1	0,024	0,001	-0,052	1

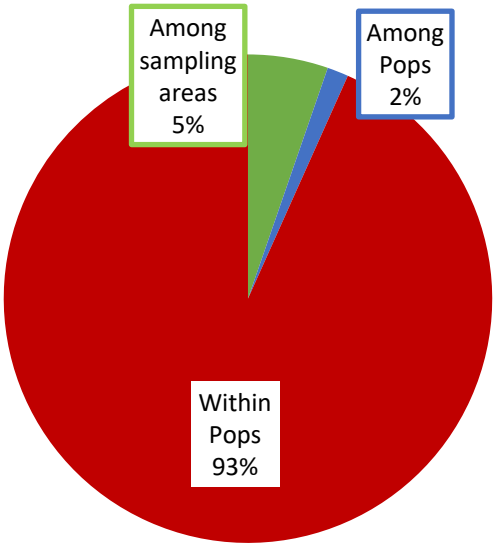


Figure 15: Analysis of molecular variance (AMOVA) within populations, among populations within sampling areas and among sampling areas. (Referring to the nuclear microsatellite data.)

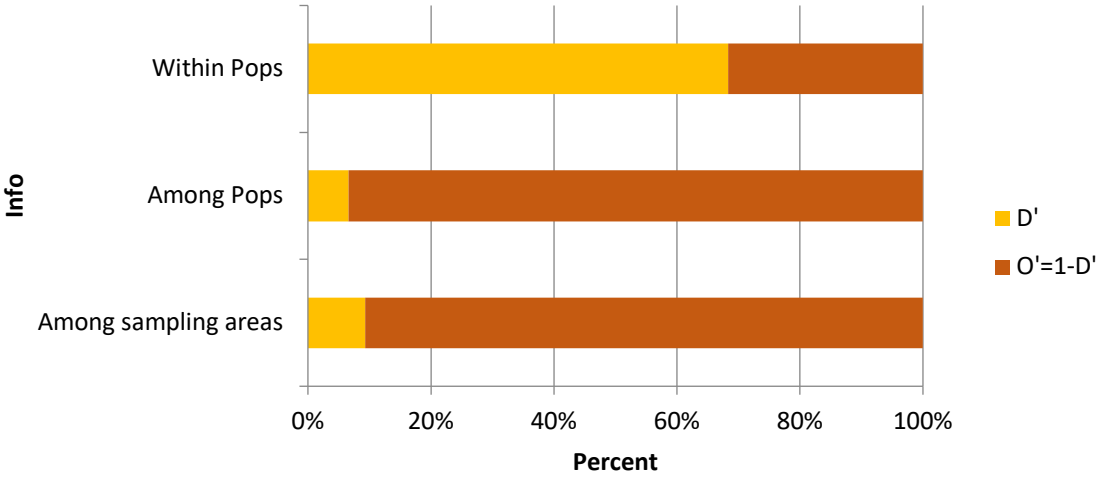
Table 9: Analysis of molecular variance (AMOVA) for nSSR, assuming a geographic population structuring based on isolation in the three sampling areas Funtensee, Sittersbach High Valley and Reiteralm. Df, degrees of freedom; SS, sum of squares; Est. Var., estimated variance; *p*, probability (based on standard permutation across the full data set).

Source of Variance	Df	SS	Est. Var.	Variation [%]	*p*
Among sampling areas	2	76,205	0,389	5%	0,001
Among populations within sampling areas	6	62,016	0,103	1%	0,002
Within populations	279	1910,557	6,848	93%	0,001

Table 10: Shannon Partition Index for diversity among sampling areas, among populations within sampling areas and within populations. Df, degrees of freedom; G-Test, log-likelihood Chi-Square; sH, Shannon Information Index; I[%], Percent of total Information; exp(sH), diversity estimates; D', [0,1] scaled diversity; O' = 1-D' scaled overlap; *p*, estimated probability was tabulated for the relevant sH based on Specialized permutation that shuffles strictly with sampling areas.

Source of Information	df	G-Test	sH	I [%]	exp(sH)	D'	O'	*p*
Among sampling areas	2	58,738	0,051	4	1,053	0,093	0,907	0,001
Among Populations	6	56,564	0,049	4	1,051	0,067	0,933	0,001
Within Populations	563	1280,076	1,120	92	3,064	0,683	0,317	1,000

Figure 16: Shannon informational scaled diversity (D' [%]) and overlap (O' [%]) within populations, among populations within sampling areas and among sampling areas.



a)

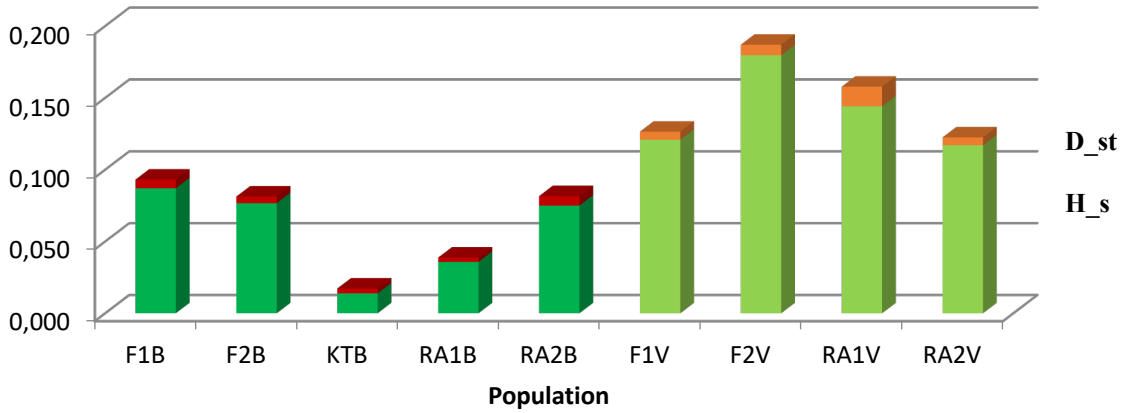
Adult Generation											
Sampling Area	Sampling Plot	N	N_A	SE	N_E	SE	h	SE	uh	SE	P [%]
Funtensee	F1_B	32	3	1	1,87	0,541	0,353	0,136	0,364	0,14	100
	F2_B	24	4	1	3,414	1,11	0,63	0,083	0,658	0,087	100
Reiteralm	RA1_B	13	3	0,707	2,057	0,554	0,396	0,149	0,429	0,161	100
	RA2_B	30	3,5	0,866	1,57	0,317	0,301	0,104	0,311	0,108	100
Sittersbach	ST_B	6	2,5	0,5	1,788	0,404	0,375	0,097	0,45	0,117	100
		105									

b)

Juvenile Generation											
Sampling Area	Sampling Plot	N	N_A	SE	N_E	SE	h	SE	uh	SE	P [%]
Funtensee	F1_V	40	3,75	1,109	2,021	0,662	0,383	0,127	0,392	0,13	100
	F2_V	59	4	0,707	2,233	0,716	0,444	0,115	0,451	0,117	100
Reiteralm	RA1_V	45	4	0,816	2,306	0,498	0,518	0,076	0,53	0,078	100
	RA2_V	39	3,5	0,866	1,949	0,74	0,318	0,15	0,326	0,154	100
		183									

Table 11: Means and standard errors of standard measures of genetic diversity for the a) adult and b) juvenile age group over 4 chloroplast microsatellite markers. N, number of genotyped individuals; N_A, number of different alleles; N_E, number of effective alleles; h, Diversity; uh, unbiased diversity; P, percentage of polymorphic loci; SE, standard error.

Figure 17: H_s, within population genetic diversity in dark green for the adult samples and light green for regeneration; D_st, amount of population genetic differentiation among the other populations in red for the adult samples and orange for the Juveniles. The sum of these two components presents the total diversity of each population (H_s) and the amount of single population genetic differentiation among the other populations (H_t = D_st + H_s).



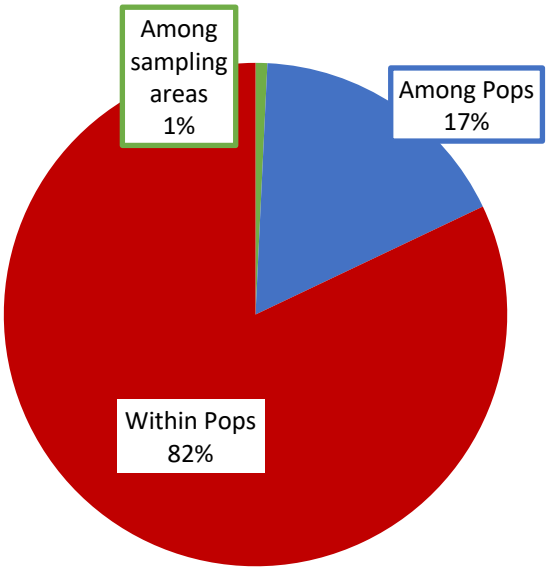


Figure 18: Analysis of molecular variance (AMOVA) for chloroplast microsatellites within populations, among populations within sampling areas and among sampling areas.

Table 14: Analysis of molecular variance (AMOVA) for chloroplast microsatellites, assuming a geographic population structuring based on isolation in the three sampling areas Funtensee, Sittersbach High Valley and Reiteralm. Df, degrees of freedom; SS, sum of squares; Est. Var., estimated variance; *p*, probability (based on standard permutation across the full data set).

Source of Variance	Df	SS	Est. Var.	Variation [%]	*p*
Among sampling areas	2	11,199	0,008	1%	0,092
Among Populations, within sampling areas	6	41,656	0,180	17%	0,001
Within Populations	279	239,996	0,860	82%	0,001

Maternity

Offspring ID	Mother ID
RA2_V32	RA2_B27
RA2_V19	RA2_B19
RA2_V18	RA2_B01
RA1_V26	RA1_B03
RA1_V22	RA1_B07
RA1_V08	RA1_B07
F2_V57	F2_B09
F2_V39	F2_B09
F2_V38	F2_B01
F2_V32	F2_B27
F2_V22	F2_B27
F1_V33	F1_B12
F1_V24	F1_B09
F1_V22	F1_B12
F1_V21	F1_B09
F1_V12	F1_B11
F1_V09	F1_B22
F1_V07	F1_B12

Paternity

Offspring ID	Father ID
RA2_V32	RA2_B27
RA2_V19	RA2_B19
RA1_V26	RA1_B03
RA1_V22	RA1_B07
RA1_V08	RA1_B07
F2_V57	F2_B09
F2_V39	F2_B09
F2_V32	F2_B27
F2_V22	F2_B27
F1_V33	F1_B12
F1_V24	F1_B09
F1_V22	F1_B12
F1_V21	F1_B09
F1_V09	F1_B22
F1_V07	F1_B12

Parent Pair

Offspring ID	First candidate ID	Second candidate ID
F2_V39	F2_B09	F2_B09
F2_V57	F2_B09	F2_B09

Table 15: Maternity, paternity and parent pair (sexes unknown) assignments of CERVUS parentage analysis at high stringency (95% confidence level).

Table 16: COLONY paternity and maternity assignments with probabilities. Overlaps between sampling areas Funtensee and Reiteralm are highlighted in green.

Paternity

Offspring ID	InferredDad1	ProbDad1	InferredDad2	ProbDad2	InferredDad3	ProbDad3
F1V07	F1_B12	10.000				
F1V22	F1_B12	10.000				
F1V33	F1_B12	10.000				
F2V02	RA2_B27	10.000				
F2V12	F2_B19	10.000				
F2V28	RA2_B15	0.8982	RA2_B14	0.0509	RA2_B16	0.0509
RA1V25	RA1_B01	10.000				
RA1V29	RA1_B03	10.000				
RA2V13	RA1_B01	10.000				

Maternity

Offspring ID	InferredMum1	ProbMum1	InferredMum2	ProbMum2	InferredMum3	ProbMum3
F1V05	F1_B09	10.000				
F1V08	F2_B21	10.000				
F1V17	RA1_B03	10.000				
F1V19	F2_B27	0.5000	F2_B26	0.5000		
F1V20	F1_B09	10.000				
F1V21	F1_B09	10.000				
F1V24	F1_B09	10.000				
F1V26	F1_B09	10.000				
F1V33	RA1_B01	10.000				
F1V40	F1_B12	10.000				
F2V13	F1_B14	0.5000	F2_B26	0.5000		
F2V22	F2_B27	0.5000	F2_B26	0.5000		
F2V25	F2_B19	10.000				
F2V31	F2_B19	10.000				
F2V32	F2_B27	0.5000	F2_B26	0.5000		
F2V40	F1_B09	10.000				
F2V46	F1_B14	0.5000	F2_B26	0.5000		
RA1V04	F2_B21	10.000				
RA1V09	RA2_B15	0.8982	RA2_B14	0.0509	RA2_B16	0.0509
RA1V14	RA1_B01	10.000				
RA1V26	RA1_B03	10.000				
RA1V27	RA1_B03	10.000				
RA1V28	RA1_B01	10.000				
RA1V31	F2_B21	10.000				
RA1V36	RA1_B01	10.000				
RA1V42	RA2_B15	0.8982	RA2_B14	0.0509	RA2_B16	0.0509
RA2V25	RA1_B01	10.000				
RA2V30	RA2_B27	10.000				
RA2V32	RA2_B27	10.000				

Figure 19: Full and half sibship assignments among all juveniles (COLONY). The upper diagonal showing full sibships in orange (N = 48), the lower diagonal showing half sibships in green (N = 383).

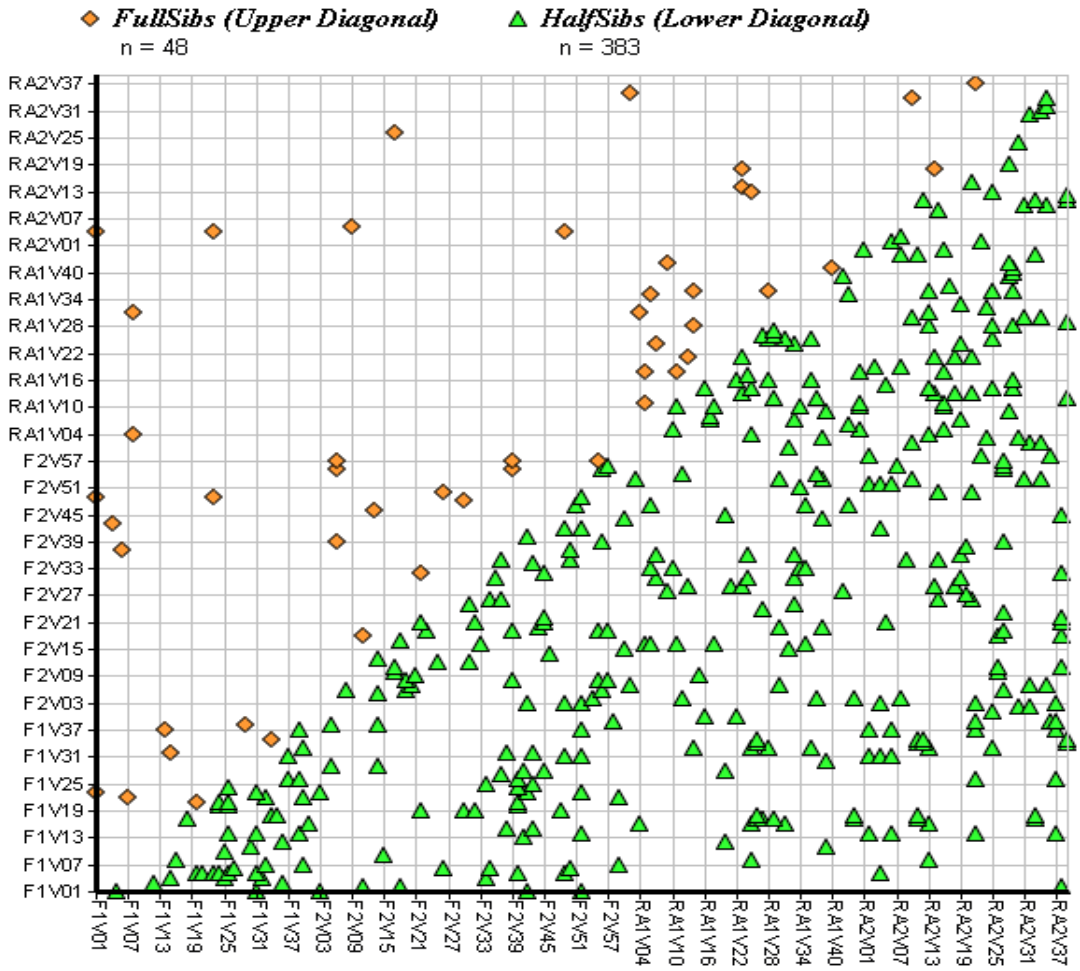


Table 17: Estimates of inbreeding (COLONY). Methods: FL_PLS, full likelihood and pairwise likelihood score combined; F_{IS}(DHW), Wright's inbreeding coefficient for departure of Hardy-Weinberg equilibrium; ID, identity disequilibrium method. Est., Estimates of inbreeding/selfing; CI95-L, lower 95% confidence interval; CI95-U, upper 95% confidence interval.

Method	Parameter	Est	CI95-L	CI95-U
FL_PLS	Inbreeding	0.0427	0.0208	0.0656
FL_PLS	Selfing	0.0820	0.0408	0.1231
F_{IS}(DHW)	Inbreeding	0.0201		
F_{IS}(DHW)	Selfing	0.0393		
ID	Selfing	0.0529		

Abstract in German – Zusammenfassung

Die Konifere *Pinus cembra* L. (Zirbelkiefer) ist eine endemische Schlüsselart des oberen subalpinen Waldgürtels. Bereits in der Vergangenheit verschiedensten negativen menschlichen Einflüssen ausgesetzt, leidet die Zirbe in der Gegenwart unter fortlaufender Fragmentierung ihres Lebensraums. Zusätzlich ist diese Baumart durch die extremen klimatischen Bedingungen in ihrem Habitat und durch artspezifische Eigenschaften sehr empfindlich auf die Auswirkungen des Klimawandels, weshalb zukünftig eine signifikante Reduktion der für die Zirbelkiefer besiedelbaren Flächen erwartet wird. Vor allem in kleinen und fragmentierten Populationen am Rande des Verbreitungsgebiets von *Pinus cembra* wurden bereits Anzeichen genetischer Verarmung, eingeschränkten Genflusses und Tendenzen zu Inzucht nachgewiesen. In der vorliegenden Studie werden drei Zirbenpopulationen untersucht, gelegen im Nationalpark Berchtesgaden in Bayern, Deutschland, am nördlichen Verbreitungsrand der Art in den Kalkalpen. Analysen von genetischen Mustern und Verwandtschaftsbeziehungen werden mithilfe von vier Chloroplasten- und zwölf nuklearen Mikrosatellitenmarkern anhand von Proben von Verjüngung und adulten Bäumen aus fünf über die drei Populationen verteilten Probeflächen durchgeführt. Die Ergebnisse deuten darauf hin, dass die drei Populationen seit Langem getrennt sind und dass genetischer Austausch hauptsächlich innerhalb der Populationen stattfindet. Das Ausmaß von genetischer Fragmentierung und Inzucht, die genetischen Strukturen und der Verjüngungserfolg variieren zwischen den drei Populationen. Daher wird erwartet, dass Vitalität und Weiterbestehen in Zukunft unterschiedlich erfolgreich für jede der Populationen ausfallen werden.