universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Genetic structure of the local endemic *Dianthus plumarius* subsp. *blandus* in the Gesäuse National Park and its regional context“

verfasst von / submitted by

Gisbert Alexander Wolfgang Bauer

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magister der Naturwissenschaften (Mag.rer.nat.)

Wien, 2018 / Vienna, 2018

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 190 445 313
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Lehramtsstudium UF Biologie und Umweltkunde UF Geschichte, Sozialkunde und Politische Bildung
Betreut von / Supervisor:	ao. Univ.-Prof. i. R. Dr. Josef Greimler

Table of Contents

Danksagungen

An dieser Stelle möchte ich all jenen Personen meinen Dank aussprechen, die maßgeblich daran beteiligt waren, mein Studium mit dieser Arbeit zu einem erfolgreichen Abschluss zu bringen!

Ich danke herzlich Herrn ao. Univ.–Prof. i. R. Dr. Josef Greimler, der mir nicht nur die Möglichkeit geboten hat eine wissenschaftliche Arbeit durchzuführen, sondern mich auch aktiv und engagiert bei meinem Forschungs- und Schreibprozess begleitet hat. Dank sei auch Herrn Mag. Dieter Reich ausgesprochen, ohne dessen geduldige Anleitung die Laborarbeit nicht durchführbar gewesen wäre und dessen Kenntnisse und Ratschläge viele Fragen und Probleme gelöst haben, die mir im Laufe meiner Arbeit begegnet sind. Auch möchte ich der Verwaltung des Nationalparks Gesäuse, für die Bereitstellung des Kartenmaterials und Übernahme der Druckkosten dieser Arbeit, ein Wort des Dankes aussprechen.

Zuletzt möchte ich all jenen Danken, die mir meine Studienzeit erleichtert und mich unterstützt haben, meinen Freunden, den alten wie den neuen, die mich mit Witz und Freude auch durch die intensivste Prüfungszeit geleitet haben und vor allem meinen Eltern, Cosima und Wolfgang Bauer, ohne deren Unterstützung und Geduld all dies nicht möglich gewesen wäre.

.

Introduction

The conservation and protection of nature and its diversity is the great challenge of our time. Human activities in the last centuries have led to a massive decline of biodiversity worldwide due to habitat loss and fragmentation. Today more than 25,000 species worldwide are considered threatened with extinction and more than 90,000 are on the IUCN red list (IUCN, 2018), even in Austria 33.4% of the occurring plant species are listed as endangered (Niklfeld et al., 1999). Studies characterize the recent rate of species loss as the sixth mass extinction in earth history (Ceballos et al., 2015; Briggs, 2017). Reacting to the rapid and on–going loss of biodiversity and its severe impact on human welfare multiple national and international organizations such as the *International Union for Conservation of Nature* (IUCN) have been founded to address these problems and to create adequate policy frameworks. With the *Convention on Biological Diversity* 196 parties joined a global political program for biodiversity conservation and sustainable use of natural resources (CBDa, 2018). The *Aichi Biodiversity Targets* are the most recent goals of the IUCN for the Strategic plan for biodiversity 2011–2020, they aim at protecting biodiversity on three different levels: safeguarding ecosystems, species and genetic diversity (CBDb, 2018).

As a consequence of rising awareness in the scientific community the discipline of conservation biology was established to investigate problems in the context of protection and conservation of ecosystems and their biodiversity and to combine science and necessary political action (Meffe and Viedermann 1995). The formation of the discipline began in the late 1970's and early 1980's when ecologists first recognized a decline in biological diversity (Meffe and Viederman, 1995). Soulé (1985) described it as a "crisis oriented [discipline which] is concerned with the long–term viability of whole systems". Conservation biology aspires an interdisciplinary approach and combines domains of biology, natural resource fields as well as social sciences and tries to address issues beyond ecology including economic and political questions (Meffe and Viederman, 1995; Soulé, 1985). The core values of the discipline are clear: high biodiversity, functioning and complex ecosystems are good and continuing evolutionary change and adaptation are favorable states (Soulé, 1985). On this basis the scientific data is interpreted and recommendations are made.

With the development of molecular tools scientists were able to directly address genetic diversity, the field of conservation genetics was established. It is "the study of genetic patterns or processes in any context that informs conservation efforts" (Avise, 2008). From this point on it was possible to gain insight into patterns of genetic diversity within and among different taxa, populations and individuals. Also genetic processes such as gene flow and genetic drift as well as selection could be investigated. The majority of studies about conservation genetics are concerned with species diversity issues, geographic variation, like genetic population structure and gene flow, and within–population genetics like inbreeding. The targets of those investigations are particular populations or species which are either rare, threatened or provide a deeper insight in certain genetic processes (Avise, 2008). "Biodiversity is, ultimately genetic diversity" (Avise, 2008) which means, by the main principles of conservation biology, genetic diversity is good and worth protecting. It is essential for evolutionary processes and the basis for natural selection to work on and to direct organisms through changing environments (Vida, 1994). Thus, studies show a positive relationship between genetic diversity and fitness traits (Leimu et al., 2006; Reed and Frankham, 2003).

One of the main concerns of nature conservationists was always the protection of rare and endemic taxa and their habitats (Schmid and Matthies, 1994). In the scientific literature a variety of definitions of rarity exists and different variables have been used to describe them. According to Kruckeberg and Rabinowitz (1985) the narrow or local endemic is the embodiment of rarity. It appears only on a small geographic range, occupies very restricted habitats and the single populations are small in size (Rabinowitz, 1981). In comparison to their common relatives rare plant species often exhibit differences in their life history traits and their genetic patterns (Gaston and Kunin, 1997). Studies show that rare plant species tend to possess lower levels of genetic variation and gene flow than their common congeners (Cole, 2003).

Gene flow, genetic drift and natural selection are the main forces that shape genetic structure and diversity within and between plant populations (Loveless and Hamrick, 1984), strategies of pollen and seed dispersal, breeding system as well as the landscape determine the effect of gene flow (Gaudel et al., 2007; Kalisz, et al., 2001). Spatial isolation leads to increasing differentiation between populations due to genetic drift (Kuss et al., 2008; Putz et al., 2015). Population size and genetic diversity are often closely related (Leimu et al., 2006). In many cases small and isolated populations suffer from the effects of genetic drift and inbreeding and exhibit low levels of genetic diversity (Ouborg et al., 2006; Honnay and Jacquemyn 2007). Also the risk of extinction increases with increasing distance between populations (Matthies et al., 2004).

The pink carnation *Dianthus plumarius* subsp. *blandus* (later referred to as *Dianthus blandus*) is one of the most famous and beautiful endemics in the Austrian national park Gesäuse. From May to June its pinkish flowers can be spotted in many ravines all over the park and some individuals can be admired right next to hiking trails. In the ravine Gseng one of the biggest and most dispersed populations accompanies the path all the way up to 1,200 m above sea level. Also the national park officials recognize *D. blandus* as one of their flagship species of the Gesäuse and are therefore concerned about the situation and wellbeing of the different populations within their responsibility.

The Gesäuse area as an ancient glacial refugium (Schönswetter et al., 2005) shelters about 30 endemic and subendemic plant species of the North–Eastern Limestone Alps (NP Gesäuse, 2018; Rabitsch and Essl, 2009). *Dianthus blandus* as one of those endemics can be characterized as a rare regional endemic according to Kruckeberg and Rabinowitz (1985). The species appears only in a small area in the North–Eastern Alps (Fischer et al., 2008). There it inhabits only a small range of habitat types on stabilized carbonate scree on sites in early stages of succession (Meusel and Mühlberg, 1979). This results in a very scattered distribution of the single populations. This pattern can also be observed in the Gesäuse region where the populations are situated in different ravines divided by rivers and ridges. Therefore geomorphologically they are well–separated from each other.

Using amplified fragment length polymorphisms (AFLP) this study addresses the following questions: (1) Does the genetic structure of the *Dianthus blandus* populations in the Gesäuse region follow any biogeographic pattern? (2) Do the populations of the Gesäuse exhibit signals of isolation or loss of genetic diversity. (3) Is there a correlation between genetic diversity, population size, and habitat type. (4) Which recommendations for conservation can be derived from these results?

To address relationships between *D. blandus* and its closest relatives in eastern Austria and Italy samples of other populations and related taxa were included.

Fig. 1: *Dianthus blandus* in the Gesäuse region.

Materials and Methods

Plant material and study design

Taxonomy of *Dianthus* sect. *Plumaria* is far from being resolved. The genus *Dianthus* itself is one of the most diverse plant groups in Europe, more than 100 species are discriminated, of which over 70 are considered endemic (Valente et al., 2010). The study of Valente et al. (2010) suggests a rapid radiation in the last 1–2 Myr leading to this high number of species. In many cases species boundaries are unclear and hybridization is frequently observed (Valente et al., 2010). Polyploidization within some groups such as *Dianthus* sect. *Plumaria* (Weiss et al., 2002) may have been a trigger for rapid diversification.

Besides *Dianthus blandus* two other closely related local and regional endemics of *Dianthus plumarius* s. str. (Fischer et al., 2008) can be found in Austria: *Dianthus plumarius* subsp. *neilreichii* (later referred to as *D. neilreichii*) is a local endemic in the mountains around Mödling near Vienna and *Dianthus plumarius* subsp. *hoppei* (later referred to as *D. hoppei*) can be found in Styria and Carinthia (Meusel and Mühlberg, 1979). Despite the poor morphological differences they are well separated by their ecological niche and different distributional areas. Two other related taxa were included: *Dianthus lumnitzeri* (later referred to as *D. lumnitzeri*) appearing in the eastern part of Austria around Hundsheim and in the Little Carpathians (Meusel and Mühlberg, 1979), and finally *Dianthus sternbergii* (later referred to as *D. sternbergii*) of the south–eastern Alps.

Thus, the presented study includes accessions of closely related taxa of section *Plumaria* (s.l.) from altogether 7 regions (**Fig. 2/B**): *Dianthus lumnitzeri* was collected in Hundsheim (Lower Austria, Austria), *D. neilreichii* in Mödling (Lower Austria, Austria), *D. hoppei* in Graz (Styria, Austria) and *D. sternbergii* in Tolmezzo (Friuli–Venezia Giulia, Italy). The main focus lies on *D. blandus* which was gathered from three additional regions in Styria: Ramsau, Gröbming and Gesäuse. Within the latter, 7 localities (**Fig. 2/A**), 3 north and 4 south of the Enns river, were sampled based on the populations as described in Köppl and Oberklammer (2015), who defined populations as all individuals co–occurring in one ravine. In some cases subpopulations were defined if ramets were grouped in clear clusters and the distance between clusters exceeds 100 m.

Collecting was performed from June to July 2017, for each sampled region a herbarium voucher was gathered and deposited in the herbarium of the Institute of Botany of the University of Vienna (WU, number and link see **Table 1**). In each population leaf material of 15-20 individuals was collected and dried in zip lock bags with silica gel. The coordinates of the populations and subpopulations were recorded (**Supplement Table 1**). Furthermore the elevation above sea level was recorded for the subpopulations in the Gesäuse region (**Supplement Table 5**). The population sizes were estimated (N_{est}) and based on those estimates sizes classes (NC_{est}) were defined as shown in **Table 1**. In addition to our estimates the population sizes estimated by Köppl and Oberklammer (2015) (N_K) were included in our data. Based on their data a separate set of size classes was defined (NC_K) as shown in **Table 1**. Habitat classes (HC) were defined according to Köppl (2016) based on the syntaxonomy in Mucina et al. (1993). Population sizes and classes as well as habitat classes and their description are shown in **Table 1**. For the population Gseng (e) the coordinates of each individual were recorded (**Supplement Table 2**). Furthermore, in two large *D. blandus* cushions 3 samples were probed, e104a/b/c and e301a/b/c, to test for the clonal component, i. e. whether the whole cushion originated from one genet (**Fig. 3**).

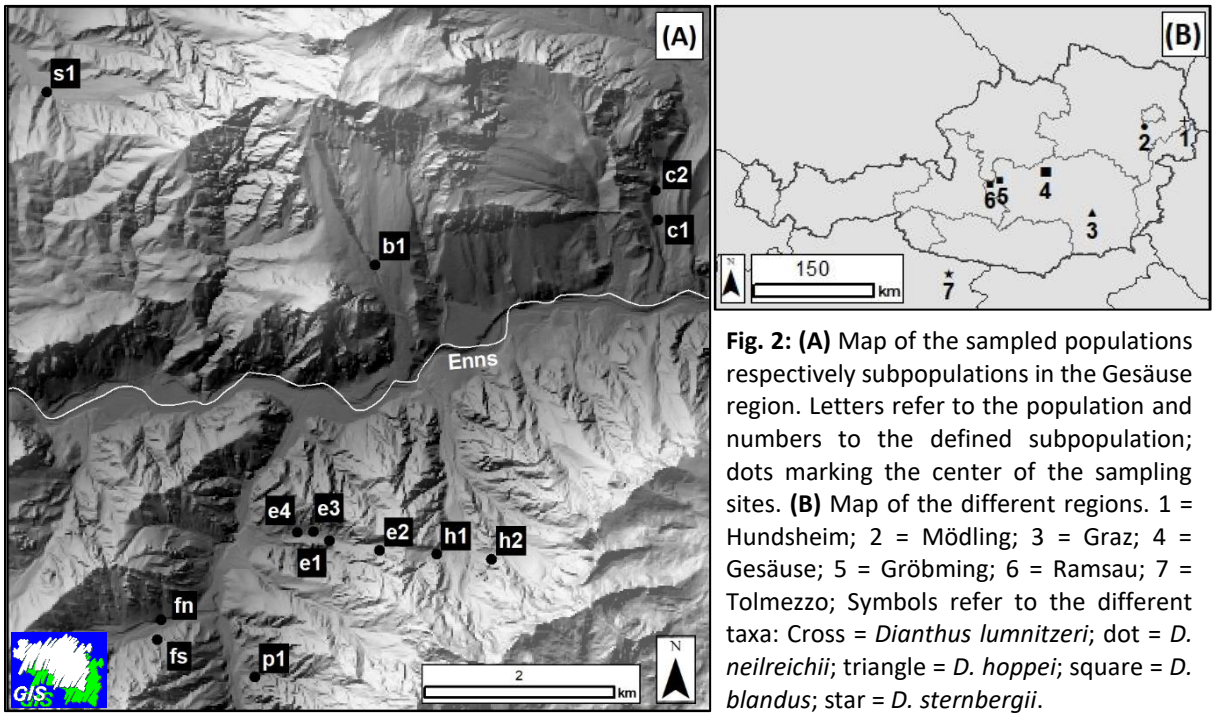


Fig. 2: (A) Map of the sampled populations respectively subpopulations in the Gesäuse region. Letters refer to the population and numbers to the defined subpopulation; dots marking the center of the sampling sites. **(B)** Map of the different regions. 1 = Hundsheim; 2 = Mödling; 3 = Graz; 4 = Gesäuse; 5 = Gröbming; 6 = Ramsau; 7 = Tolmezzo; Symbols refer to the different taxa: Cross = *Dianthus lumnitzeri*; dot = *D. neilreichii*; triangle = *D. hoppei*; square = *D. blandus*; star = *D. sternbergii*.

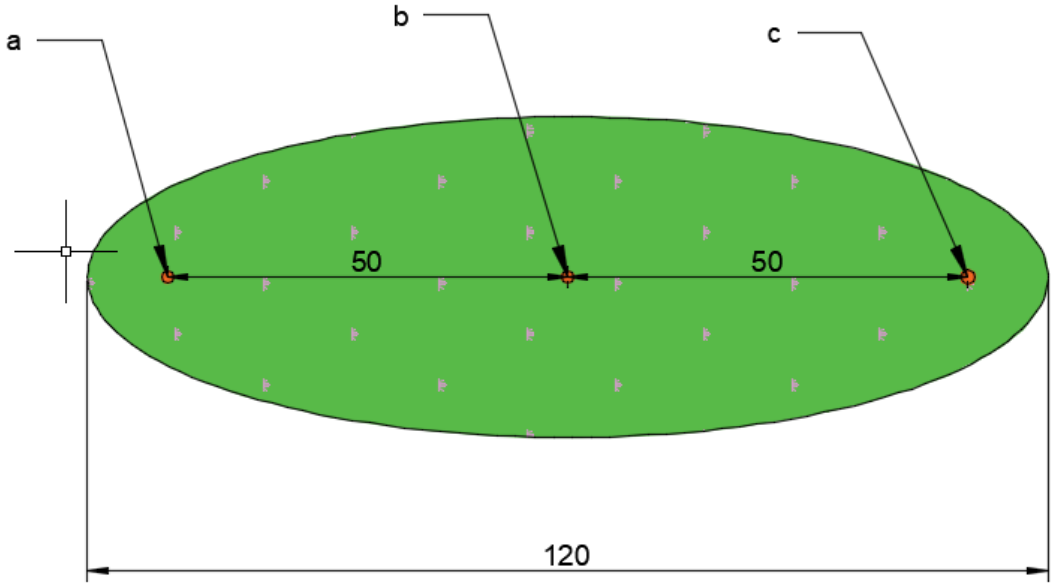


Fig. 3: The probing pattern of two *Dianthus blandus* cushions e104a/b/c and e301a/b/c to investigate possible clones. Letters indicate the three samples of each cushion; distances in cm.

DNA extraction and AFLPs

DNA extraction was performed using the CTAB-based method (Doyle and Doyle, 1987) with some modifications as described in Schönswetter et al. (2009). The quality of the extractions was checked on a 1% TAE agarose gel.

An amplified fragment length polymorphism (AFLP) was conducted according to the protocol of Vos et al. (1995) with modifications as described in Schönswetter et al. (2009). The same primer combinations were used as mentioned in Putz et al. (2015). The results were purified using Sephadex G-50 fine (GE Health care Bio-Sciences, Uppsala, Sweden). The AFLP-products were run on a 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, CA, USA). GeneScan 500 ROX (Applied Biosystems, Foster City, CA, USA) was used as an internal size standard. Two negative controls were included throughout the whole laboratory procedure and to assess reproducibility approximately 10% of the samples were repeated.

Scoring and statistical analysis

GeneMarker V1.95 (Softgenetics, State College, PA, USA) was used to score the resulting AFLP bands. The program automatically created a binary matrix for each selective primer combination. Present fragments were counted as 1 and absent fragments as 0. The output was manually checked and corrected for scoring errors. Only fragments within a size range of 50 and 350 bp were included in the final data matrix. The three primer combinations were combined in one table. Based on the replicates included in the AFLP-analysis the error rate was calculated as described in Bonin et al. (2004).

Clones

The two sample sets of the suspected clones (e104a/b/c and e304a/b/c) were analyzed using the R script AFLPdat (Ehrich, 2006). According to the calculated error rate the E value was set to 10. For visualization a neighbor net analysis was executed using the standard settings of Splitstree4 version 4.14.6 (Huson and Bryant, 2006) including all samples. The samples e104b/c and e304b/c were excluded from further analyses.

Genetic Structure

To reveal the grouping of the remaining samples a neighbor net analysis was calculated using Splitstree4 version 4.14.6 as mentioned above. FAMD 1.31 (Schlüter and Harris, 2006) was employed to create a majority rule consensus tree based on Jaccard coefficients. The number of bootstrap replicates was set to 1,000. Additionally two principal coordinate analyses (PCoA) were performed with GenAlEx 6.5 (Peakall and Smouse, 2012; Peakall and Smouse, 2006). The first calculation contained all samples and the second the samples of the Gesäuse region. The first three axes were visualized. Furthermore the program STRUCTURE 2.3.4 was employed using the Bayesian method (Falush et al., 2007, Pritchard et al., 2000) to test for genetic grouping. Two runs were performed. The first one included the samples of the Gesäuse region. K was set from 2-12 with 10 replicates for each K, the admixture model and independent allele frequencies among populations were assumed. Each run had 500,000 iterations and a burn-in period of 50,000. The second run contained all samples. This time the K was set from 2-10 with 10 replicates for each K, again the admixture model and independent allele frequencies were assumed. The number of the iterations was set to 10^6 and the number of the burn-in

was set to 10^5. The results of the two runs were analyzed using the online tool Structure Harvester (Earl et al., 2012). The most likely K was evaluated following the method purposed by Evanno et al. (2005). The best alignment of the clusters was calculated with the program CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007). The final data was visualized using Distruct 1.1 (Rosenberg, 2004).

The results of the PCoA and STRUCTURE suggested to split the population Langgries (f) into a norther (fn) and a southern (fs) population. The following calculations were performed with this design.

The software Arlequin ver 3.5 (Excoffier et al., 2005) was employed to perform several hierarchical and nonhierarchical analysis of molecular variance (AMOVA) with the number of permutations set to 1,000. Four different AMOVAs were performed including all populations. For the first one no additional grouping was set. For the second run 7 groups were assumed according to the different sampling regions. The third was grouped according to the different taxonomic classifications. The last run combined the three subspecies of *Dianthus plumarius* into one group, the second group contained *D. lumnitzeri* and the third contained *D. sternbergii*. Another 5 runs were performed including only populations of the Gesäuse region. The first run assumed no additional grouping. For the second AMOVA a northern (b, c, s) and a southern group (e, fn, fs, h, p) were defined divided by the river Enns. The third run excluded the population Hartlschütt (s) from the northern group into an own group. This was done because a high degree of isolation from the remaining Gesäuse was suspected. For the fourth run two groups were assumed: Hartlschütt (s) and the remaining Gesäuse. The last run was grouped according to the results of STRUCTURE for the region Gesäuse. Pairwise F_{st} were calculated for all populations using Arlequin ver 3.5. The number of permutations was set to 1,000.

Two Mantel tests were executed in GenAlEx 6.5. The first one contained only the populations of the region Gesäuse and the second one contained all samples. For the calculations the coordinates of the subpopulations were used and the number of permutations was set to 999. A spatial autocorrelation was performed using the same software. The individuals of the population Gseng (e) and their coordinates were included in the calculations. The number of permutations was set to 999 and the number of bootstraps to 1,000. To assess an even distribution of values over distance classes following classes were defined: 0–100, 101–250, 251–600, 601–900 m.

Genetic diversity

Several parameters were calculated for each population to assess genetic diversity: AFLP-SURV 1.0 (Vekemans, 2002) was employed to calculate the number of fragments per population ($Frag_n$) and percentage of polymorphic fragments ($Frag_{poly}$). PopGene 1.32 (Yeh et al., 1999) was employed to calculate Nei's gene diversity (H_j) and Shannon's information index (IHS) with the assumption of Hardy–Weinberg equilibrium. Arlequin ver 3.5 was used to calculate the mean number of pairwise differences (PI) for each population. The number of permutations were set to 1,000. GeneAlEx 6.5 was used to calculate the number of private fragments (Priv).

In IBM SPSS Statistics 24 (IBM, Armork, NY, USA) Spearman's rank correlations for genetic diversity parameters, population sizes, population classes and habitat classes as shown in **Table 1** were calculated.

Furthermore the same diversity parameters were calculated for the subpopulations of the Gesäuse region as described above. This time percentage of polymorphic fragments ($Frag_{poly}$) was calculated with PopGene 1.32. The parameters and the measured elevation of the subpopulations as shown in **Supplement Table 5** were used to perform a Spearman's rank correlation with IBM SPSS Statistics 24.

Results

The final dataset contained 151 samples, after excluding 4 suspected clonal ramets and 12 replicates. The populations of the Gesäuse region amounted to 94 samples. The number of samples per population ranged from 6 to 20. In total 14 populations were defined. The size of fragments ranged from 52 to 316 bp. The data matrix contained 200 loci and the error rate amounted to 4.67% with the error rate per sample ranging from 1% to 14%.

Genetic diversity

The genetic diversity parameters are summarized in **Table 1**. The number of fragments for each population ranged from 87 in (c) to 121 (b). The highest number of private fragments was found in Tolmezzo (t) with 5 and Gröbming (w) with 4. The populations (fn), (fs) and (c) had no private fragments. The percentage of polymorphic fragments ($Frag_{poly}$) ranged from 43.5% in populations (c) to 63% in population (m). Shannon index (IHS) ranged from 0.117 to 0.214 with the lowest values in population (p), (s) and (fs). The highest value of IHS appeared in population (fn), (e), and (r). Nei's gene diversity (H_j) ranged from 0.076 in population (fs) to 0.142 in populations (fn). The mean number of pairwise differences (PI) ranged from 20.85 in Petergstamm (p) to 32.93 in Langgries North (fn). The populations Langgries South (fs), Petergstamm (p) and Hartlschütt (s) showed in all cases low biodiversity values. Langgries North (fn) in contrary showed high values. The correlation analysis showed no significant connection between genetic biodiversity parameters, population sizes and habitat classes (**Supplement Table 4 A/B**).

The elevation of the different subpopulations in the Gesäuse region ranged from 660 m to 1,180 m. The lowest subpopulation was (c1) and the highest (h2). H_j ranged between 0.082 in (c1) and 0.142 in (fn). IHS ranged from 0.116 in (e4) to 0.214 in (fn). $Frag_{poly}$ ranged from 22.0% (e4) to 43.0% in (fn). PI ranged from 20.85 in (p) to 32.93 in (fn). There was no significant correlation found between diversity parameters and elevation (**Supplement Table 6**).

Genetic structure

All regions

The neighbor net analysis (**Fig. 4**) did not resolve all populations as consistent groups. The members of the Tolmezzo group (t) showed the clearest differentiation, followed by the populations of the Graz (z) and Mödling (m) regions. The samples of Hundsheim (u) also group together but are not clearly separated from Ramsau (r) and Gröbming (w). The samples of Ramsau and Gröbming build a mixed group with some members of Ramsau grouped entirely separate next to the Tolmezzo group. Most of the populations of the Gesäuse region are not clearly differentiated from each other. The samples of the population Kühgraben (b) form a consistent group next to population (m). The majority of samples of the northern Langgries (fn) are grouped right next to them. The majority of the samples of the population Weißenbachgraben (c) are combined in one cluster next to the population Gseng (e). Gseng (e) does not include all samples of the population. The samples of the Hartlschütt (s) emerge right next to the individuals of Ramsau. The remaining populations (fs, p, h) did not form consistent clusters.

Table 1: Summary of the investigated *Dianthus* populations. A = Austria; I = Italy; Pop = acronym of the population; Ind_n = number individuals; $Frag_n$ = number of fragments per population; $Frag_{poly}$ = % of polymorphic fragments; Priv = number of private fragments; IHS = Shannon's information index; H_j = Nei's gene diversity; PI = mean number of pairwise differences; S.D. = standard deviation; N_k = population size estimated by Köppl and Oberklammer (2015); NC_k = size classes based on N_k (0-199 = 1; 200–499 = 2; 500–999 = 3; 1000–1999 = 4; 2000–4999 = 5; ≥5000 = 6); N_{est} = estimated population size; NC_{est} = size classes based on N_{est} (0–50 = 1; 50–99 = 2; 100–199 = 3; 200–299 = 4; 300–499 = 5; ≥500 = 6); HC = habitat classes (1 = stabilized scree (Petasition); 2 = stabilized scree and grassland patches (Petasition, Seslerion); 3 = stabilized scree and dwarf shrubs (Petasition, Ericetalia); 4 = small forest clearing (Pinion sylvestris, Piceetalia); 5 = rocks, dry grassland, sun exposed (Stipo-Festucetalia pallentis); 6 = rocks, dry grassland under tree cover, north exposed (Stipo-Festucetalia pallentis)) .

Taxon	Region	Site (Pop)	Ind_n	$Frag_n$	$Frag_{poly}$	Priv	IHS	H_j	PI (±S.D.)	N_k	NC_k	N_{est}	NC_{est}	HC
D. blandus	Gesäuse (A)*	Kühgraben (b)	9	121	60.5	2	0.171	0.113	27.11 (±13.141)	200	2	75	1	3
		Weißenbachgraben (c)	14	87	43.5	0	0.179	0.116	26.43 (±12.338)	1000	4	200	4	2
		Gseng (e)	20	114	57.0	1	0.191	0.121	28.35 (±12.953)	6000	6	700	6	1
		Langgries North (fn)	8	121	60.5	0	0.214	0.142	32.93 (±16.111)	1500	4	100	3	3
		Langgries South (fs)	6	89	44.5	0	0.117	0.076	21.60 (±11.145)	1500	4	100	3	4
		Haindlkar (h)	13	99	49.5	1	0.177	0.114	27.62 (±12.944)	2000	4	250	4	2
		Petergstamm (p)	14	92	46.0	1	0.133	0.083	20.85 (±9.801)	10000	6	400	5	3
		Hartlschütt (s)	10	98	49.0	2	0.145	0.094	22.47 (±10.829)			250	4	3
	Ramsau (A)*	Ramsau (r)	11	106	53.0	1	0.193	0.127	28.76 (±13.650)			150	3	2
	Gröbming (A)*	Gröbming (w)	9	97	48.5	4	0.175	0.117	27.22 (±13.194)			150	3	2
D. neilreichii	Mödling (A)*	Mödling (m)	11	126	63.0	2	0.185	0.121	27.49 (±13.061)			150	3	6
D. hoppei	Graz (A)*	Graz (z)	9	119	59.5	3	0.176	0.117	26.39 (±12.800)			25	1	6
D. lumnitzeri	Hundsheim (A)*	Hundsheim (u)	7	94	47.0	2	0.160	0.107	25.52 (±12.792)			250	4	5
D. sternbergii	Tolmezzo (I)*	Tolmezzo (t)	10	111	55.5	5	0.188	0.126	29.67 (±14.193)			150	3	3

*Voucher collected for each region.

Gesäuse (A) WU 0100299.
Ramsau (A) WU 0099035.
Gröbming (A) WU 0099033.
Mödling (A) WU 0099036.
Graz (A) WU 0099034.
Hundsheim (A) WU 0093649.
Tolmezzo (I) WU 0099037.

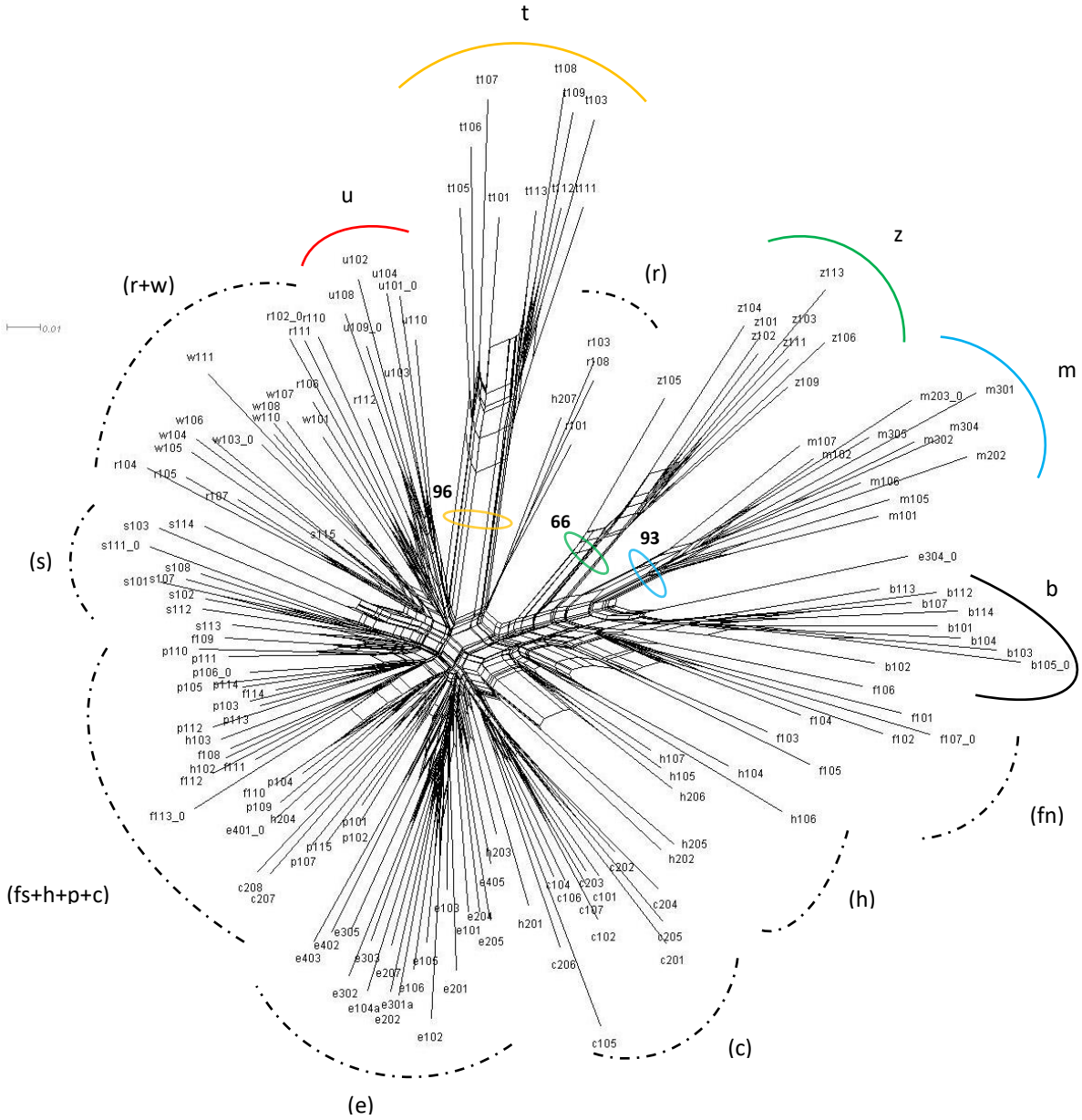


Fig. 4: Neighbor net including all sampled populations. Numbers and ellipses on the base of branches symbolize the bootstrap support (shown> 65%) calculated for the majority rule consensus tree; solid arcs indicate that all samples of one population group together; dashed arcs and letters in brackets indicate a fragmentary and/or mixed population cluster; letters = populations represented in the cluster; arc colors indicate different taxa: black = *Dianthus blandus*, blue = *D. neilreichii*, green = *D. hoppei*, orange = *D. sternbergii*, red = *D. lumnitzeri*.

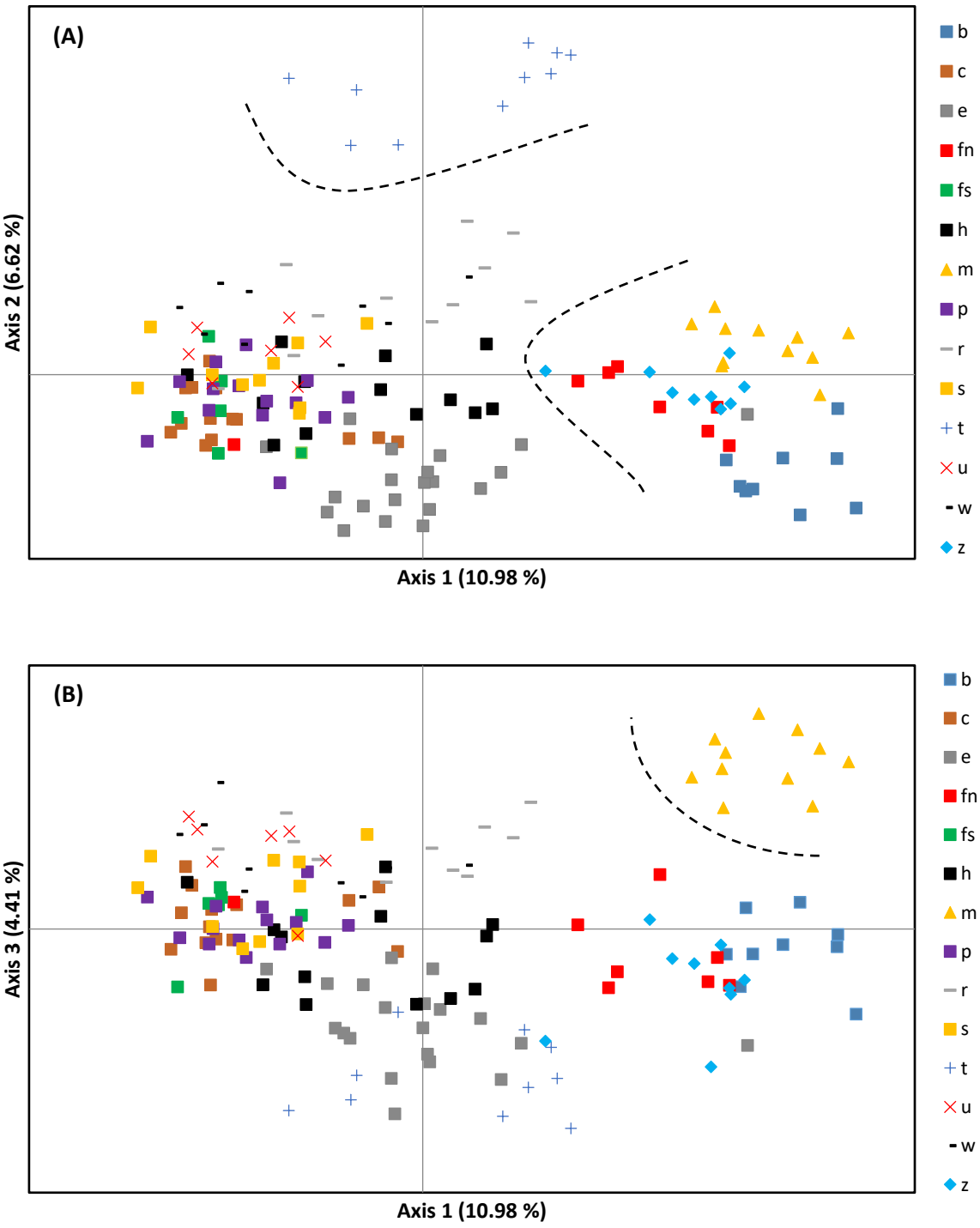


Fig. 5: (A) PCoA ordination of the first two axes showing all sampled populations. **(B)** PCoA of axis 1 and axis 3. Samples of different regions are shown with different symbols: squares = Gesäuse (*Dianthus blandus*); long dashes = Ramsau (*D. blandus*); short dashes = Gröbming (*D. blandus*); triangles = Mödling (*D. neilreichii*); diamonds = Graz (*D. hoppei*); plus = Tolmezzo (*D. sternbergii*); red cross = Hundsheim (*D. lumnitzeri*).

The majority rule consensus tree supported the clusters of *Dianthus sternbergii* (t) with 96%, followed by *D. neilreichii* (m) with 93%, *D. hoppei* (z) with 66% and the Gesäuse population (b) of *D. blandus* with 53%. The remaining samples collapsed to one big cluster including *D. blandus* populations and the population of *D. lumnitzeri* (u). The support for the clusters is shown in **Fig. 4**.

In total the first three axes of the PCoA explained 22.02% (PC1 10.98%, PC2 6.62 and PC3 4.41%). The PCoA with all samples included did not separate populations and taxa (**Fig. 5**). Along axis 1 the *Dianthus blandus* populations (b), (fn) together with *D*. *neilreichii* of Mödling (m) and *D*. *hoppei* of Graz (z) are separated from a large group containing the majority of the Gesäuse populations (c, e, fs, h, p, s) and the populations of Ramsau (r). Gröbming (w) as well as *D. lumnitzeri* of Hundsheim (u). Notably the Gesäuse samples of Langgries were separated into two different groups corresponding to a population north of the ravine (fn) and another population south of it (fs). Some Ramsau samples group in between the majority of the *D. blandus* populations and the *D. sternbergii* population (t). Along the second axis only *D. sternbergii* (t) was clearly separated from the other populations and taxa (**Fig. 5/A**). The first and the third axis showed the Mödling population (m) separated from the other samples (**Fig. 5/B**).

The STRUCTURE analysis of the whole dataset (**Supplement Figure 1**) supported the results of the PCoA. K = 4 got the highest support. STRUCTURE put *Dianthus sternbergii* (t) in a unique group different from all other populations. The Langgries (f) was again divided into a northern and a southern group, therefore treated as two populations. The population (r) showed low admixture with population (t).

The pairwise F_{st} calculations resulted in values between 0.085 for population (p) and (fs), and 0.504 for population (b) and (t). The populations Tolmezzo (t), Graz (z), Mödling (m) and Kühgraben (b) showed high F_{st}. The values of population (t) ranged between 0.327 with population Ramsau (r) and 0.504 with population (b). The values of population (z) ranged from 0.299 with population Langgries North (fn) and 0.452 with (t). The values for population (m) ranged between 0.289 with (fn) and 0.457 with population (t). The values of (b) ranged between 0.214 with (fn) and 0.504 with (t). Within the Gesäuse populations (b) showed the highest F_{st} values.

Table 2: The results of the AMOVA for all populations. Letters in brackets symbolize population in the same group. (1) = grouping according to regions; (2) = grouping according to taxonomic status; (3) = grouping according to taxonomic status with *Dianthus plumarius* subspecies combined in one group; N = number of groups; d.f. degrees freedom; SS = mean sum of squares; F_{st} = general fixation index.

Grouping	N	Source of variation	d.f.	SS	Variance [%]	Fixation index
no groups	14	Among groups	13	1036	31.74	F_{st} = 0.317*
		Within populations	137	1827	68.26	
(1) [b, c, e, fn, fs, h, p, s] [r] [w] [m] [z] [u] [t]	7	Among groups	6	594	14.36	
		Among populations within groups	7	442	20.92	F_{st} = 0.244*
		Within populations	137	1827	64.72	
(2) [b, c, e, fn, fs, h, p, r, s, w] [m] [z] [u] [t]	5	Among groups	4	474	18.74	
		Among populations within groups	9	562	20.03	F_{st} = 0.388*
		Within populations	137	1827	61.22	
(3) [b, c, e, fn, fs, h, m, p, r, s, w, z] [u] [t]	3	Among groups	2	214	13.39	
		Among populations within groups	11	822	25.45	F_{st} = 0.388*
		Within populations	137	1827	61.16	

*$p < 0.001$.

The results of the AMOVA for all 14 populations are presented in **Table 2**. When no grouping was applied the fixation index (F_{st}) amounted to 0.317, therefore 68.26% of the variation occurred within populations and 31.74% between populations. The lowest F_{st} was calculated when the populations where grouped according to the sampling regions. The highest value appeared when the populations where grouped according to their taxonomic status. The grouping *Dianthus blandus* [b. c. e. fn. fs. h. p. r. s. w]. *D. neilreichii* [m]. *D. hoppei* [z]. *D. lumnitzeri* [u] and *D. sternbergii* [t] revealed an F_{st} of 0.388 and the variance among groups amounted to 18.74% which was the highest value calculated compared to the other groupings. Also the combination of the *D. plumarius* subspecies into one group amounted to a similar F_{st} value and a variance among groups of 13.39%.

The results of the pairwise F_{st} calculations are shown in **Supplemet Table 3.** The values ranged from 0.085 between (p) and (fs) to 0.504 between (t) and (b). *Dianthus sternbergii* obtained high values ranging between 0.327 with (r) and 0.504 with (b). The F_{st} values of *D. neilreichii* and *D. hoppei* were also high in comparison to the F_{st}s between *D. blandus* populations, nearly all values were over 0.3. *D. neilreichii* shared the lowest F_{st} (0.289) with population (fn) and the highest with *D. sternbergii* (0.457). *D. hoppei* shared the lowest value (0.299) with population (fn) and the highest (0.452) with *D. sternbergii*. Within the *D. blandus* populations only the Kühgraben population (b) showed high F_{st} values, nearly all of them over 0.350. Population (b) shared the lowest F_{st} (0.214) with population (fn).

The Mantel–test including all populations resulted in a correlation coefficient (R^2) of 0.25 ($P < 0.005$) and showed a connection between genetic and geographic distance. The results are shown in **Fig. 6/A**.

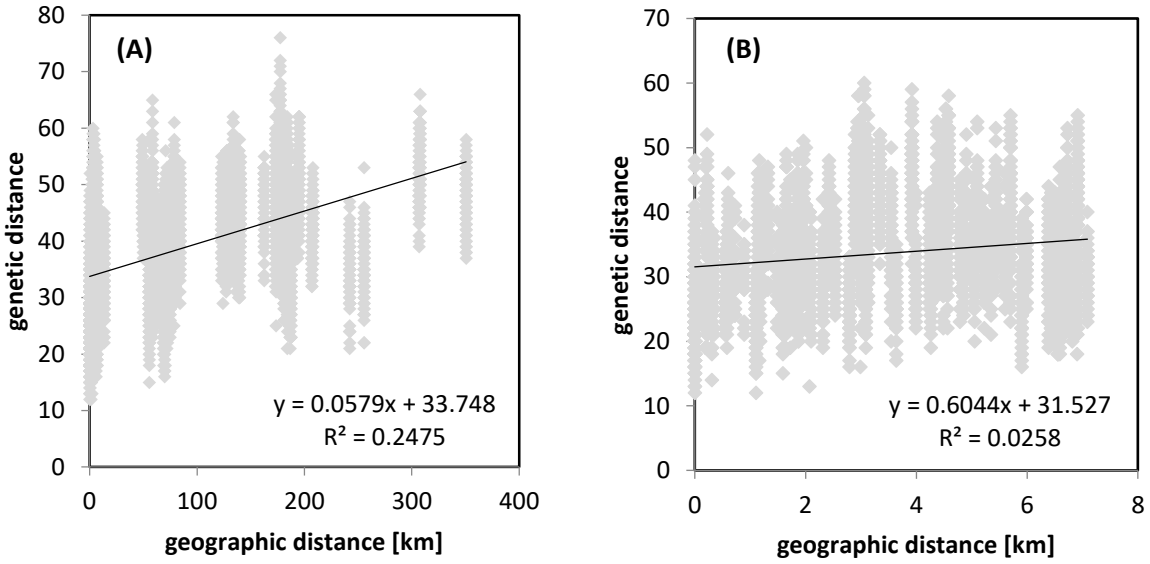


Fig. 6: (A) Mantel–test for all populations ($p < 0.005$). **(B)** Mantel–test for the Gesäuse populations ($p < 0.005$). R^2 = correlation coefficient.

Gesäuse Region

The STRUCTURE analysis for the Gesäuse region (**Fig. 7)** revealed K = 3 as the most likely group number. Again Langgries (f) belonged to two different groups. Therefore the population was split as mentioned above. The first group included the populations Langgries North (fn) and Kühgraben (b). The second group contained Langgries South (fs) together with Weißenbachgraben (c), Petergstamm (p), Hartlschütt (s) and Haindlkar (h). The population (h) showed some admixture between the first and second group. The population Gseng (e) formed a third group also exhibiting also some admixture with the first and second group.

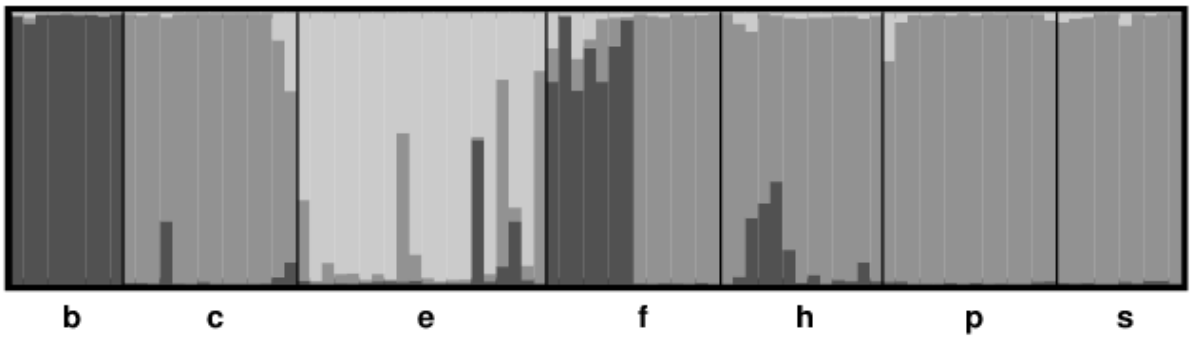


Fig. 7: Results of the STRUCTURE analysis for the Gesäuse region with K = 3. Langgries (f) is shown as one population instead of Langgries North (fn) and Langgries South (fs).

In total the first three axes of the PCoA of the entire Gesäuse region **(Fig. 8)** explain 24.67% of the variation (PC1 13.40%, PC2 6.36%, PC3 4.92%). The first two axes (**Fig. 8/A**) show the differentiation into the same three groups as calculated with STRUCTURE. Axis 2 and 3 (**Fig. 8/B**) exhibit a slight differentiation between the group (b, c, s) and the group (e, fn, fs, h, p). This division is congruent with the biogeographic separation in groups N and S of the river Enns. Also in this analysis the individuals of population (e) are slightly separated from the remaining southern individuals.

The AMOVA for the populations of the Gesäuse region is shown in **Table 3**. The different groupings showed very similar F_{st} values ranging between 0.230 (no groups) and 0.290 (grouping according to STRUCTURE). Most of the variance occurs within populations and amounts in all calculations between 71.04% (grouping according to the STRUCTURE results) and 75.03% (no groups). Almost no variance (0.65%) occurs among groups if the populations are arranged in a group north [b, c, s] and a group south the Enns river [e, fn, fs, h, p]. Most variance among groups appears when sorted according to STRUCTURE, the value amounts to 14.99%.

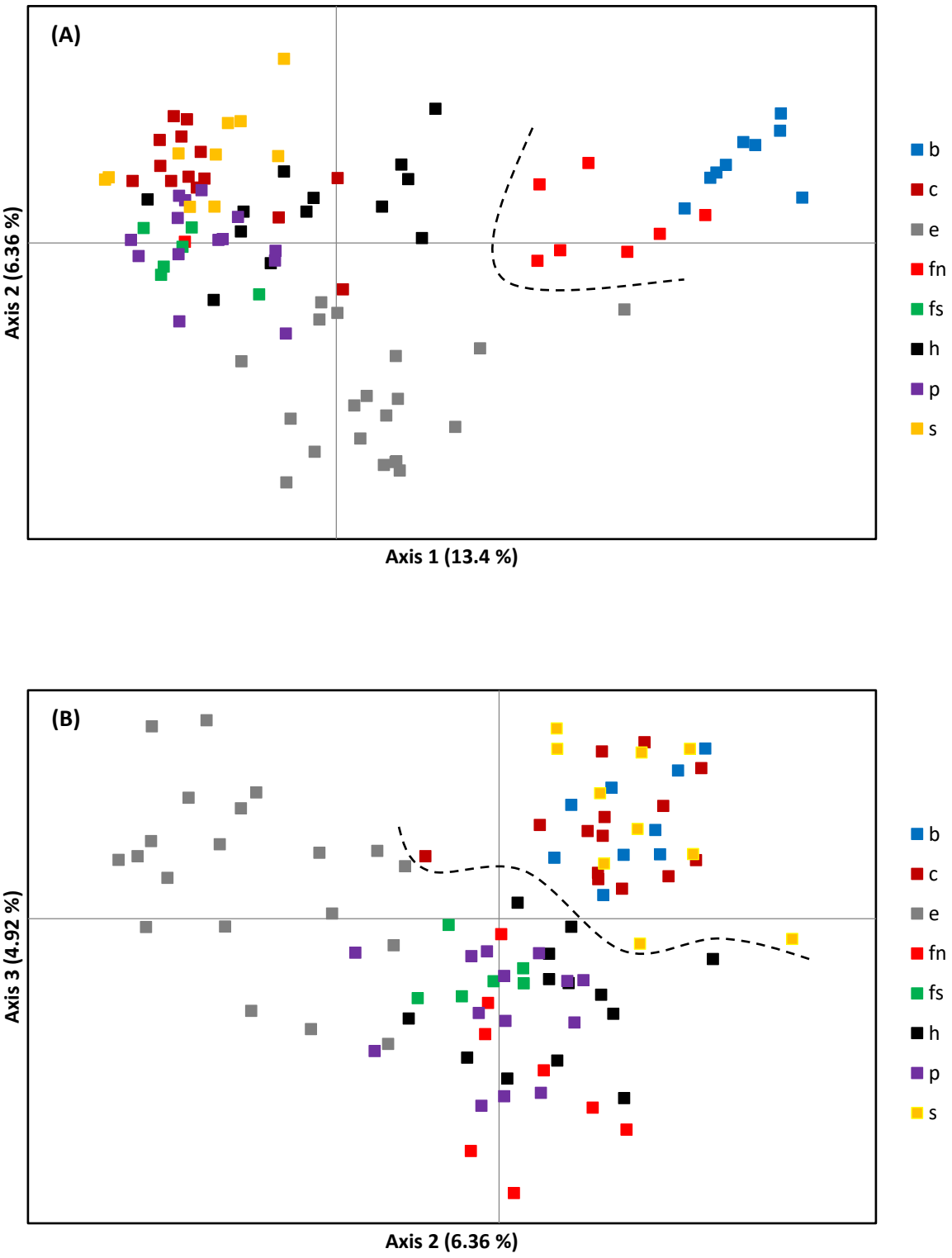


Fig. 8: PCoA ordination including the populations of the Gesäuse region. **(A)** shows the first two axes of the PCoA **(B)** shows axis 2 and axis 3 of the PCoA.

Table 3: The results of the AMOVA for the populations of the Gesäuse region. Letters in brackets symbolize population in the same group. (1) = Grouping north/south; (2) = Grouping north/south/Hartlschütt; (3) = grouping Gesäuse/Hartlschütt; (4) = grouping according to STRUCTURE; N = number of groups; d.f. degrees freedom; SS = mean sum of squares; F_{st} = general fixation index.

Grouping	N	Source of variation	d.f.	SS	Variance [%]	Fixation index
no groups	8	Among groups	7	442	24.97	F_{st} = 0.230*
		Within populations	86	1121	75.03	
(1) [b, c, s] [e, fn, fs, h, p]	2	Among groups	1	71	0.65	
		Among populations within groups	6	371	24.55	F_{st} = 0.252*
		Within populations	86	1121	74.81	
(2) [b, c] [e, fn, fs, h, p] [s]	3	Among groups	2	133	1.26	
		Among populations within groups	5	308	24.1	F_{st} = 0.254*
		Within populations	86	1121	74.65	
(3) [b, c, e, fn, fs, h, p] [s]	2	Among groups	1	61	1.02	
		Among populations within groups	6	381	24.54	F_{st} = 0.256*
		Within populations	86	1121	74.44	
(4) [e] [b, fn] [c, fs, h, p, s]	3	Among groups	2	240	14.99	
		Among populations within groups	5	202	13.97	F_{st} = 0.290*
		Within populations	86	1121	71.04	

*p < 0.001.

The Mantel test showed with R^2 = 0.02 (P < 0.005) almost no correlation between genetic and geographic distance (**Fig. 6/B**). **Fig. 9** shows the spatial autocorrelation of all 20 individuals of the population (e). Each size class included between 41 and 54 values. The results suggest that there is no significant gene flow above a distance of approx. 100 m.

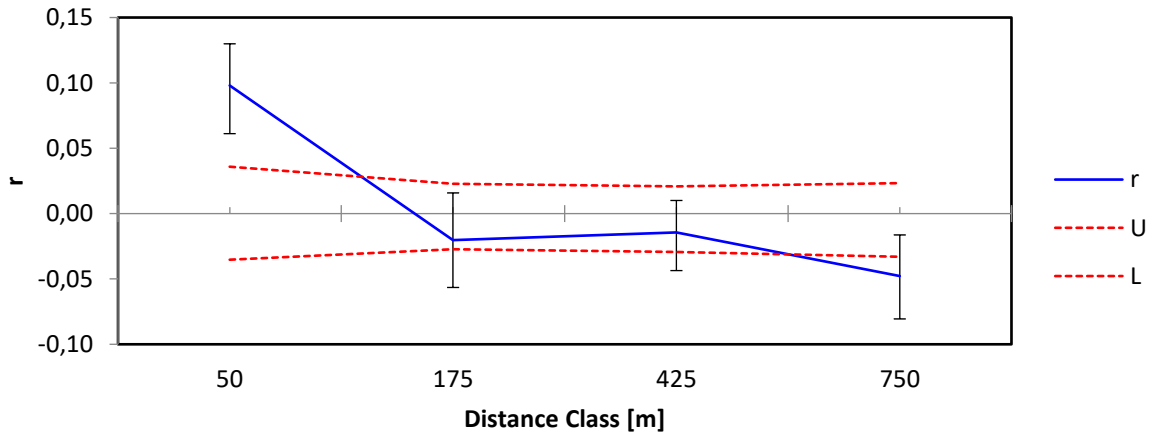


Fig. 9: Spatial autocorrelation of the individuals of the Gseng population (e). Error bars are situated in the middle of each class. U and L = upper and lower confidence limits bound the 95% confidence interval about the null hypothesis of No spatial structure for the combined data set as determined by permutation; error bars bound the 95% confidence interval about r as determined by bootstrap resampling.

The investigation of two large *Dianthus blandus* cushions (e104a/b/c and e301a/b/c) showed following results: The calculations suggest that all samples of cushion (e301) belong to the same genet due to

less than 10 differences between the three samples. 6 differences between loci were found between (e301a) and (e301b) and 3 differences were found between (e301a) and (e301c) as well as between (e301b) and (e301c). The samples (e104b) and (e104c) of cushion (e104) descend from the same genet and showed 2 differences over all loci. **Fig. 10** pictures a detail of the generated neighbor net. The sample triplet of cushion e301 grouped close together but sample e104a emerged slightly separated from sample e104b and e104c.

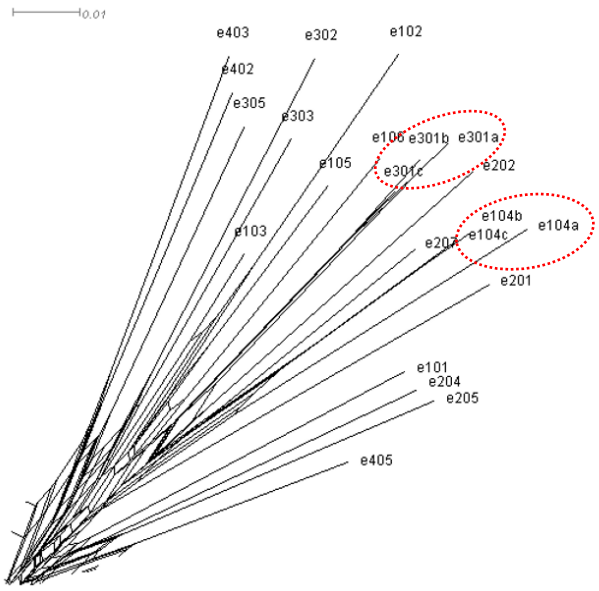


Fig. 10: Detail of the neighbor net analysis including the suspected clones.

Discussion

Genetic structure in the *Dianthus* sect. *Plumaria* (s. l.) in eastern Austria

The analysis of genetic structure did not entirely resolve the different populations according to their geographic distribution or their taxonomic status. Only *Dianthus sternbergii* in Italy showed a clear genetic differentiation from the other populations. The second axis of the PCoA, the STRUCTURE calculations as well as the results of the majority rule consensus tree support a separation of population (t) from all other samples. Population (t) also possessed the highest number of private fragments (5) which can also be seen as an indication for genetic differentiation. At least in the neighbor net analysis *D. neilreichii* and *D. hoppei* appear as moderately to well supported groups. Also the majority rule consensus tree and the pairwise F_{st} calculations support these two taxa. The populations of *D. neilreichii* and *D. hoppei* only exhibited a moderate number of private alleles.

There was no differentiation between the *Dianthus lumnitzeri* population of Hundsheim and the *D. blandus* populations of the Gesäuse region. The geographical distance between the two sites would have suggested a significant difference in the genetic variation. Due to these results, the Hundsheim population also introduces bias into the calculations of the Mantel–test which would otherwise show a higher correlation between genetic and geographic distance. Altogether these data do not provide support for the classification as applied in Fischer et al. (2008).

The three different *Dianthus blandus* regions Gröbming, Ramsau and Gesäuse showed no major differentiation from each other. The Gröbming population obtained a surprisingly high number of private fragments (4) in comparison to the other populations investigated, this could indicate a higher degree of isolation. The STRUCTURE analysis showed minor admixture between the Ramsau population of *D. blandus* and the Tolmezzo population of *D. sternbergii*. Also in the ordination of the PCoA some Ramsau samples were plotted between the Gesäuse and the Tolmezzo populations. There is some morphological evidence for higher similarity between the Ramsau populations of *D. blandus* and the Southern Alps *D. sternbergii* (Greimler, 1998).

The low resolution in the overall data gives a hint at the close relationship between the investigated taxa even though further data is necessary to clarify the taxonomic situation. In this context also homoplasy could have biased our data, especially the close relationship of *Dianthus lumnitzeri* and the Gesäuse populations seems to be implausible.

Genetic patterns of *Dianthus blandus* in the Gesäuse region

Although the populations in the Gesäuse region appeared to be quite isolated from one another due to high relief energy the present investigation showed no clear differentiation among populations. The Mantel–test showed no isolation by distance although the maximum distance between two sampled populations adds up to 6.8 km. Also the river Enns, which was assumed to be a significant barrier for gene flow did not appear to have a strong impact on the genetic structure. All analyses agree in low differentiation and less than 1% of the variation occurred between these two groups separated by the river (AMOVA, **Table 3**). F_{st} values denoting the differentiation among populations within groups were always found between 0.25 and 0.29 regardless the grouping in all three level AMOVAs.

In comparison to the study of Reisch and Bernhardt–Römermann (2014) the F_{st} value was low but still within the limits for a plant species which can be characterized as rare, but typical for plants with mixed mating systems (Reisch and Bernhardt–Römermann, 2014). Many plant populations who are considered as highly isolated or endangered by inbreeding depression show F_{st}–values of 0.40 or higher (Gaudeul et al., 2000; Travis et al., 1996; Cardoso et al., 2003). In this study the degree of differentiation among populations can in general be considered as moderate, this implies that a certain amount of gene flow still occurs and there is no evidence for elevated inbreeding.

Although no obvious biogeographic pattern could be detected, there was some structure on the small scale. A clear division among the samples within the Langgries ravine was observed in the PCoA as well as the STRUCTURE analysis. The two groups are separated by a 50 to 100 m wide and east to west oriented debris stream which leads to the Johnsbach river. This valley appears to be a strong barrier for gene flow, neither pollen, respectively pollinators, nor diaspores are likely to cross this narrow barrier mostly bare of any vegetation. In general diaspores of *Dianthus blandus* are only dispersed over short distances (Meusel and Mühlberg, 1979), thus it seems unlikely for them to successfully cross the debris stream. The two populations show distinct differences in their genetic diversity parameters. All values are clearly lower for the southern population. In contrary the diversity parameters for the northern population are high, even in comparison to the other investigated populations in this study. Additionally the norther Langgries population shows a strong relationship to the Kühgraben population which is located north of the river Enns without an obvious geographical connection.

The Kühgraben population has also some outstanding characteristics within the investigated Gesäuse populations. It exhibits a high degree of isolation with F_{st} values ranging from 0.34 to 0.47 in comparison to the other populations of the region, which mostly show values lower than 0.3. However, the Kühgraben populations and Langgries North seem to be closely related to each other, they share the lowest F_{st} with 0.214. The distance between the two populations amounts to 4.5 km and they are divided by the river Enns as mentioned above. Thus, one can only speculate about the reasons for the close relationship between the two populations, especially when taking into consideration, that the southern Langgries population shows no genetic similarity to its northern counterpart, although they are only separated by 100 m of debris. One explanation can be anthropogenic dispersal of plant parts or seeds, most of the ramets of Langgries North are found along an old gravel road and under power supply lines and plant material could have been transferred during forestry or road construction works or other human activities. The Kühgraben population shows a similar situation along to a gravel road and could therefore have the same origin or history as the Langgries population.

Also the Gseng population has shown outstanding genetic characteristics in comparison to the other populations of the region. Although it shows some admixture, the Gseng complex is slightly separated from all other investigated populations including the geographically close Haindlkar population. Therefore, the mountain ridge between the Gseng ravine and Haindlkar seems to be a very effective barrier for gene flow. Although different the pairwise F_{st}s do not show higher degrees of isolation from other Gesäuse populations. Within the Gseng population the spatial autocorrelation suggest unrestricted gene flow occurring up to about 100 m. The Gseng is not only one of the largest populations, it also shows relatively high genetic diversity values in contrary to other populations like Hartlschütt or Petergstamm. These populations are considered as large populations as well but they show only low genetic diversity.

In general observed genetic diversity parameters were lower in comparison to other studies on rare and closely related species like *Dianthus gratianopolitanus* (Putz et al., 2015) or *D. callizonus* (Gabel et

al., 2017) but the values are within the range of rare perennial plants with mixed mating systems (Reisch and Bernhardt–Römermann, 2014). The comparison of the diversity parameters showed low values for the populations Hartlschütt, Petergstamm and Langgries South. Surprisingly the populations Petergstamm and Hartlschütt were two of the largest populations in the Gesäuse region. Population genetic diversity, however, did not show any correlation with population size, or elevation and habitat type. In many cases large populations possess more genetic diversity than small ones as recent studies suggest (Gabel et al., 2017; Leimu et al., 2006; Ouborg et al., 2006; Putz et al., 2015;). On the other hand, in many alpine and subalpine plant species population size and genetic diversity can be independent from each other (Gabel et al., 2017). Investigations on the subalpine plant species *Epilobium fleischeri* showed no connection between population size and diversity which lead to the assumption that clonal reproduction may have an important role in population growth (Kuss et al., 2008).

In *Dianthus blandus* the ability of clonal reproduction by creating large plant cushions and runners has been observed (Meusel and Mühlberg, 1979; Köppl, 2016). Also in the present investigation the ability to create large cushions from a single genet which can at least overcome a distance of 1 m was observed. So it is possible that clonal growth and the disaggregation of genets due to relief energy accounted for the overall growth of populations. Additionally in situ sowing experiments by Köppl (2016) assessed high seed drains and low establishing rates for seedlings of *D. blandus* in the region which would also emphasize the importance of clonal reproduction for *D. blandus*.

AFLPs in polyploid plants – possible shortcomings and pitfalls

In the last decades the AFLP technology was a very popular tool to investigate genetic variation and structure of closely related taxa and populations, especially in plants. It is ideal for rapid generation of data when no prior sequence information is available and was used on diploid as well as on polyploid study organisms (Meudt and Clarke, 2007). However, there still remain problems when using AFLP marker on polyploid plant species like *Dianthus blandus* and its closest relatives.

The core issue of AFLPs is and has ever been homoplasy, fragments which appear to have the same size but are of different origin. There are many sources for this quasi homology, it can derive from scoring errors, co migration of non–homologous fragments or independent loss of fragments. This could result in underestimation of genetic diversity and loss of resolution of the AFLP data (Meudt and Clarke, 2007; Reisch and Berhardt–Römermann, 2014). Homoplasy increases with increasing taxonomic rank of the investigated individuals and with decreasing fragment size (Meudt and Clarke, 2007). Polyploid species and species with large genomes also tend to have a higher number of fragments, they contain a large number of repetitive DNA and retrotransposons. This can lead to many low intensity peaks who are difficult to score (Dufresne et al., 2013; Meudt and Clarke, 2007).

All investigated taxa included in this study are hexaploids (Dobeš and Greimler, unpublished). Elevated homoplasy due to high ploidy level of the *Dianthus* taxa could have resulted in an underestimation of the differentiation between the *Dianthus plumarius* subspecies and their close relatives. Especially the similarity between *D. lumnitzeri* and the *D. blandus* is unexpected and could be the result of homoplasy.

Conclusions and consequences for conservation

This study allows no major conclusions regarding relationships in the group of *Dianthus* sect. *Plumaria*. The AFLP method is probably inappropriate for certain larger scale investigations especially when polyploids are involved. There are however no practicable alternatives. Also within the Gesäuse region AFLP data provided poor resolution. However, no evidence for elevated inbreeding (as far as can be concluded from these data) was found for the populations in the Gesäuse. Only the Kühgraben population showed a high degree of differentiation from all other populations which could be a clue for strong isolation. Furthermore the Langgries ravine showed a clear separation between Langgries North and Langgries South. In general there is no immediate threat to the entire Gesäuse meta–populations from a genetic point of view. Gene flow seems to take place within reasonable distances. Many of the populations are large and do not show evidence of strong isolation. Local extinction, however, may occur in those segments or sub–populations that are trapped in closing forest gaps.

References

Literature

Avise, J.C., 2008. The history, purview, and future of conservation genetics, in: Carroll, S.P., Fox, C.W. (Eds.), Conservation biology: evolution in action. Oxford University Press, Oxford/New York, pp. 5–15.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F. Brochmann, C., Taberlet, P., 2004. How to track and assess genotyping errors in population genetics studies. Mol. Ecol. 13, 3261–3273.

Briggs, J.C., 2017. Emergence of a sixth mass extinction. Biol. J. Linn. Soc. 122, 243–248.

Cardoso, M.A., Provan, J., Powell, W., Ferreiram P.C.G., de Oliveira, D.E., 2003. High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae–Caesalpinioideae). Mol. Ecol. 7, 601–608.

CBDa. History of the Convention. https://www.cbd.int/history/ (accessed 26 June 2018).

CBDb. Aichi Biodiversity Targets. https://www.cbd.int/sp/targets#GoalC (accessed 26 June 2018).

Ceballo, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M., Palmer, T.M., 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Sci. Adv. 1, e1400253.

Cole, T.C., 2003. Genetic variation in rare and common plants. Annu. Rev. Ecol. Evol. Syst. 34, 213–237.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Dufresne, F., Stift, M., Vergilino, R., Mable, B.K., 2014. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state–of–the–art molecular and statistical tools. Mol. Ecol. 23, 40–69.

Earl, D.A., von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.

Ehrich, D., 2006. AFLPdat: a collection of R functions for convenient handling of AFLP data. Mol. Ecol. 6, 603–604.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. 1, 47–50.

Falush, D., Stephens, M., Pritchard, J.K., 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. 7, 574–578.

Fischer, M.A., Oswald, K., Adler, W. (Eds.), 2008. Exkursionsflora für Österreich, Liechtenstein und Südtirol, third ed. Biologiezentrum der Oberösterreichischen Landesmuseen, Linz.

Gabel, A., Sattler, J., Reisch, C., 2017. Genetic variation and performance of the alpine plant species *Dianthus callizonus* differ in two elevational zones of the Carpathians. Alpine Bot. 127, 65–74.

Gaston, K.J., Kunin, W.E., 1997. Rare–common differences: an overview, in: Kunin, W.E., Gaston, K.J. (Eds.), The biology of rarity. Chapman & Hall, London/Weinheim/New York/Tokyo/Melbourne/Madras, pp. 12–22.

Gaudeul, M., Stenøien, H.K., Ågren, J., 2007. Landscape structure, clonal propagation, and genetic diversity in Scandinavian populations of *Arabidopsis lyrata* (Brassicaceae). Am. J. Bot. 94, 1146–1155.

Gaudeul, M., Taberlet, P., Till–Bottraud, I., 2000. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Mol. Ecol. 9, 1625–1637.

Greimler, J., 1998. Vergleichende Untersuchungen an Populationen von *Dianthus plumarius* agg. (Caryophyllaceae). Bot. Jahrb. Syst. 120, 87–117.

Honnay, O., Jacquemyn, H., 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conserv. Biol. 21, 823–831.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

IUCN. The IUCN red list of threatened species. https://www.iucn.org/theme/species/our-work/iucn-red-list-threatened-species (accessed 11 June 2018).

Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 23, 1801–1806.

Kalisz, S., Nason, J.D., Hanzawa, F.M., Tonsor, S.J., 2001. Spatial population genetic structure in Trillium grandiflorum: the roles of dispersal, mating, history, and selection. Evolution. 55, 1560–1568.

Köppl, W., 2016. Gefährdungsanalyse der Populationen einer Flagship–Species, der zierlichen Federnelke (*Dianthus plumarius* subsp. *blandus*) im Nationalpark Gesäuse. Master's thesis Universität Wien, Wien.

Köppl, W., Oberklammer, I., 2015. Monitoring der zierlichen Federnelke *Dianthus plumarius* subsp. *blandus* im Nationalpark Gesäuse, Arbeitsbericht. http://www.nationalpark.co.at/forschung/veroeffentlichungen/pflanzen-pilze-und-flechten/3820-2015-monitoring-der-zierlichen-federnelke-dianthus-plumarius-subsp-blandus-im-nationalpark-gesaeuse-3820 (accessed 1 July 2018).

Kruckeberg, A.R., Rabinowitz, D., 1985. Biological aspects of endemism in higher plants. Ann. Rev. Ecol. Syst. 16, 447–479.

Kuss, P., Pluess, A.R., Ægisdóttir, H.H., Stöcklin, J., 2008. Spatial isolation and genetic differentiation in naturally fragmented plant populations of the Swiss Alps. J. Pant Ecol. 1, 149–159.

Leimu, R., Mutikainen, P., Koricheva, J., Fischer, M., 2006. How general are positive relationships between plant population size, fitness and genetic variation. J. Ecol. 94, 942–952.

Loveless, M., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. 15, 65–95.

Matthies, D., Bräuer, I., Maibom, W., Tscharntke, T., 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos. 105, 481–488.

Meffe, G.K., Viederman, S., 1995. Combining science and policy in conservation biology. Wildl. Soc. Bull. 23, 327–332.

Meudt, H.M., Clarke, A.C., 2006. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends Plant Sci. 12, 106–117.

Meusel, H., Mühlberg, M.H., 1979. Unterfamilie Silenoideae (Lindl.) A. Br., in: Rechinger, K.H. (Ed.), Illustrierte Flora von Mitteleuropa, Band 3, Teil 2, Pteridophyta Spermatophyta, Angiospermae Dicotyledones 1, second ed. Verlag Paul Parey, Berlin/Hamburg, pp. 947–1153.

Mucina, L., Grabherr, G., Ellmauer (Eds.), T., 1993. Die Pflanzengesellschaften Österreichs Teil 1–3. Gustav Fischer Verlag, Jena/Stuttgart/New York.

NP Gesäuse. Endemiten–Juwelen im Gesäuse. https://www.nationalpark.co.at/de/naturraum/besonderheiten/354-endemiten (accessed 5 June 2018).

Niklfeld, H. (Ed.), 1999. Rote Listen gefährdeter Pflanzen Österreichs, second ed. Austria Medien Service, Graz.

Ouborg, N.J., Vergeer, P., Mix, C., 2006. The rough edges of the conservation genetics paradigm for plants. J. Ecol. 94, 1233–1248.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics. 28, 2537–2539.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. 6, 288–295.

Pritchard, J.K., Stephens, M., Donnelly, O., 2000. Inference of population structure using multilocus genotype data. Genetics. 155, 945–959.

Putz, C.M., Schmid, C., Reisch, C., 2015. Living in isolation – population structure, reproduction, and genetic variation of the endangered plant species *Dianthus gratianopolitanus* (Cheddar pink). Ecol. Evol. 5, 3610–3621.

Rabinowitz, D., 1981. Seven forms of rarity, in: Synge, H. (Ed.), The biological aspects of rare plant conservation. John Wiley & Sons, Chichester/New York/Brisbane/Toronto, pp. 205–2017.

Rabitsch, W., Essl, F.(Eds.), 2009. Endemiten – Kostbarkeiten in Österreichs Pflanzen– und Tierwelt. Naturwissenschaftlicher Verein für Kärnten und Umweltbundesamt GmbH, Klagenfurt/Wien.

Reisch, C., Bernhardt–Römermann, M., 2014. The impact of study design and life history traits on genetic variation of plants determined with AFLPs. Plant Ecol. 215, 1493–1511.

Reed, D.H., Frankham, R., 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17, 230–237.

Rosenberg, N.A., 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. 4, 137–138.

Schlüter, P.M., Harris, S.A., 2006. Analysis of multilocus fingerprinting data sets containing missing data. Mol. Ecol. 6, 569-572.

Schmid, B., Matthies, D., 1994. Seltenheit und Gefährdung–populationsbiologische Grundlagen des Artenschutzes. Sci. Nat. 81, 283–292.

Schönswetter, P., Stehlik, I. Holderegger, R. Tribsch, A., 2005. Molecular evidence for glacial refugia of mountain plants in the European Alps. Mol. Ecol. 14, 3547–3555.

Schönswetter, P., Solstad, H., García, P.E., Elven, R., 2009. A combined molecular and morphological approach to the taxonomically intricate European mountain plant *Papaver alpinum* s.l. (Papaveraceae) –taxa or informal phylogeographical groups. Taxon. 58, 1326–1343.

Soulé, M.E., 1985. What is conservation biology. BioScience. 35, 727–734.

Travis, S.E., Maschinski, J., Keim, P., 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. Mol. Ecol. 5, 735–745.

Valente, L.M., Savolainen, V., Vargas, P., 2010. Unparalleled rates of species diversification in Europa. Proc. R. Soc. B. 277, 1489–1496.

Vekemans, X., 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale. Université Libre de Bruxelles. Belgium.

Vida, G., 1994. Global issues of genetic diversity, in: Leoschke, V., Tomiuk, J., Jain, S.K. (Eds.), Conservation Genetics. Birkhäuser Verlag, Basel/Boston/Berlin, pp. 9–19.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J. Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407–4414.

Weiss, H., Dobeš, C., Schneeweiss, G.M., Greimler, J., 2002. Occourrence of tetraploid and hexaploid cytotypes between and within populations in *Dianthus* sect. *Plumaria* (Caryophyllaceae). New Phytol. 156, 85–94.

Yeh, F.C., Yang, R., Boyle, T.J., Ye, Z., Xiyan, J.M., 1999. PopGene32. Microsoft Window-based Freeware for Population Genetic Analysis Version 1 32. Molecular Biology and Biotechnology Centre. University of Alberta, Edmonton, Alberta, Canada.

Maps

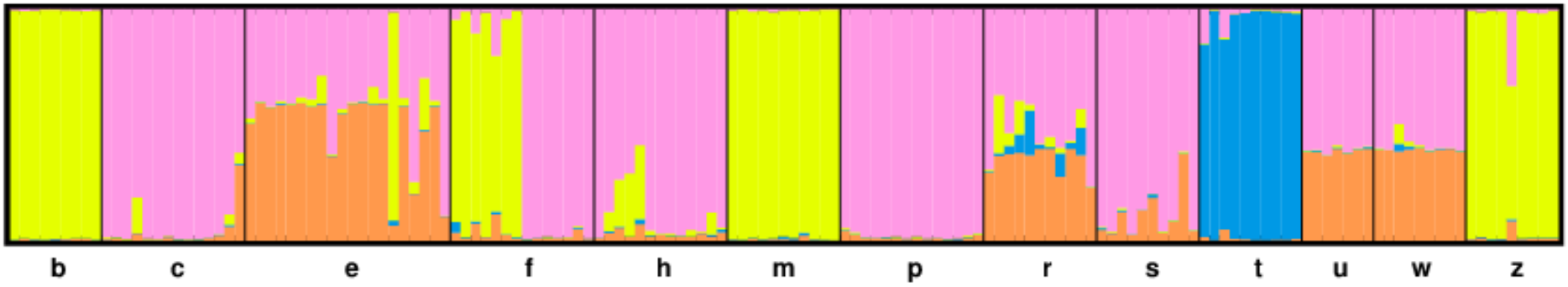
Natural Earth. www.naturalearthdata.com (accessed 4 July 2018).

GIS Steiermark. www.gis.steiermark.at (accessed 5 July 2018).

Voucher

Region	**Number**	**Link** (accessed 4 July 2018)
Gesäuse (A)	WU 0100299	http://herbarium.univie.ac.at/database/detail.php?ID=1382143
Ramsau (A)	WU 0099035	http://herbarium.univie.ac.at/database/detail.php?ID=1382143
Gröbming (A	WU 0099033	http://herbarium.univie.ac.at/database/detail.php?ID=1305607
Mödling (A)	WU 0099036	http://herbarium.univie.ac.at/database/detail.php?ID=1305695
Graz (A)	WU 0099034	http://herbarium.univie.ac.at/database/detail.php?ID=1305694
Hundsheim (A)	WU 0093649	http://herbarium.univie.ac.at/database/detail.php?ID=1168607
Tolmezzo (I)	WU 0099037	http://herbarium.univie.ac.at/database/detail.php?ID=1305610

Supplement Figure 1



Results of the STRUCTURE analysis for all samples with K = 4. Langgries (f) is shown as one population instead of Langgries North (fn) and Langgries South (fs).

Supplement Table 1

Coordinates of the subpopulations.

Site (Pop)	Subpop	Coordinates (WGS 84)	
Kühgraben (b)	b1	47.59451 N	14.602145 E
Weißenbachgraben (c)	c1	47.598915 N	14.641821 E
	c2	47.601703 N	14.641469 E
Gseng (e)	e1	47.56826 N	14.595896 E
	e2	47.56734 N	14.603015 E
	e3	47.569107 N	14.593695 E
	e4	47.569054 N	14.591436 E
Langgries North (fn)	fn	47.560689 N	14.572364 E
Langgries South (fs)	fs	47.558744 N	14.571794 E
Haindlkar (h)	h1	47.567007 N	14.610995 E
	h2	47.566583 N	14.618709 E
Petergstamm (p)	p1	47.555266 N	14.585561 E
Hartlschütt (s)	s1	47.610761 N	14.55585 E
Ramsau (r)	r1	47.433331 N	13.679162 E
Gröbming (w)	w1	47.468612 N	13.84945 E
Mödling (m)	m1	48.079336 N	16.26738 E
	m2	48.081002 N	16.275343 E
	m3	48.08211 N	16.276847 E
Graz (z)	z1	47.105012 N	15.383057 E
Hundsheim (u)	u1	48.123613 N	16.931665 E
Tolmezzo (t)	t1	46.406115 N	13.043054 E

Supplement Table 2

Coordinates of the individuals of the Gseng population (e).

Subpop	Individual	Coordinates (WGS 84)	
e1	e101	47.568196 N	14.595697 E
e1	e102	47.568205 N	14.59567 E
e1	e103	47.568214 N	14.595723 E
e1	e104a	47.568214 N	14.595829 E
e1	e105	47.568233 N	14.595936 E
e1	e106	47.568215 N	14.596029 E
e2	e201	47.56724 N	14.602777 E
e2	e202	47.56724 N	14.602817 E
e2	e204	47.567249 N	14.602817 E
e2	e205	47.567249 N	14.60283 E
e2	e207	47.567222 N	14.602817 E
e3	e301a	47.569107 N	14.593695 E
e3	e302	47.569287 N	14.593694 E
e3	e303	47.569314 N	14.593694 E
e3	e304_0	47.569314 N	14.59376 E
e3	e305	47.569359 N	14.593693 E
e4	e401_0	47.569549 N	14.591698 E
e4	e402	47.569549 N	14.591631 E
e4	e403	47.569369 N	14.5915 E
e4	e405	47.568874 N	14.591503 E

Supplement Table 3

Pairwise F_{st} including all populations. High values are highlighted in red and low values are highlighted in green; *D. p. blandus* populations are highlighted in bold; bland = *D. p. blandus*; hopp = *D. p. hoppei*; lumni = *D. p. lumnitzeri*; neilr = *D. p. neilreichii*; sternb = *D. p. sternbergii*; G = Gesäuse; Grö = Gröbming; Hu = Hundsheim; Möd = Mödling; Ra = Ramsau; To = Tolmezzo.

Dianthus		**bland G**	**bland G**	**bland G**	**bland G**	**bland G**	**bland G**	neilr Möd	**bland G**	bland Ra	**bland G**	sternb To	lumni Hu	bland Grö	hopp Graz
	Pop	b	c	e	fn	fs	h	m	p	r	s	t	u	w	z
bland G	b														
bland G	c	0.422*													
bland G	e	0.343*	0.221*												
bland G	fn	0.214*	0.305*	0.208*											
bland G	fs	0.467*	0.174*	0.185*	0.271**										
bland G	h	0.351*	0.147*	0.160*	0.161*	0.096*									
neilr Möd	m	0.361*	0.437*	0.368*	0.289*	0.438*	0.370*								
bland G	p	0.472*	0.172*	0.195*	0.284*	0.085*	0.117*	0.447*							
bland Ra	r	0.367*	0.233*	0.273*	0.210*	0.219*	0.163*	0.336*	0.227*						
bland G	s	0.463*	0.190*	0.269*	0.326*	0.196*	0.182*	0.453*	0.219*	0.240*					
sternb To	t	0.504*	0.424*	0.414*	0.373*	0.420*	0.353*	0.457*	0.429*	0.327*	0.427*				
lumni Hu	u	0.498*	0.269*	0.295*	0.337*	0.256*	0.220*	0.440*	0.275*	0.238*	0.309*	0.424*			
bland Grö	w	0.437*	0.226*	0.278*	0.293*	0.218*	0.186*	0.411*	0.213*	0.164*	0.149*	0.371*	0.224*		
hopp Graz	z	0.380*	0.425*	0.332*	0.299*	0.439*	0.322*	0.368*	0.427*	0.388*	0.429*	0.452*	0.436*	0.387*	

*p < 0.001.
**p < 0.005.

Supplement Table 4 A

Spearman's rank correlation with the parameters from **Table 1**. Significant correlations are highlighted in bold.

		Priv	h_j	IHS	$Frag_{poly}$	PI	N_k	N_{est}	NC_k	NC_{est}	HC
Priv	Correlation Coefficient										
	Sig. (2-tailed)										
	N										
h_j	Correlation Coefficient	0.097									
	Sig. (2-tailed)	0.741									
	N	14									
IHS	Correlation Coefficient	-0.099	**.960****								
	Sig. (2-tailed)	0.735	0.000								
	N	14	14								
$Frag_{poly}$	Correlation Coefficient	0.304	**.625***	**.559***							
	Sig. (2-tailed)	0.291	0.017	0.038							
	N	14	14	14							
PI	Correlation Coefficient	0.036	**.908****	**.925****	**.598***						
	Sig. (2-tailed)	0.902	0.000	0.000	0.024						
	N	14	14	14	14						

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Supplement Table 4 B

Spearman's rank correlation with the parameters from **Table 1**. Significant correlations are highlighted in bold.

		Priv	h_j	IHS	$Frag_{poly}$	PI	N_k	N_{est}	NC_k	NC_{est}	HC
N_k	Correlation Coefficient	0.117	-0.036	-0.036	-0.100	-0.090					
	Sig. (2-tailed)	0.803	0.939	0.939	0.831	0.848					
	N	7	7	7	7	7					
N_{est}	Correlation Coefficient	-0.154	-0.226	-0.080	-0.411	-0.141	**.855***				
	Sig. (2-tailed)	0.599	0.438	0.785	0.145	0.631	0.014				
	N	14	14	14	14	14	7				
NC_k	Correlation Coefficient	-0.065	0.100	0.100	-0.251	-0.120	**.905****	**.905****			
	Sig. (2-tailed)	0.891	0.832	0.832	0.587	0.799	0.005	0.005			
	N	7	7	7	7	7	7	7			
NC_{est}	Correlation Coefficient	-0.360	-0.267	-0.097	-0.494	-0.195	**.807***	**.964****	**.913****		
	Sig. (2-tailed)	0.207	0.357	0.741	0.072	0.505	0.028	0.000	0.004		
	N	14	14	14	14	14	7	14	7		
HC	Correlation Coefficient	0.277	-0.246	-0.369	0.217	-0.398	-0.245	-0.455	-0.355	-0.444	
	Sig. (2-tailed)	0.337	0.397	0.194	0.457	0.158	0.596	0.102	0.435	0.112	
	N	14	14	14	14	14	7	14	7	14	

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Supplement Table 5

Diversity indices and elevation of the different subpopulation in the Gesäuse region. Subpop = subpopulation; Ind_n = number of samples; $Frag_n$ = number of fragments per subpopulation; H_j = Nei's gene diversity; IHS = Shannon's information index; $Frag_{poly}$ = percentage of polymorphic fragments; PI = mean number of pairwise differences; Alt = elevation.

Subpop	Ind_n	$Frag_n$	H_j	IHS	$Frag_{poly}$	PI	Alt [m]
b1	9	121	0.113	0.171	33.5	27.11	1,060
c1	6	89	0.082	0.126	25.5	22.67	660
c2	8	94	0.114	0.172	34.0	27.68	700
e1	6	100	0.091	0.138	27.5	24.27	830
e2	5	101	0.089	0.135	27.0	25.60	1,080
e3	5	111	0.099	0.152	31.0	28.60	800
e4	4	91	0.077	0.116	22.0	23.50	760
fn	8	121	0.142	0.214	43.0	32.93	780
fs	6	89	0.076	0.117	24.0	21.60	800
h1	6	104	0.104	0.157	31.0	27.67	1,140
h2	7	102	0.101	0.155	32.5	26.29	1,180
p1	14	92	0.083	0.133	33.5	20.85	740
s1	10	98	0.094	0.145	31.5	22.47	980

Supplement Table 6

Spearman's rank correlation based on the data of **Supplements Table 5**. Significant correlations are highlighted in bold. Ind_n = number of samples; H_j = Nei's gene diversity; IHS = Shannon's information index; $Frag_{poly}$ = percentage of polymorphic fragments; PI = mean number of pairwise differences; Alt = elevation.

		Ind_n	H_j	IHS	$Frag_{poly}$	PI	Alt
Ind_n	Correlation Coefficient						
	Sig. (2-tailed)						
	N						
H_j	Correlation Coefficient	0.413					
	Sig. (2-tailed)	0.160					
	N	13					
IHS	Correlation Coefficient	0.439	**.995****				
	Sig. (2-tailed)	0.134	0.000				
	N	13	13				
$Frag_{poly}$	Correlation Coefficient	**.776****	**.829****	**.835****			
	Sig. (2-tailed)	0.002	0.000	0.000			
	N	13	13	13			
PI	Correlation Coefficient	-0.131	**.830****	**.813****	0.479		
	Sig. (2-tailed)	0.669	0.000	0.001	0.097		
	N	13	13	13	13		
Alt	Correlation Coefficient	-0.059	0.256	0.270	0.010	0.187	
	Sig. (2-tailed)	0.849	0.399	0.373	0.975	0.541	
	N	13	13	13	13	13	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Abstract

Today, the conservation of nature and organisms is more important than ever, thus it is of utmost necessity to gain knowledge of the condition of the habitat, life history traits and genetic patterns of rare and possibly endangered species. Especially rare species with fragmented and isolated populations often suffer from loss of genetic diversity and increasing differentiation between the populations due to genetic drift. The Austrian endemic *Dianthus plumarius* subsp. *blandus* is a species with fragmented populations due to its special niche, i. e. open habitats on stabilized scree. This investigation aimed at resolving the genetic patterns within and among the populations in the Gesäuse region. Additionally, closely related taxa of the *Dianthus* sect. *Plumaria* in eastern Austria were included to enlighten the genetic relationship between these poorly defined taxa. However, the employed method of AFLPs only partly resolved the relationship between the different taxa or the populations of the Gesäuse. Within the Gesäuse region no evidence for high degrees of isolation in most of the single populations was found. One population was found to be highly differentiated from all others in the region. Polyploidy in the investigated Dianthus species may have resulted in elevated amounts of homoplasy and lower resolution in the AFLP data.

Zusammenfassung

Das Thema Artenschutz ist heute wichtiger als jemals zuvor und nur durch sorgfältiges Studium der Lebensräume, Biologie und Genetik der Organismen können Prognosen für das Fortbestehen von Tier– und Pflanzenarten getroffen und entsprechende Strategien entwickelt werden. Besonders seltene Arten mit weit verstreuten Populationen leiden oft unter den Effekten von genetischer Drift und Isolation, was zur Folge hat, dass die genetische Diversität innerhalb der einzelnen Populationen abnimmt und die genetischen Unterschiede zwischen den Populationen zunehmen. Aufgrund seiner engen ökologischen Nische hat der österreichische Endemit *Dianthus plumarius* subsp. *blandus* eben solch ein Verbreitungsmuster. Das Vorkommen der Pflanze beschränkt sich vor allem auf Ruhschuttflächen und offene Standorte mit geringem Konkurrenzdruck. Das Hauptziel dieser Studie war die genetische Strukturierung und Diversität der Populationen im Gesäuse aufzuklären und darauf aufbauend eine Einschätzung für die Gefährdung der Pflanze abzuleiten. Weiters wurden nahverwandte Taxa der *Dianthus* Sect. *Plumaria* aus dem östlichen Österreich und Norditalien in die Untersuchungen inkludiert, um einen Einblick in die Verwandtschaftsverhältnisse dieser schwach differenzierten Gruppe zu erhalten. Mit der gewählten Methode der AFLPs konnte nur eine geringe Auflösung der Daten erzielt werden. Die Beziehungen der verschiedenen Taxa zueinander konnten nur eingeschränkt rekonstruiert werden. Auch innerhalb des Gesäuses zeigte sich keine klare genetische Struktur der verschiedenen Populationen. Generell scheinen die Populationen in diesem Gebiet nicht hochgradig voneinander isoliert zu sein. Weder konnte eine hohe Differenzierung zwischen den Populationen festgestellt werden noch scheinen die Populationen einen Verlust an genetischer Diversität erlitten zu haben. Nur eine einzige Population im Gesäuse wies einen hohen Grad an Differenzierung auf. Es ist möglich, dass die Polyploidie der untersuchten *Dianthus* Taxa zu erhöhter Homoplasie und damit geringerer Auflösung der AFLP Daten geführt haben.

List of Figures

Supplements

List of Tables

Supplements