Tuexenia 42: 297–319. Göttingen 2022.
doi: 10.14471/2022.42.010, available online at www.tuexenia.de

Population genetic study of *Alopecurus rendlei* in Luxembourg, Belgium, France and Germany

Populationsgenetische Untersuchungen zu *Alopecurus rendlei*-Populationen in Luxemburg, Belgien, Frankreich und Deutschland

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Abstract

In recent decades considerable changes in agricultural practices (especially increased fertilisation, early and increased cutting) have led to the decline of many characteristic grassland species. Formerly widespread species have become rare, and many grassland species are considered to be nowadays threatened and are on Red Lists. The continuous loss of species-rich grassland habitats remains unabated and affects even species that are still relatively widespread and abundant. The associated fragmentation and reduced gene flow will therefore impact the genetic diversity of local populations also of these species.

The present study concerns a formerly common grassland species that has experienced a sharp decline in large parts of the study area (Saar-Lor-Lux) within the last decades. *Alopecurus rendlei*, is an annual grass occurring in fragmented and sometimes very small populations on periodically humid to wet grassland sites with moderate nutrient supply. The aims of the study were to analyse genetic diversity and population genetic structure to study the potential consequences of habitat fragmentation and to determine if there is a relationship between genetic differentiation and geographical distance (IBD pattern). Leaf samples were collected along 25 meter transects and genotyped after DNA extraction using 13 newly designed microsatellite markers. Genetic variation within populations was assessed as N_P (private alleles), N_{AP} (alleles per population), N_E (effective alleles), H_O (observed heterozygosity), uH_E (unbiased expected heterozygosity) and F_{IS} (fixation index). Population structure was analysed by principal coordinate analysis (PCoA), AMOVA and STRUCTURE and a Mantel test was performed to analyse the relationship between geographic distance and genetic differentiation.

The studied populations showed a high genetic diversity (mean $N_P = 0.026$, $N_{AP} = 5.16$, $N_E = 3.17$, $H_O = 0.52$, $uH_E = 0.65$) and a weak genetic structure (STRUCTURE analysis). The assumption that at least some effects of genetic drift were already detectable in the studied populations could be confirmed, particularly in the highly isolated and very small population D11 in Germany (uHe = 0.56). Weak genetic differentiation among regions (1%) and a low differentiation of populations within regions (5%) strongly suggest that the populations were only recently fragmented, and that genetic drift remains largely unnoticeable mostly due to very large population sizes. Another non-exclusive explanation for the high genetic diversity and the weak genetic structure could be that there is ongoing gene flow between the extant populations, as shown by the significant isolation by distance pattern.

Manuscript received 16 June 2022, accepted10 October 2022
Published online 12 December 2022
Co-ordinating Editor: Brigitta Erschbamer

Conservation measures will be necessary to counteract a further decline of *A. rendlei* and the generally strong species loss in grassland. Ensuring extensive agricultural practices adapted to the species' habitat requirements plays an important role. Likewise, measures to counteract further fragmentation and decline of current population sizes to prevent a reduction of gene flow are needed to assure the long-term conservation of the species.

Keywords: conservation genetics, genetic variation, Greater Region, habitat fragmentation, new rare species, old rare species, threatened plant species

Erweitere deutsche Zusammenfassung am Ende des Artikels

1. Introduction

Structural changes in agriculture with their accompanying changes in grassland management, especially increased fertilisation and more frequent cutting regimes, have led to an increased homogenisation and a significant loss of biodiversity in grassland biotopes in recent decades. These changes have been at the expense of early-flowering, insect-pollinated species while favouring cut-tolerant and highly competitive species (DIERSCHKE & BRIEMLE 2008, WESCHE et al. 2012). Certain plant populations have disappeared completely or have been reduced to small, fragmented populations, so that the degree of isolation between populations has increased (HEINKEN 2009). Small and isolated plant populations with reduced gene flow among them are usually threatened by two population genetic consequences: genetic drift and/or inbreeding depression (ELLSTRAND & ELAM 1993, YOUNG et al 1996). Random genetic drift causes genetic diversity to decrease within a population, leading to reduced heterozygosity, and to an increase of genetic differentiation among populations (ELLSTRAND & ELAM 1993). A loss of genetic diversity usually leads to a reduced ability to adapt to changing environmental conditions, such as anthropogenically-induced changes in the environment or changes related to climate change (e.g. increased temperatures and water stress), and reduces the evolutionary potential of a population (FRANKHAM et al. 2017). Thus, fragmented, and small populations suffer a higher risk of extinction (MATTHIES et al. 2004). In small populations with low gene flow among them, inbreeding depression is another population genetic consequence that can occur (ELLSTRAND & ELAM 1993). Inbreeding affects the fitness of individuals and often leads to restricted reproduction and a reduced seed set in plants, lower dispersal potential and reduced germination and/or offspring survival (HEINKEN & WEBER 2013, FRANKHAM et al. 2017). Decline and fragmentation do not only affect rare species threatened with extinction, but increasingly also species classified so far as not or near threatened. In some cases, the latter can even record the greatest decline (BRULHEIDE et al. 2020). For this reason, it is even more important to take a closer look at these species, which have so far received less attention relating to nature conservation, and to halt their decline.

The present study deals with one such grassland species, Rendle's meadow Foxtail, *Alopecurus rendlei* EIG (synonym *Alopecurus utriculatus* (L.) SM.). This annual grass, which flowers between May and June, can be easily recognised by its bulbous upper leaf sheath, a relatively short ovate to elongated-ovate spike and a low height (maximum 50 cm) (JÄGER 2011, LAMBINON & VERLOOVE 2015; Fig. 1). *Alopecurus rendlei* prefers to colonise base-rich loam and clay soils that have moist, periodically humid to wet conditions with a moderate nutrient supply (SCHNEIDER 2019, BESCH et al. 2021).

Fig. 1. *Alopecurus rendlei*: **a)** Habitus (Photo: L. Besch, 01.04.2020), **b)** spike and **c)** the eponymous inflated leaf sheath (Photos: S. Schneider, 28.05.2008).

Abb. 1. *Alopecurus rendlei*: **a)** Habitus (Foto: L. Besch, 01.04.2020), **b)** Ähre und **c)** namensgebende aufgeblasene Blattscheide (Fotos: S. Schneider, 28.05.2008).

The species' main distribution area is southern and western Europe, but it is also found in Asia Minor and North Africa (LAMBINON & VERLOOVE 2015, GBIF 2021). The limit of its north-eastern range runs through Luxembourg, southern Belgium and the extreme west of Germany (Van ROMPAEY & DELVOSALLE 1979). Here, occurrences of *A. rendlei* show very different population sizes (Table 1). On the one hand, the species can cover large areas and be recognised from a distance by its reddish-brown colouration (Fig. 2), and on the other hand, individuals may be found only in well-defined small sub-areas, where they often grow in herds (REMACLE 2013, SCHNEIDER 2011, 2019, BESCH et al. 2021). As an annual plant, *A. rendlei* is highly influenced by competitive pressure and, accordingly, occurs more frequently in favourable sub-areas, in so-called microhabitats, which are characterised, for example, by higher moisture, such as in small flood depressions, and a generally patchy stand of surrounding vegetation (BESCH et al. 2021). In Belgium, Rendle's meadow Foxtail can be observed increasingly in pastures (REMACLE 2013). In Luxembourg, it prefers meadows or mowed pastures (SCHNEIDER 2019, BESCH et al. 2021); about 25 sites are currently confirmed for Luxembourg (BESCH et al. 2021, MNHNL 2000-).

Alopecurus rendlei is considered a differentiating species of two *Calthion* communities (SCHNEIDER 2011) in Luxembourg, where the species occurs mainly in the south-west (SCHNEIDER 2019). In the Red List of Luxembourg, *A. rendlei* is classified as near threatened (COLLING 2005); in Belgium, the species is considered to be critically endangered (SAINTENOY-SIMON et al. 2006). In France, the species is classified as near threatened (UICN FRANCE FCBN, AFB & MNHN 2018); in the Lorraine region, PARENT (2004) lists *A. rendlei* as rare, found only in certain areas, but then appearing in large numbers. In Germany, the species is considered to be critically endangered (METZING et al. 2018) – with only one currently known occurrence in Saarland. There are some historical observations

Fig. 2. View of one of the study sites in Luxembourg (L11) – the population can be recognized from a considerable distance by its reddish-brown coloration (Photo: L. Besch, 19.04.2021).

Abb. 2. Blick auf eine Untersuchungsfläche in Luxemburg (L11) – schon von Weitem ist die Population an der rot-bräunlichen Färbung zu erkennen (Foto: L. Besch, 19.04.2021).

of *A. rendlei* in Saarland (e.g. in Primstal) and in Rhineland-Palatinate (e.g. in the Moselle valley near Trier or in the Upper Rhine valley near Bad Dürkheim) (HAFFNER 1990, LANG & WOLFF 1993, HAND et al. 2016). Today, the occurrence between Schwalbach and Hülzweiler is the last known site in Germany (WEICHERDING & STAUDT 2006). In recent years, a strong decline of the species has been recorded by REMACLE (2013) for Belgium. A recent study confirmed a similar decline in Luxembourg (BESCH et al. 2021).

The present work is a follow-up of a morphological study of *A. rendlei* populations (BESCH et al. 2021). The aim of the present work was to analyse the genetic diversity and population genetic structure of *A. rendlei* populations in four geographical regions (Lorraine in France, Wallonia in Belgium, Gutland in Luxembourg and Saarland in Germany) in order to study the potential genetic consequences of habitat fragmentation, and to infer management recommendations to halt the decline of the extant populations. We asked the following questions: (1) Is there a relationship between genetic differentiation and geographical distance (isolation by distance pattern), assuming that the extant fragmented populations were once part of a larger, contiguous distribution area? (2) Does the genetic diversity within populations already show any negative effects of habitat fragmentation?

2. Methods

2.1 Study area

The study area is located in the Greater Saar-Lor-Lux region and covers an area stretching from the Grand Duchy of Luxembourg to the Saarland in Germany, Lorraine in France and Wallonia in Belgium (Fig. 3). The sampling site selection was largely based on the populations of a previous plant morphology study (BESCH 2020, BESCH et al. 2021). For various reasons three former sampling sites had to be substituted in 2021 (e.g. due to grazing at the time of the planned sampling). Nine national clusters of local populations were distinguished *ad hoc* by country and region: three clusters in Luxembourg (L1–L3), three clusters in Belgium (B1–B3), two clusters in France (F1–F2) and one cluster in Germany (D1). Four to five populations were sampled in each cluster, except for Germany, where only a single site is currently known. The populations were named using the code of the cluster and a serial number (e.g. L11, L12, L13, L14, L15; see Table 1, Supplement E1). A total of 38 populations were included in this study (Supplement E1). Sampling took place between 19 and 30 April 2021.

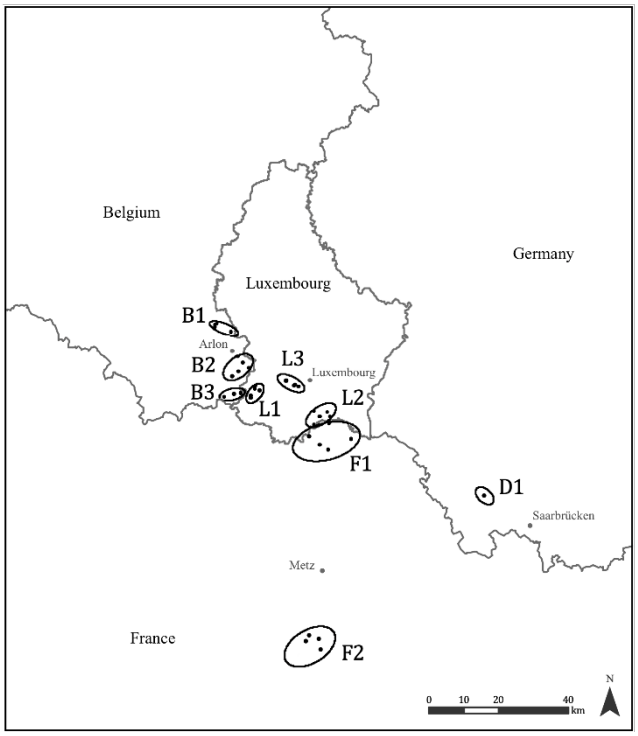

Fig. 3. Location of the studied 38 populations and national clusters of *Alopecurus rendlei* within the study area (B1 to B3 [Belgium], L1 to L3 [Luxembourg], F1 and F2 [France] and D1 [Germany]). Source map: Administration du Cadastre et de la Topographie Luxembourg, Landesamt für Vermessung, Geoinformation und Landentwicklung des Saarlandes and Département de la Géomatique du Service public de Wallonie.

Abb. 3. Lage des Untersuchungsgebietes mit den nationalen Clustern der 38 analysierten *Alopecurus rendlei*-Populationen (B1 bis B3 [Belgien], L1 bis L3 [Luxemburg], F1 und F2 [Frankreich] sowie D1 [Deutschland]). Quelle Kartengrund: Administration du Cadastre et de la Topographie Luxembourg, Landesamt für Vermessung, Geoinformation und Landentwicklung des Saarlandes und Département de la Géomatique du Service public de Wallonie.

Table 1. Locations and genetic diversity indices of the 38 studied populations of *Alopecurus rendlei* (abbreviations of population cluster and plots in Supplement E1) (N = population size, n = sampling size, N_P = private alleles, N_{AP} = alleles per population, N_E = effective alleles, H_O = observed heterozygosity, uH_E = unbiased expected heterozygosity, F_{IS} = fixation index, n. a. = not applicable).

Tabelle 1. Lage der 38 untersuchten *Alopecurus rendlei*-Populationen (Untersuchungsfläche im jeweiligen Cluster, Abkürzungen der Populationen in Anhang E1) und genetische Diversitätsindizien (N = Populationsgröße, n = Probenahmegröße, N_P = Anzahl an privaten Allelen, N_{AP} = Anzahl an Allelen pro Population, N_E = Anzahl an effektiven Allelen, H_O = berechnete Heterozygotie, uH_E = fehlerfreie erwartete Heterozygotie, F_{IS} = Fixation Index, n. a. = nicht angegeben).

Population	Lat. (°N)	Long. (°O)	N	n	N_P	N_{AP}	N_E	H_O	uH_E	F_{IS}
B11	5.7568	49.7533	3 951 360	15	0.000	5.077	3.055	0.436	0.641	0.283
B12	5.7519	49.7486	628 800	15	0.000	4.846	2.894	0.496	0.630	0.185
B13	5.7500	49.7523	n. a.	15	0.000	4.846	2.927	0.512	0.634	0.208
B14	5.8134	49.7357	5 755 860	15	0.077	5.462	3.482	0.521	0.673	0.200
B21	5.8393	49.6714	2 602 600	15	0.000	4.538	3.146	0.508	0.655	0.201
B22	5.8436	49.6321	350 240	15	0.000	5.385	3.297	0.537	0.668	0.178
B23	5.8186	49.6199	4 168 220	15	0.000	5.385	3.295	0.559	0.687	0.153
B24	5.8841	49.6408	9 508 500	15	0.000	5.154	3.384	0.532	0.686	0.192
B25	5.8597	49.6563	n. a.	15	0.000	4.923	3.005	0.582	0.652	0.092
B31	5.8528	49.5772	67 392	8	0.000	3.846	2.514	0.558	0.584	-0.043
B32	5.8248	49.5752	9 075 360	15	0.000	5.154	3.307	0.546	0.651	0.099
B34	5.7833	49.5655	n. a.	15	0.000	4.385	2.709	0.456	0.588	0.205
D11	6.8173	49.3109	80	15	0.000	3.538	2.297	0.477	0.555	0.094
F11	6.2048	49.5003	116 440	15	0.231	5.846	3.372	0.451	0.671	0.315
F12	6.2000	49.4319	3 585 040	15	0.000	5.923	3.523	0.536	0.683	0.160
F13	6.1680	49.4418	1 131 000	15	0.000	5.308	3.087	0.579	0.628	0.077
F14	6.1241	49.4653	6275 680	15	0.077	5.462	3.133	0.518	0.643	0.173
F15	6.2934	49.4583	3 010 200	15	0.000	5.385	2.947	0.562	0.651	0.102
F22	6.1693	48.9147	374 916	15	0.000	5.154	3.268	0.513	0.663	0.174
F23	6.1642	48.9416	5 094	15	0.077	5.462	3.261	0.544	0.680	0.151
F24	6.1245	48.9516	22 714 160	14	0.077	5.615	3.578	0.527	0.692	0.194
F25	6.1636	48.9253	n. a.	15	0.077	6.077	3.384	0.538	0.647	0.127
F26	6.1133	48.9446	30	15	0.000	5.385	3.460	0.631	0.685	0.012
L11	5.9295	49.5844	8 013 500	15	0.000	5.769	3.474	0.542	0.705	0.194
L12	5.9136	49.5964	164 256	15	0.000	5.692	3.375	0.503	0.670	0.203
L13	5.9106	49.5897	525 600	15	0.154	4.846	3.148	0.498	0.652	0.193
L14	5.8925	49.5717	238 986	15	0.077	6.077	3.767	0.552	0.678	0.145
L15	5.8949	49.5649	1 072 567	15	0.077	5.462	3.706	0.528	0.690	0.204
L21	6.1474	49.5304	55 608	15	0.000	4.462	2.810	0.513	0.621	0.136
L22	6.1992	49.5289	1 522 326	15	0.000	4.846	3.063	0.441	0.640	0.274
L23	6.1483	49.4973	16 660	13	0.077	5.077	3.148	0.508	0.662	0.202
L24	6.1691	49.5167	164 866	15	0.000	5.769	3.419	0.493	0.676	0.217
L25	6.2101	49.5173	67 403	15	0.000	4.462	2.721	0.448	0.589	0.216
L31	6.0663	49.5969	1 019 704	15	0.000	5.308	3.323	0.475	0.682	0.278
L32	6.0683	49.5983	3 434	11	0.000	4.462	2.864	0.555	0.654	0.103
L33	6.0827	49.5956	4 922 700	15	0.000	4.923	3.042	0.597	0.636	0.038
L34	6.0353	49.6098	21 888	15	0.000	5.308	3.153	0.513	0.654	0.172
L35	6.0344	49.6085	18 662	15	0.000	5.462	2.940	0.474	0.649	0.233

2.2 Sampling

Within the sampling sites – meaning the entire land use plot – *Alopecurus rendlei* often occurred only locally in specific sub-areas. Sampling took place in one sub-area per sampling site. For this purpose, a transect with a length of 25 m was laid out and leaves of 25 individuals (one leaf/individual) were randomly collected at regular distances. In small populations (a few m^2 in area, e.g. D11), the transect was laid out to the maximum possible length and leaves were collected from all individuals. GPS coordinates (WGS85 lat/long) of the start and end point of the transect were recorded for each population (Supplement E1). In order to obtain a homogeneous and uniform data set for the genetic analyses, usually 15 randomly selected samples (Table 1) from each population were used for DNA extraction and amplification in the laboratory.

The estimation of population sizes took place in 2020 in the previous study (BESCH et al. 2021; Table 1). At some study sites, population size could not be determined (e.g. grazing at the time of the planned sampling). We assumed that population sizes had not changed significantly within one year (study years 2020/2021).

2.3 DNA-extraction and amplification

A Maxwell® Rapid Sample Concentrator 48 (RSC 48) extraction robot was used for DNA extraction and purification from the collected leaves. It can perform 48 automated nucleic acid purifications (PROMEGA GMBH 2022) simultaneously, ensuring a fast and precise DNA extraction and purification process. We used the Maxwell® RSC Plant DNA Kit, which makes use of paramagnetic particles to bind the nucleic acids and purify the samples (PROMEGA GMBH 2022, LOTEN et al. 2018). The Go Taq® G2 Hot Start Master Mix (PROMEGA GMBH 2022) was then used to perform a polymerase chain reaction (PCR) to amplify the samples. A total of 13 loci were amplified using PCR. The primers of the individual microsatellite loci were specifically designed for *A. rendlei* (Table 2). The total PCR mix of 5 µl consisted of 0.5 µl of a 1:10 dilution of genomic DNA, 1.25 µl of the Go Taq® G2 Hot Start Master Mix (PROMEGA GMBH 2022) reaction mix, 2.75 µl distillate water and 0.5 µl of the primer (2µM) (Supplement E2). Reactions were performed using a Mastercycler Nexus PCR Thermocycler (Eppendorf SE, Hamburg, Germany). Cycling conditions consisted of an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles with denaturation at 95 °C for 30 seconds, an annealing at 60 °C for 90 seconds and 30 minutes of extension at 72 °C. A final extension of 10 minutes at 68 °C was followed by a cooling cycle up to 4 °C (Supplement E3). PCR products were separated using an automated sequencer (3730xl DNA Analyzer, Applied Biosystems, Warrington UK). The data were analysed using Geneious 11.1.5 (https://www.geneious.com, KEARSE et al. 2012).

2.4 Population genetic study

A total of 556 plant individuals from 38 *A. rendlei* populations were analysed and genotyped using 13 newly designed microsatellite markers (Tables 1, 2). Data analysis was carried out using the population genetics software GenAlEx 6.5 (PEAKALL & SMOUSE 2006, 2012), an add-in running in Microsoft Excel. The raw dataset was converted to a final matrix and processed in GenAlEx. With GenAlEx, certain genetic diversity indices were calculated, such as unique alleles (private alleles (N_P)), alleles per locus and population (N_{AL}, N_{AP}), effective alleles (N_E), the observed heterozygosity (H_O), the unbiased expected heterozygosity (uH_E) and the fixation index (F_{IS}). A two-dimensional PCoA (Principal Coordinates Analysis) was calculated, both at the individual and the population level. We used AMOVA (Analysis of molecular variance) to calculate the proportion of genetic variation due to differences among regions, among populations within regions, among individuals within populations and within individuals. A Mantel test was performed to examine whether there was a relationship between pairwise geographical distances and pairwise genetic differentiation among populations. The genetic and the geographical distance matrices, the latter calculated from the GPS coordinates (Table 1), were used as input data. Finally, a linear regression analysis was performed to investigate whether there was a correlation between population size and genetic diversity (unbiased expected heterozygosity uH_E).

Table 2. Characteristics of the developed 13 microsatellite primers for *Alopecurus rendlei*. bp = base pairs, Tº = annealing temperature.

Tabelle 2. Merkmale der 13 Mikrosatelliten-Marker von *Alopecurus rendlei.* bp = Basenpaare, Tº = „Annealing"-Temperatur.

Locus	Primer sequence (5'–3')	Allele size range (bp)	Repeat motif	Fluorescence dye used	Tº
Alo 11	F AACTGAAATAACGGAAATAAACTGC R AGTCTGCCATCTCGCTCTG	358-374	(AG)10	ROX	60 °C
Alo 42	F GCACACTCTTCTGCCACTTG R TCATGATGCACGCTGAAATAC	249-273	(TCG)8	HEX	60 °C
Alo 52	F TCGATCACGTGAGCTTGTTC R AGTGCACACACAGCTTACAAGTTAC	195-219	(GA)13	HEX	60 °C
Alo 89	F TTGGTCCCACATGTCTTGG R CGCAATGGTTGATGATGAAG	353-363	(CT)11	TAMRA	65 °C
Alo 93	F ATGCATTTGATGTTCCCAAAC R GAGCGGTGTGCTCAGTACTTC	131-165	(CT)21	FAM	60 °C
Alo 95	F GGAATAACTGTGGCGAGTGG R AA TGGAGAACGACGACGAAC	142-238	(AAT)14	ROX	60 °C
Alo 122	F ATTCGTGCTCATCGTCGTC R CATGCAGGTTTGACCCAAG	220-228	(AC)10	TAMRA	63 °C
Alo 22	F GCCACCTACTGCTCCATTTC R ATAGCTCCACCGGAAGACAG	221-245	(CT)19	ROX	60 °C
Alo 080	F ACTTACCAGCGAATGCCAAG R TGGCATGATTGTTTGGATTG	243-251	(TC)10	ATTO565	60 °C
Alo 80	F GATGCGCTTAGGGTTGACAC R AATGGAGGGCATACCATGTG	224-250	(GA)18	FAM	63 °C
Alo 867	F AGGGATTTGAATTCCACACG R TACGTCTTGCCTTGTTGCTG	175-220	(TTG)15	FAM	60 °C
Alo 98	F GCAAGCTGGTGAATGGAATC R AAATAGGATGGACACACGAAGG	233-279	(TA)20	FAM	59 °C
Alo 64	F GACAGAGGTACGTCAAGGAACTG R GGTTATGTAAACGACAGCCCTAAC	270-306	(GAA)17	FAM	60 °C

The genetic structure of *A. rendlei* populations was also investigated using STRUCTURE 2.3.4 (PRITCHARD et al. 2000). To determine the number of genetic clusters (*K*), ten independent runs with $K = 1–20$ were performed with a Markov Chain Monte Carlo (MCMC) iteration of 10^6 and a burn-in period of 10^5. A model with correlated allele frequency assuming admixture was used. The selection of the *K* value that best described the structure of the *A. rendlei* populations was made using the delta *K* method of EVANNO et al. (2005) (Supplement E4). This selection is based on the individual *K*-values and their standard deviations and was carried out in Clumpak (KOPELMAN et al. 2015). Clumpak was also used to plot the individual barplots of cluster membership.

3. Results

3.1 Genetic diversity

The number of alleles per locus (N_{AL}) varied between 1 and 11 alleles. Within nine of the 38 studied populations, private alleles (N_P) could be found, with the highest number of N_P observed in populations F11 ($N_P = 0.231$) and L13 ($N_P = 0.154$) (Table 1). The mean number

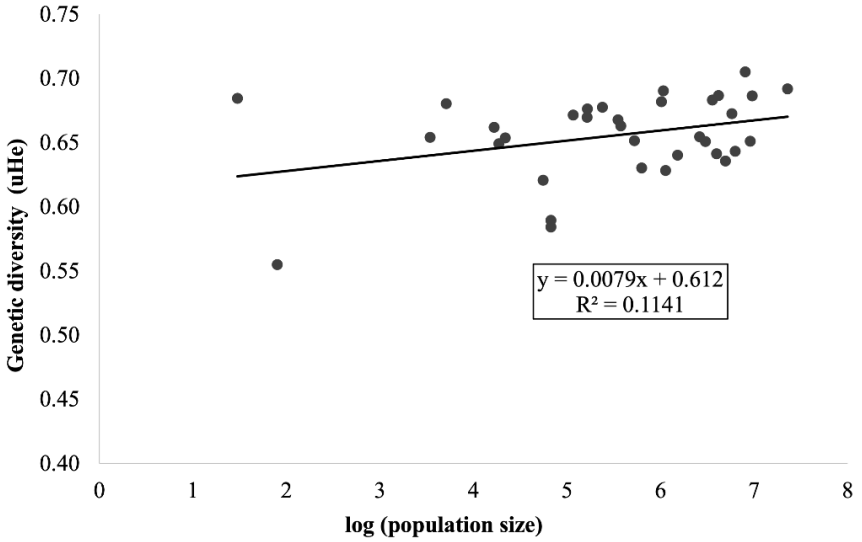

Fig. 4. Relationship between population size (log scale) and genetic diversity (unbiased expected heterozygosity uH_E); $r = 0.34$; $p < 0.05$.

Abb. 4. Beziehung zwischen logarithmierter Populationsgröße und der genetischen Diversität (fehlerfreie erwartete Heterozygotie uH_E) der *Alopecurus rendlei*-Populationen; $r = 0{,}34$; $p < 0{,}05$.

of alleles per population N_{AP} varied from 3.54 to 6.08 (overall mean 5.16) and the mean number of effective alleles N_E ranged from 2.30 to 3.77 (overall mean 3.17). The unbiased expected heterozygosity uH_E was only slightly overestimated and varied between 0.56 and 0.71 (mean 0.65) in comparison to the observed heterozygosity H_O with a mean of 0.52 (values between 0.44 and 0.63). The fixation index F_{IS}, also called the inbreeding coefficient, ranged between -0.04 and 0.32 with a mean value of 0.17 (Table 1). There was a significant relationship ($r = 0.34$, $p < 0.05$) between log population size and genetic diversity uH_E (Fig. 4). The trend line indicated that genetic diversity increases only slightly with population size (slope $m = 0.0079$). Some individual populations (B31, D11, L24) demonstrated lower values for the genetic diversity indices (Table 1, Supplement E5). The very small population D11 had the lowest genetic diversity ($uH_E = 0.56$). The Belgian population B31 was also characterized by a reduced genetic diversity ($uH_E = 0.58$).

3.2 Population genetic structure

The PCoA analysis at the individual level was based on the pairwise individual-by-individual genetic distance matrix and mapped all *Alopecurus rendlei* individuals of all populations (Supplement E6). The first axis explained 6.06%, the second axis 4.50% and the third axis explained 4.20% of the variance. Individuals of separate populations were widely distributed in the graph and there was no clear genetic structure reflecting geographic origin. A second PCoA, based on the pairwise F_{ST} genetic distance matrix, was performed at the population level (Fig. 5). The first axis explained 12.12%, the second 10.10% and the third 8.47% of the variance. Most of the populations were close to axis 1, with populations B11,

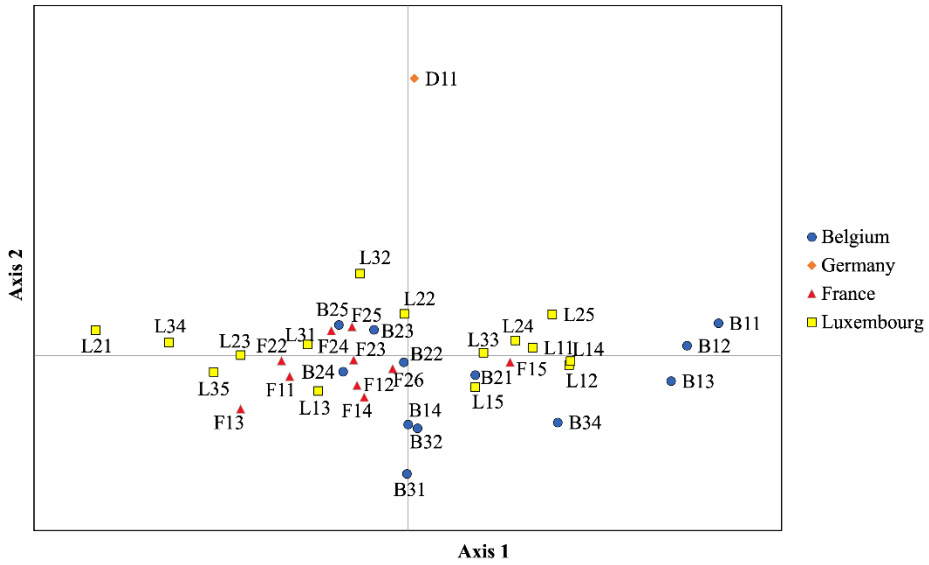

Fig. 5. Two-dimensional principal coordinates analysis (PCoA) of the 38 studied populations with different colours for different regions (for abbreviations see Supplement E1). The first axis explains 12.12% and the second axis explain 10.10% of the variation.

Abb. 5. Biplot zur PCoA auf Populationsebene. Abbildung der 38 Populationen mit unterschiedlichen Farben für die verschiedenen Regionen (Abkürzungen siehe Anhang E1). Die erste Achse erklärt 12,12 % und die zweite 10,10 % der Varianz.

B12 and B13 in Belgium slightly off on the positive side, at a marked distance from population B14 from the same cluster. The same was true for population L21, located on the negative side of axis 1, with a considerable distance to the remaining L2 populations. Population D11, situated in the upper positive part along the 2nd axis, stood out from the rest of the populations. Population B31 was slightly off in the opposite direction to D11 on the negative part of the 2nd axis.

The AMOVA showed only a very weak differentiation among regions (1%) and the differentiation of populations within regions was low (5%; Table 3). The F_{ST} value of 0.05 was significantly different from zero ($p < 0.001$), revealing a significant genetic differentiation among the studied *A. rendlei* populations. The molecular variation among individuals within populations was 21%. The highest proportion of molecular variation was due to the variance within all *A. rendlei* individuals within the populations (73%).

In the analysis of the population structure by STRUCTURE, the delta *K* method of EVANNO et al. (2005) suggested $K = 3$ as the best possible upper hierarchical level, which most closely describes the genetic structure of the populations (Supplement E4). Most populations showed a high level of admixture and a clear population structure could not be distinguished (Fig. 6). However, based on the dominant cluster per population, it was possible to group some populations together, genetically. There was a Belgian group, with populations B11, B12, B13 and population B31 dominated by the violet cluster. A group with population D11 in Germany and population L21 in Luxembourg showed a dominance of the blue cluster. A last group with the population L13, distanced from populations L11, L12, L14, L15 of the same cluster L1, had a high dominance of the orange cluster.

Table 3. Analysis of molecular variance (AMOVA) of the studied *Alopecurus rendlei* populations (df = degrees of freedom, Sum. Sq. = sum of squares, Mean Sq. = mean sum of squares, Est. Var. = estimated variance).

Tabelle 3. AMOVA (Analysis of molecular variance) der untersuchten *Alopecurus rendlei*-Populationen (df = Freiheitsgrade, Sum. Sq = Quadratsumme, Mean Sq. = Mittelwert Quadratsumme, Est. Var. = geschätzte Varianz).

	df	Sum. Sq.	Mean Sq.	Est. Var.	%
Among regions	3	81.4	27.1	0.1	1
Among populations	34	386.0	11.4	0.2	5
Among individuals	518	2716.5	5.2	0.9	21
Within individuals	556	1868.0	3.4	3.4	73
Total	1111	5051.8		4.6	100

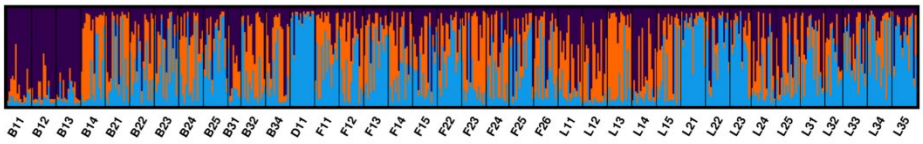

Fig. 6. Genetic structure of the analysed *Alopecurus rendlei* populations. STRUCTURE results for $K = 3$.

Abb. 6. Genetische Struktur der *Alopecurus rendlei*-Populationen; aus den Ergebnissen von STRUCTURE für $K = 3$.

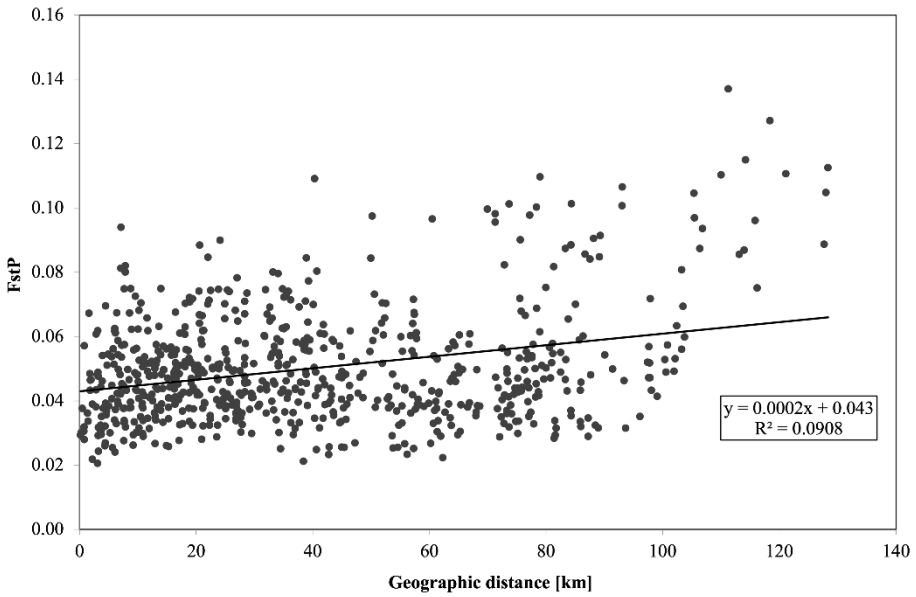

Fig. 7. Relationship between the pairwise geographical and genetic distances among all studied *Alopecurus rendlei* populations ($R^2 = 0.0908$, $p = 0.014$; Mantel test).

Abb. 7. Verhältnis der paarweisen geografischen Distanzen zu den paarweise genetischen Distanzen zwischen allen untersuchten *Alopecurus rendlei*-Populationen ($R^2 = 0{,}0908$, $p = 0{,}014$; Mantel-Test).

3.3 Isolation by distance

The Mantel test showed a weak but significant relationship between geographical distance among populations and genetic differentiation (isolation by distance, IBD; $p = 0.014$) (Fig. 7). The trend line of the linear regression showed a very flat slope of $m = 0.0002$, indicating a low isolation by distance and a high gene flow among populations. This can also be seen in the low F_{ST} values, which increased to a maximum of 0.140 with an intercept of 0.043. With a coefficient of determination $R^2 = 0.0908$, the linear model explained only about 9% of the variation in the data.

4. Discussion

Overall, the genetic diversity within the studied *Alopecurus rendlei* populations was high. Comparing the diversity values with those from similar studies, such as that of DUWE et al. (2018) on wind pollinated *Crepis mollis* (Jacq.) Asch populations, DUTECH et al. (2004) on *Vouacapoua americana* Aubl., BYARS et al. (2009) on *Poa hiemata* Vickery or LAST et al. (2013) on *Dactylis glomerata* L., the *A. rendlei* populations can be classified as genetically diverse. High genetic diversity typically occurs in large populations (ELLSTRAND & ELAM 1993). Our results showed a weak but significant positive correlation between population size and genetic diversity. Overall, most of the analysed populations showed very large population sizes (mean N = 2 487 084); only four populations (D11, F23, F26, L32) were smaller than 10 000 individuals (see Table 1). In a few populations (B31, D11 and L24), genetic diversity was reduced. As expected, the lowest genetic diversity occurred in one of the smallest populations (D11, N = 80). In other, less isolated populations (such as B31 or L24), there was a less pronounced decrease of genetic diversity. The assumption that the effects of genetic drift may already be observable in very small and isolated populations (like D11) could be confirmed. Interestingly, the smallest (N = 30) population F26 had the highest H_o (Table 1). One explanation could be that this very small population (still) stores "old" genetic variation from a time when the population was much larger. However, such time lags between population size reduction and reduction of genetic diversity, have mainly be discussed for long-lived species, where "old individuals" still carry "historical" genetic variation (KROPF 2012). This phenomenon however is not likely in an annual species like *A. rendlei*. Another reason could be, that in this population a massive decline took place quite recently and that genetic drift have not yet a chance to take effect. This could be an explanation as during the sampling, constructions were going on nearby the sampling site. High genetic diversity within populations may increase the evolutionary potential, that is the ability of a species to adapt to changing environmental conditions. This adaptability is an important function of animal and plant species to cope with any abiotic changes and to adapt if necessary (FRANKHAM et al. 2017). For *A. rendlei*, this could mean that this species with genetically diverse populations and a short generation time (annual species) may be able to locally adapt to changes, especially under the scenario of climate change and other changes in environmental conditions. However, changes in management practices inducing an increase in competition due to ever higher loads of mineral fertiliser and early silage cutting may jeopardize the long-term survival of the extant populations. Private alleles could only be found in nine of 38 populations (24%), a rate which can be classified as low (see DUWE et al. 2018).

Our results showed that the *A. rendlei* populations were only weakly differentiated, genetically. Based on the results of the AMOVA, the partitioning of the genetic variability among regions was only 1% whereas the differentiation among populations within regions was 5%. The genetic variation among populations with an F_{ST} value of 0.05, was significant, but small (see DUTECH et al. 2004, CLARKE et al. 2013). By far most of the genetic variation was within populations. The results of the PCoA and the STRUCTURE analysis confirmed that the small and isolated population in Germany is genetically distinct and that genetic drift already played a role here. The same was true for three out of four populations (B11, B12, B13) from the Belgian cluster B1. The genetic structure of the fourth population B14 from cluster B1 was more similar to populations in the neighbouring clusters B2 and B3. These statements are also consistent with the results of the PCoA: population D11 stands out from the rest of the populations. Within the Belgian B1 cluster the population B14 is genetically most distant from the populations B11, B12, and B13.

In comparison to other rare or increasingly rare plant species in Luxembourg such as *Pulsatilla vulgaris*, with a very strong and clearly delineated genetic structure (BERNARDONI 2020), the genetic differentiation among *A. rendlei* populations is very weak. The generally shallow genetic structure can be an indicator for a relatively recent habitat fragmentation where its negative effects are not very strong yet. In a former study, it was already assumed that the study area once contained large contiguous populations (BESCH 2020, BESCH et al. 2021). Historical records confirm this assumption with regards to the area containing the last known population in Germany, despite the species always being rare, even in the past. Between 1934 and 1986, the species could still be sighted in the Primstal valley in Saarland and in the Mosel valley in Rhineland-Palatinate (HAFFNER 1990); on the Lower Prim, BETTINGER (1996) reported the disappearance of a known population as late as 1996.

Fragmentation and increased geographical distance between populations usually lead to restricted gene flow (FRANKHAM et al 2017). Such a relationship could be observed for the widespread wind-pollinated *Fagus sylvatica* L. (JUMP & PEÑUELAS 2006). Over the last 600 years, increased fragmentation of forest stands in Central Europe has led to significant negative consequences in terms of higher inbreeding rates and loss of genetic diversity (JUMP & PEÑUELAS 2006). A similar phenomenon was observed in the rare wind-pollinated species *Stipa capillata* (HENSEN et al. 2010). Despite a considerable, recent fragmentation of the investigated *A. rendlei* populations, we found a weak, but significant correlation between genetic differentiation and geographical distance (IBD pattern), indicating gene flow among populations.

Gene flow between plant populations can occur via the transport of pollen and the dispersal of seeds (LOVELESS & HAMRICK 1984) or other diaspores (HEINKEN 2009), whereby pollen transport – in contrast to seed exchange – plays a superior role in gene flow (ELLSTRAND 1992). The degree of isolation of a population as well as the type of pollen transport (wind/insect pollination) and the dispersal potential of the seeds are decisive for the genetic exchange between populations (HEINKEN 2009). In general, pollen dispersal increases with decreasing spatial distance from the donor population, both for insect-pollinated species and for wind-pollinated species, to which *A. rendlei* belongs (ROGNLI et al. 2000, BUTCHER et al. 2020). Pollen dispersal by wind mainly depends on wind direction, wind speed, duration of the wind (DAMIALIS et al. 2005), neighbouring vegetation and topography of the area (ROGNLI et. al 2000). Pollen reception decreases with increasing geographical distance from the donor plant (COPELAND & HARDIN 1970). In our study area, some of the populations were very close to each other, separated by only a few (one to 10) km as the crow flies,

except population clusters D1 and F2. The pollen collection efficiency of the closely related *Alopecurus pratensis* is estimated to range between 5% and 20% and is strongly dependent on the size and morphology of the spike as well as the spike's position in relation to the ground (CRESSWELL et al. 2010). As the spike of *A. rendlei* is much shorter, pollen collection efficiency is expected to be lower. *Alopecurus rendlei* can reach a maximum height of 50 cm and occasionally flowers as early as the end of April. As an annual plant, it can be highly affected by competition; the species is often already in the flowering stage when the surrounding vegetation is still in the vegetative phase. Comparing the wind-pollinated species *A. rendlei* with an insect-pollinated species such as *Arnica montana* L., the latter showed a clear correlation between geographical and genetic distance and thus a clear IBD (MAURICE et al. 2016) in comparison to the weak IBD found in *A. rendlei*.

An explanation as to why *A. rendlei* populations are genetically very similar could therefore be that gene flow among populations due to wind pollination is still high. Based on the weak IBD, the location of the populations, their overall huge population sizes, as well as the early period of flowering and the conditions of the surrounding vegetation, it can be assumed that there is still sufficient pollen dispersal and pollen collection by *A. rendlei* in the study area. Thus, there may still be sufficient genetic exchange between most of the populations. However, a definite statement would only be possible based on pollen dispersal experiments, which are very time-consuming and complex.

The effects of fragmentation may increase in the future and the consequences of genetic drift will increase over time when populations become much smaller and isolated. In the case of *A. rendlei*, a strong decline of the species occurrences has been recorded in Luxembourg (BESCH et al. 2021) as well as in Belgium (REMACLE 2013) and in Germany (BETTINGER 1996, WEICHERDING & STAUDT 2006) in the last decades. Older floristic publications showed that *A. rendlei* used to be more widespread (KOLTZ 1873, KROMBACH 1875, HAFFNER 1990, BETTINGER 1996, PARENT 2004). It can be assumed that the species once occurred over a large contiguous area, that the study populations were formerly part of a larger metapopulation and have only recently been separated from each other through habitat fragmentation. *Alopecurus rendlei* may therefore be described as an example of a new rare species. In contrast, old rare species like *Astragalus exscapus* occur naturally in very isolated and limited areas (BECKER et al. 2011). Populations of the grass species *Poa badensis* Haenke ex Willd. in Western Germany showed low genetic diversities but still had high reproductive success (PLENK et al. 2019). Our results indicate that in *A. rendlei,* the effects of genetic drift are mainly detectable in the very small and isolated German population; in a few others (e.g. B31, L24) there are some indications that genetic drift may affect the genetic structure in the future if habitat fragmentation persists or increases and/or population sizes decrease. Therefore, from a conservation point of view the latter negative developments as well as a further reduction of gene flow among populations should be avoided to counteract the negative consequences of genetic drift or inbreeding depression. For this reason, it is imperative to establish a long-term habitat management concept, to connect extant *A. rendlei* populations and create habitat corridors to conserve and optimise gene flow. Establishment of new populations and reintroductions through seed transfer may also play a crucial role here.

5. Conclusions and conservation management

Alopecurus rendlei should be considered as an endangered or even critically endangered plant species in Luxembourg as well as in Belgium and in Germany, as it partly occurs in very fragmented and isolated populations, and especially because the number of populations has strongly diminished in recent decades (BESCH et al. 2021). Our initial concern that these populations may already show negative consequences of genetic drift, could only be partially confirmed. Based on our results, there are two explanations that can support the assumption that *A. rendlei* may be considered as a new rare species where the negative effects of fragmentation are not yet observable: there is still sufficient gene flow among populations as the fragmentation of the populations has occurred only recently, and that so far, negative effects of genetic drift are only detectable in a few small and isolated populations because of otherwise still large population sizes.

Agricultural intensification has led to the disappearance of well-developed species-rich meadows (DIERSCHKE 1997, RUTHSATZ et al. 2004). Accordingly, it is no longer only the oligotrophic grassland communities whose conservation must be ensured by suitable measures, but also increasingly the distinctive or species-rich meadows and their flora. It is important to stop the further decline of characteristic grassland species – like *Alopecurus rendlei* – and to prevent the extant populations from becoming even smaller and genetic drift from continuing its negative effects. It is also important to prevent further fragmentation of populations and gene flow from being interrupted. Our results show that it is not too late to take conservation measures, but they should be implemented as soon as possible. Since the seed bank of grasses and especially of annual *Poaceae* is poorly developed and/or very short-lived, a potentially rapid loss of local populations must be expected. Once a population of *A. rendlei* has become extinct, a new population can be established by seed dispersal or can be initiated through artificial re-introduction.

The creation and maintenance of stepping stones in the biotope network are also of importance. A good option is to establish populations in extensive grassland sites that fulfil all necessary site-ecological criteria through seed transfer, either via transmission of hay or sowing. It also makes sense to re-establish the species at historical sites if the target site remains suitable or can be restored; since the population differentiation among populations is shallow and the fact that populations are genetically very similar, a mix of several populations could be used as source material to increase genetic diversity while establishing new populations. Above all, the highly isolated population D11, which is threatened with genetic drift, should be enlarged by reinforcement. For this purpose, new *Alopecurus rendlei*-populations should be established in the vicinity of the extant populations to conserve regional genetic diversity and maintain gene flow.

In addition, it is important to adapt the habitat management to the specific requirements of the characteristic grassland species at all sites, especially where the greatest recent decline has been recorded, as well as at newly established sites. In general, extensive grassland management with late first mowing and very little – ideally no – mineral fertilisation is aimed for. For the annual species *A. rendlei*, an increase in competition from tall or fast-growing species in the scenario of increased fertilisation can be expected to occur in a very short time. Contractual nature conservation is an appropriate instrument to avoid the negative effects of increased mineral fertilisation and early silage cutting (MÉMORIAL 2017, WOLFF et al 2020). In order to be able to react to possible population changes at an early stage, it is important to maintain a regular monitoring scheme. The sites should be visited

regularly, e.g. every six years, and population sizes should be estimated. As fragmentation progresses, genetic diversity can decrease in small populations due to genetic drift, so genetic diversity within populations should be kept under close observation.

Interesting research questions would be, for example, to determine the breeding system of the species, as it is unknown if *A. rendlei* is outcrossing or selfing. Another idea is to analyse the fitness of the individuals in the populations on the basis of seed set and germination rate, in order to investigate further aspects in population biology and genetics. Large-scale studies comparing north-eastern populations in Luxembourg, Belgium, France and Germany with those from the Mediterranean region (GBIF 2021) could also provide new insights about the genetic population structure of the species at the edge of its distribution range.

Regarding the severe loss of species in species-rich grassland, it is evident that for some (highly) endangered species we can only halt further loss with great difficulty and that for species such as *A. rendlei* it is even more important to take protective measures at present and thus ensure the conservation of local populations of this species. Here, the effort is worthwhile – the survival of the small grass species can be made possible by relatively simple and efficient measures. Nevertheless, it remains to be seen how ongoing changes in grassland management – with increasing intensification – as well as increasing urban sprawl and resulting fragmentation will affect the populations of *A. rendlei*. It is very much hoped that the conservation measures will be successful.

Erweiterte deutsche Zusammenfassung

Einleitung – Der landwirtschaftliche Strukturwandel mit den einhergehenden Nutzungsänderungen der Grünlandbewirtschaftung führte in den letzten Jahrzehnten vermehrt zu einer Homogenisierung der Grünlandbiotope und dem signifikanten Rückgang der Artenvielfalt dieser Lebensräume. Pflanzenpopulationen sind zum Teil vollständig verschwunden und/oder wurden zu kleinen, fragmentierten Restpopulationen zurückgedrängt, sodass sich der Isolationsgrad zwischen den einzelnen Populationen erhöht hat. Kleinen und isolierten Pflanzenpopulationen mit reduziertem Genfluss zwischen den Populationen drohen in der Regel zwei populationsgenetische Konsequenzen: Genetische Drift und/oder Inzuchtdepression.

Nicht nur seltene vom Aussterben bedrohte Arten sind zunehmend vom Rückgang der Populationen und ihrer zunehmenden Fragmentierung betroffen, sondern auch Arten, die bisher als nicht gefährdet oder potentiell gefährdet eingestuft wurden. Die vorliegende Untersuchung beschäftigt sich mit einer solchen Grünlandart, dem Aufgeblasenen Fuchsschwanz. Dieses einjährige Fuchsschwanzgras mit seiner markanten bauchigen oberen Blattscheide (Abb. 1) besiedelt vorzugsweise basenreiche Lehm- und Tonböden, die feuchte, wechselfeuchte bis wechselnasse Bedingungen mit einer mäßigen Nährstoffversorgung aufweisen. Seine nordöstliche Arealgrenze verläuft durch Luxemburg, Südbelgien und den äußersten Westen Deutschlands. Die Vorkommen von *Alopecurus rendlei* zeigen dort meist sehr unterschiedliche Populationsgrößen und -dichten; einige sind schon von weitem an der rot-bräunlichen Färbung der Ähre zu erkennen (Abb. 2). In Luxemburg und Frankreich wird die Art als potentiell gefährdet eingestuft; in Belgien gilt die Art sogar als stark gefährdet (COLLING 2005, SAINTENOY-SIMON et al. 2006, UICN FRANCE FCBN, AFB & MNHN 2018). In Deutschland hat die Art – mit nur noch einem rezenten Vorkommen im Saarland – die höchste Gefährdungsstufe und gilt als „vom Aussterben bedroht" (METZING et al. 2018).

In den letzten Jahren ist ein starker Rückgang der Art in Belgien zu verzeichnen, der durch eine rezente Untersuchung auch in Luxemburg bestätigt werden konnte (REMACLE 2013, BESCH et al. 2021). Die vorliegende Arbeit schließt sich an die Untersuchungen zur Morphologie von *Alopecurus rendlei*-Beständen an (BESCH et al. 2021). Unser Ziel war es, die genetische Vielfalt und populationsgenetische Struktur von *A. rendlei*-Populationen in vier Regionen (Wallonien in Belgien, Lothringen in

Frankreich, Gutland in Luxemburg und Saarland in Deutschland) zu analysieren, um Rückschlüsse auf die Folgen der Lebensraumfragmentierung zu ziehen und Managementempfehlungen zu erarbeiten. Wir stellten folgende Fragen: (1) Besteht ein Zusammenhang zwischen genetischer Differenzierung und geografischer Entfernung („isolation by distance"), wenn man davon ausgeht, dass die vorhandenen fragmentierten Populationen einst Teil eines größeren, zusammenhängenden Verbreitungsgebiets waren? (2) Zeigt die genetische Vielfalt innerhalb der Populationen bereits negative Auswirkungen der Lebensraumfragmentierung?

Methoden und Untersuchungsgebiet – Das Untersuchungsgebiet liegt in der Großregion Saar-Lor-Lux. Die Flächenauswahl basiert auf den Untersuchungen zur Morphologie der Art (BESCH et al. 2021; Abb. 3). Insgesamt wurden 38 Untersuchungsflächen in Transekten (25 m Länge) beprobt, in denen pro Untersuchungsfläche Pflanzenblätter von 25 unterschiedlichen *Alopecurus rendlei*-Individuen gesammelt wurden. Die DNA-Extraktion und Reinigung fand mit dem Extraktionsroboter Maxwell® Rapid Sample Concentrator 48 (RSC 48) und dem Maxwell® RSC Plant DNA Kit statt, auf die dann eine PCR zur Amplifikation der Proben folgte. Insgesamt wurden 556 Pflanzenindividuen aus 38 *A. rendlei*-Populationen mithilfe von 13 neu entwickelten Mikrosatelliten-Markern untersucht und genetisch typisiert (Tabelle 1, 2). Die Datenauswertung erfolgte mithilfe von GenAlEx (PEAKALL & SMOUSE 2006, 2012). Es wurden verschiedene genetische Diversitätsindizes (private Allele N_P, Allele pro Locus und per Population N_{AL}, N_{AP}, effektive Allele N_E, beobachtete Heterozygotie H_O, fehlerfreie erwartete Heterozygotie uH_E, Fixationsindex F_{IS}) berechnet sowie eine zweidimensionale PCoA (auf Individuen- und Populationsebene) und eine AMOVA durchgeführt, um den Anteil der genetischen Variation zu bestimmen, der auf Unterschiede zwischen Regionen, Populationen und Individuen, innerhalb der Regionen, Populationen und innerhalb von Individuen zurückzuführen ist. Es erfolgte die Berechnung eines Mantel-Tests, um zu bestimmen, ob es einen Zusammenhang zwischen den paarweisen geografischen Distanzen und der paarweisen genetischen Differenzierung zwischen den Populationen gibt. Außerdem wurde eine lineare Regressionsanalyse durchgeführt, um zu ermitteln, ob eine Korrelation zwischen der Populationsgröße und der genetischen Vielfalt besteht. Die genetische Struktur der *A. rendlei*-Populationen wurde anhand von STRUCTURE 2.3.4 untersucht. Die Auswahl des *K*-Wertes, der die Struktur der *A. rendlei*-Populationen am besten beschreibt, erfolgte nach der Delta *K*-Methode von EVANNO et al. (2005) (Anhang E4).

Ergebnisse – ***Genetische Diversität*** – Die Anzahl der Allele pro Locus (N_{AL}) variierte zwischen 1 und 11 und die mittlere Anzahl der Allele pro Population (N_{AP}) zwischen 3,54 und 6,08 (Mittelwert 5,16). Innerhalb neun von 38 Populationen konnten private Allele gefunden werden, wobei der größte Anteil privater Allele in den Populationen F11 (N_P = 0,231) und L13 (N_P = 0,154) verzeichnet werden konnte (Tabelle 1). Die fehlerfreie erwartete Heterozygosität uH_E wurde nur leicht überschätzt und variierte zwischen 0,56 und 0,71 (Mittelwert 0,65) im Vergleich zur berechneten Heterozygosität H_O mit einem Mittelwert von 0,52 (Werte zwischen 0,44 und 0,63). Der Fixationsindex F_{IS} lag zwischen -0,04 und 0,32, mit einem Mittelwert von 0,17 (Tabelle 1). Es bestand ein signifikant positives Verhältnis (r = 0,34; p < 0,05) zwischen der logarithmierten Populationsgröße und der genetischen Diversität uH_E (Abb. 4). Die genetische Diversität nahm nur geringfügig mit der Populationsgröße zu (Steigung m = 0,0079). Einzelne Populationen wiesen niedrigere Werte für die Indizes der genetischen Diversität auf (Tabelle 1, Anhang E5). Die sehr kleine Population D11 hatte im Vergleich zu größeren Populationen die geringste genetische Diversität (uH_E = 0,56).

Populationsgenetische Struktur – Die PCoA-Analyse auf individueller Ebene basierte auf der in GenAlEx erstellten paarweisen genetischen Distanzmatrix, die alle *A. rendlei*-Individuen aller Populationen abbildet (Anhang E6). Die erste Achse erklärt 6,06 %, die zweite 4,50 % und die dritte 4,20 % der Varianz. Die Individuen der einzelnen Populationen waren in der PCoA weit verteilt und es war keine klare genetische Struktur erkennbar. Die zweite PCoA auf Populationsebene (Abb. 5) ergab eine erklärte Varianz der ersten Achse von 12,12 %, die zweite Achse erklärte 10,10 % und die dritte 8,47 % der Varianz. Der größte Teil der Populationen liegt nahe der Achse 1, wobei die Populationen B11, B12, B13 höhere Werte annehmen und sich abgrenzen. Die Population D11 liegt deutlich abseits im positiven Bereich der Achse 2.

Die AMOVA zeigte nur eine sehr geringe molekulare Differenzierung zwischen den Regionen (1 %) und die Differenzierung der Populationen innerhalb der Regionen war gering (5 %; Tab. 3). Der F_{ST}-Wert von 0,05 unterschied sich signifikant von Null ($p < 0{,}001$), was eine signifikante genetische Differenzierung zwischen den untersuchten *A. rendlei*-Populationen erkennen lässt. Die molekulare Differenzierung zwischen Individuen innerhalb der Populationen betrug 21 %. Der höchste Anteil der molekularen Variation war auf die Varianz zwischen allen *A. rendlei*-Individuen innerhalb der Populationen zurückzuführen (73 %; Tab. 3). Die Delta *K*-Methode nach EVANNO et al. (2005) schlug $K = 3$ als bestmögliche Gruppierung vor (Anhang E4). Die meisten Populationen wiesen ein hohes Maß an Vermischung auf und eine klare Populationsstruktur war nicht zu erkennen (Abb. 6). Dennoch konnten Populationen aufgrund von genetischen Ähnlichkeiten gruppiert werden.

Isolation nach Entfernung – Es konnte ein schwacher, aber signifikanter Zusammenhang zwischen der geografischen Entfernung zwischen den Populationen und ihrer genetischen Differenzierung nachgewiesen werden (Abb. 7). Die geringe Steigung der Trendlinie der linearen Regression kommt typischerweise bei Arten vor, die in vermehrtem Genfluss zueinanderstehen. Die genetische Distanz zwischen den Populationen nahm mit der geografischen Entfernung nur geringfügig zu („isolation by distance“ $p = 0{,}014$).

Diskussion – Im Allgemeinen war die genetische Vielfalt innerhalb der untersuchten *Alopecurus rendlei*-Populationen hoch. Vergleicht man die Diversitätswerte mit denen aus ähnlichen Studien, wie denen von DUTECH et al. (2004), DUWE et al. (2018) u. a., können die *A. rendlei*-Populationen als genetisch vielfältig eingestuft werden. Eine hohe genetische Vielfalt tritt typischerweise in großen Populationen auf (ELLSTRAND & ELAM 1993). Unsere Ergebnisse zeigten eine schwache, aber signifikante Korrelation zwischen der Populationsgröße und der genetischen Vielfalt. Insgesamt zeigten die meisten der untersuchten Populationen hohe Populationsgrößen (Mittelwert N = 2 487 084), nur vier Populationen sind kleiner als 10 000 Individuen (siehe Tab. 1). In einigen wenigen Populationen war die genetische Vielfalt reduziert; die geringste genetische Vielfalt konnte wie erwartet in einer der kleinsten Populationen (D11, N = 80) bestätigt werden. Die ursprüngliche Vermutung, dass die Auswirkungen genetischer Drift in sehr kleinen und stark isolierten Populationen bereits zu beobachten sind, konnte somit bestätigt werden.

Eine hohe genetische Vielfalt erhöht die Möglichkeit der Anpassungsfähigkeit gegenüber veränderten Umweltbedingungen; was eine wichtige Funktion von Tier- und Pflanzenarten ist, um mit jeglichen abiotischen Veränderungen zurechtzukommen und sich gegebenenfalls anzupassen (FRANKHAM et al. 2017). Auch wenn die von uns untersuchten Mikrosatelliten neutral sind, könnte dies für *A. rendlei* bedeuten, dass – aufgrund der vorhandenen großen genetischen Diversität innerhalb der Populationen und der kurzen Generationszeit (einjährige Pflanze) – es möglich sein wird, sich relativ schnell an Veränderungen anzupassen, insbesondere im Hinblick auf den Klimawandel und andere veränderte Umweltbedingungen. Private Allele konnten nur in neun von 38 Populationen (24 %) nachgewiesen werden; eine Rate, die als gering einzustufen ist (siehe DUWE et al. 2018).

Unsere Ergebnisse (AMOVA) zeigten, dass die *A. rendlei*-Populationen nur schwach differenziert waren (zwischen Regionen: 1 %, Populationen innerhalb Regionen: 5 %) und die höchste Variation innerhalb der Populationen bestand. Die Ergebnisse der PCoA und der STRUCTURE-Analyse bestätigten, dass sich die isolierte Population in Deutschland genetisch stärker von den anderen Populationen abgrenzen lässt und Effekte genetischer Drift bereits sichtbar werden. Das Gleiche galt für drei von vier Populationen (B11, B12, B13) aus dem belgischen Cluster B1. Die allgemein schwache genetische Struktur von *A. rendlei* deutet daraufhin, dass die Auswirkungen einer vermutlich erst relativ rezenten Habitatfragmentierung bisher noch nicht sehr stark sind. Speziell für die einzige Population in Deutschland kann die Fragmentierung auch anhand der letzten historischen Artsichtungen bestätigt werden, wenngleich die Art auch früher relativ selten war. Fragmentierung und größere geografische Entfernung zwischen Populationen führen in der Regel zu eingeschränktem Genfluss (FRANKHAM et al 2017). Trotz sichtbarer Fragmentierung der untersuchten *A. rendlei*-Populationen in jüngster Zeit fanden wir eine schwache Korrelation zwischen genetischer Differenzierung und geografischer Entfernung (IBD). Eine Erklärung dafür, dass die Populationen von *A. rendlei* genetisch sehr ähnlich sind, könnte daher sein, dass der Genfluss zwischen den Populationen aufgrund der

Windbestäubung immer noch hoch war. Aufgrund vor allem der schwach ausgeprägten IBD und der im allgemeinen großen Populationsgrößen, kann davon ausgegangen werden, dass im Untersuchungsgebiet noch ausreichend Pollenflug und -sammlung zwischen den Pflanzen stattfindet und somit Genfluss herrscht. Eine endgültige Aussage wäre jedoch nur auf der Grundlage von Pollenexperimenten möglich.

Es kann angenommen werden, dass die Art früher in einem großen zusammenhängenden Gebiet vorkam (BESCH 2020, BESCH et al. 2021) und dass die untersuchten Populationen, die früher Teil einer größeren Metapopulation waren, erst kürzlich voneinander durch Fragmentierung getrennt wurden („new rare species"). Unsere Ergebnisse deuten darauf hin, dass sich die Effekte der genetischen Drift derzeit vor allem in der sehr kleinen und isolierten *A. rendlei*-Population in Deutschland zeigen. In einigen anderen Populationen gibt es Hinweise darauf, dass die genetische Diversität und Struktur in Zukunft durch genetische Drift beeinträchtigt werden könnten, wenn die Lebensraumfragmentierung anhält oder zunimmt und/oder die Populationsgrößen abnehmen. Eine weitere Zunahme der Fragmentierung der Populationen, eine Verringerung des Genflusses und eine weitere Reduktion der Populationsgrößen sollten daher vermieden werden, um den negativen Folgen von genetischer Drift oder Inzuchtdepression entgegenzuwirken. Aus diesem Grund ist es erforderlich, ein langfristiges Konzept zum Habitatmanagement zu erstellen und die Populationen von *A. rendlei* miteinander zu verbinden sowie Korridore zu schaffen, um den Genfluss zu halten und zu optimieren. Neu- und Wiederansiedlungen durch Samentransfer könnten dabei eine entscheidende Rolle spielen.

Fazit – Der Gefährdungsstatus von *Alopecurus rendlei* sollte angepasst werden. Es ist wichtig, den weiteren Rückgang der Art aufzuhalten und zu verhindern, dass die bestehenden Populationen noch fragmentierter und kleiner werden sowie die genetische Diversität durch genetische Drift weiter reduziert wird. Unsere Ergebnisse zeigen, dass es nicht zu spät ist, Schutzmaßnahmen zu ergreifen; diese sollten jedoch schnellstmöglich umgesetzt werden. Eine gute Möglichkeit ist hier die Neugründung von Populationen durch Samentransfer – sei es durch Mahdgutübertragung oder Aussaat – im Extensivgrünland, das alle notwendigen standortökologischen Parameter erfüllt. Auch wenn die Populationsdifferenzierung gering ist und die Populationen genetisch sehr ähnlich sind, sollte eine Mischung aus mehreren Populationen als Ausgangsmaterial verwendet werden, um die genetische Vielfalt weiter zu erhöhen. Vor allem sollte die stark isolierte und durch genetische Drift gefährdete Population D11 gestützt werden. Dazu könnten in ihrer Umgebung neue *A. rendlei*-Populationen angesiedelt werden.

Außerdem ist es wichtig, an allen Fundorten inklusive der Populations-Neugründungen die Bewirtschaftungsform an die speziellen Anforderungen dieser Art anzupassen. Hierbei wird generell eine extensive Grünlandnutzung mit verspätetem ersten Mahdzeitpunkt und einer sehr geringen – im Idealfall keiner – Mineraldüngung angestrebt. Der Vertragsnaturschutz stellt hier sicherlich das geeignete Instrument dar (WOLFF et al 2020). Um auf eventuelle Bestandsveränderungen frühzeitig reagieren zu können, ist es wichtig, ein engmaschiges Monitoring aufrechtzuerhalten.

Im Hinblick auf die starken Artenverluste im artenreichen Grünland wird deutlich, dass es bei vom Rückgang betroffenen Arten umso wichtiger ist, Schutzmaßnahmen zu ergreifen und damit den Fortbestand des Aufgeblasenen Fuchsschwanzes durch relativ einfache und effiziente Maßnahmen zu sichern.

Acknowledgements

We thank Harald Meimberg and Thapasya Vijayan from the University of Natural Resources and Life Sciences Vienna for generating the raw genetic data using NGS (next generation sequencing) for the development of the species-specific microsatellites. We thank Laura Daco for her support in data analysis and Gunther Backes for accepting this topic as project work (University of Kassel - Witzenhausen) of the first author. Additional thanks go to the nature conservation syndicate SICONA, which made it possible to carry out this study with financial support from the Ministry of Sustainability. We thank Caroline Grounds for linguistic correction. Our thanks go to the two reviewers as well as the associate editor Brigitta Erschbamer for their valuable comments. The relevant permissions to study the species were available (Luxembourg: N/Réf 95460 CD/ne; Belgium: Réf 2020/RS/10; Germany: AZ: 3.1/25361/1.1.8.2/SWB/DD).

Author contributions

The initiative to study the species was taken by S. Schneider, who also formulated the original idea. L. Besch did the sampling and performed the analysis, which was executed by her within the scope of her Bachelor thesis as a part of her academic project work under the supervision of the two co-authors. S. Hermant undertook the molecular biological analyses. L. Besch led the writing with S. Schneider and G. Colling. L. Glesener commented on the text.

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Supplements

Additional supporting information may be found in the online version of this article.

Zusätzliche unterstützende Information ist in der Online-Version dieses Artikels zu finden.

Supplement E1. Location information of study populations within the nine clusters with GPS coordinates and length of each transect.

Anhang E1. Lageinformationen der untersuchten Populationen innerhalb der neun Cluster mit GPS-Koordinaten und Länge der jeweiligen Transekte.

Supplement E2. Composition of the PCR mix (ddH_2O = distilled water, ADN = diluted (1:10) genomic DNA).

Anhang E2. Zusammensetzung des PCR-Mix (ddH_2O = destilliertes Wasser, ADN = verdünnte (1:10) genomische DNA).

Supplement E3. PCR protocol.

Anhang E3. PCR-Protokoll.

Supplement E4. Selection of $K = 3$ (delta K method of EVANNO et al. 2005) as the best number of clusters within studied *Alopecurus rendlei* populations.

Anhang E4. $K = 3$ als bestmögliche Anzahl von Clustern innerhalb der analysierten *Alopecurus rendlei*-Populationen (Methode nach EVANNO et al. 2005).

Supplement E5. Patterns of genetic diversity indices of the 38 studied *Alopecurus rendlei* populations.

Anhang E5. Muster der genetischen Diversitäts-Indizes der 38 untersuchten *Alopecurus rendlei*-Populationen.

Supplement E6. Two-dimensional principal coordinates analysis (PCoA) of the 556 individuals of all populations, national cluster membership is shown as symbol.

Anhang E6. Biplot zur PCoA auf Individuenniveau. Abbildung der 556 Individuen aller Populationen, die Zugehörigkeit zu den nationalen Clustern ist als Symbol dargestellt.

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Supplement E1. Location information of study populations within the nine clusters with GPS coordinates and length of each transect.

Anhang E1. Lageinformationen der untersuchten Populationen innerhalb der neun Cluster mit GPS-Koordinaten und Länge der jeweiligen Transekte.

Country	Cluster	Plot	Transect [m]	Transect beginning		Transect end	
				Lat. (ºN)	Long. (ºO)	Lat. (ºN)	Long. (ºO)
Belgium	B1	B11	25	49.7533	5.7568	49.7534	5.7570
		B12	25	49.7486	5.7519	49.7484	5.7518
		B13	25	49.7523	5.7500	49.7523	5.7497
		B14	25	49.7357	5.8134	49.7356	5.8131
	B2	B21	25	49.6714	5.8393	49.6712	5.8393
		B22	25	49.6321	5.8436	49.6319	5.8434
		B23	25	49.6199	5.8186	49.6197	5.8186
		B24	25	49.6408	5.8841	49.6409	5.8843
		B25	25	49.6563	5.8597	49.6564	5.8600
	B3	B31	15	49.5772	5.8528	49.5772	5.8525
		B32	25	49.5752	5.8248	49.5752	5.8251
		B34	25	49.5655	5.7833	49.5655	5.7830
Germany	D1	D11	14	49.3109	6.8173	49.3110	6.8173
France	F1	F11	25	49.5003	6.2048	48.5004	6.2051
		F12	25	49.4319	6.2000	49.4320	6.1996
		F13	25	49.4418	6.1680	49.4416	6.1683
		F14	25	49.4653	6.1241	49.4651	6.1238
		F15	25	49.4583	6.2934	49.4581	6.2933
	F2	F22	25	48.9147	6.1693	48.9147	6.1690
		F23	25	48.9416	6.1642	48.9419	6.1642
		F24	25	48.9516	6.1245	48.9515	6.1247
		F25	25	48.9253	6.1636	48.9251	6.1634
		F26	12	48.9446	6.1133	48.9446	6.1131
Luxembourg	L1	L11	25	49.5844	5.9295	49.5844	5.9292
		L12	25	49.5964	5.9136	49.5965	5.9133
		L13	25	49.5897	5.9106	49.5898	5.9103
		L14	25	49.5717	5.8925	49.5719	5.8923
		L15	25	49.5649	5.8949	49.5648	5.8946
	L2	L21	25	49.5304	6.1474	49.5303	6.1476
		L22	25	49.5289	6.1992	49.5290	6.1989
		L23	21	49.4973	6.1483	49.4972	6.1481
		L24	25	49.5167	6.1691	49.5167	6.1695
		L25	25	49.5173	6.2101	49.5303	6.1476
	L3	L31	25	49.5969	6.0663	49.5176	6.2101
		L32	14	49.5983	6.0683	49.5982	6.0681
		L33	25	49.5956	6.0827	49.5956	6.0824
		L34	20	49.6098	6.0353	49.6097	6.0356
		L35	25	49.6085	6.0344	49.6085	6.0348

Supplement E2. Composition of the PCR mix (ddH_2O = distilled water, ADN = diluted (1:10) genomic DNA).

Anhang E2. Zusammensetzung des PCR-Mix (ddH_2O = destilliertes Wasser, ADN = verdünnte (1:10) genomische DNA).

Reagent	Volume for 1 sample (µl)
GoTaq® G2 Hot Start Master Mix	1.25
ddH_2O	2.75
Primer (2 µM)	0.50
ADN (1:10)	0.50
Total	5.00

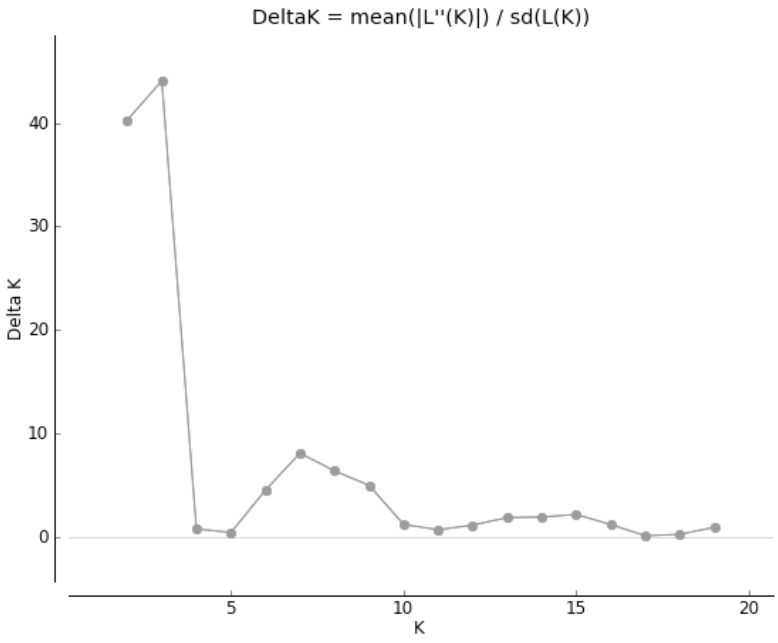
Supplement E3. PCR protocol.

Anhang E3. PCR-Protokoll.

Phase		Temperature	Time	Repetition (cycles)
Step 1	Initial denaturization	95°C	5´	1x
Step 2	Denaturization	95°C	30´´	
	Annealing	59-65°C	1'30´´	35x
	Elongation	72°C	30´	
Step 3	Final elongation	68°C	10´	1x
Step 4	Cooling	4°C	∞	1x

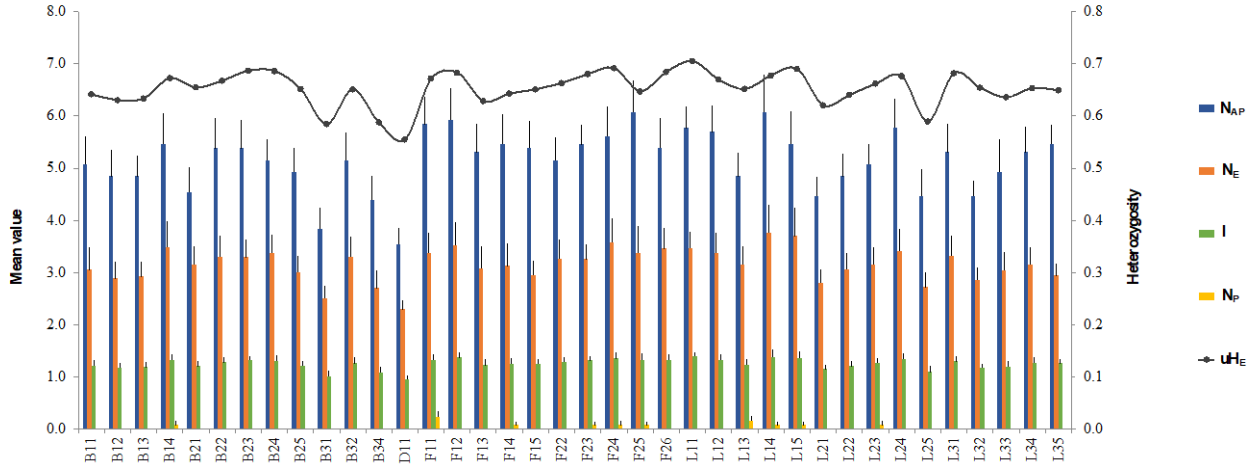
Supplement E4. Selection of *K* = 3 (delta *K* method of EVANNO et al. 2005) as the best number of clusters within studied *A. rendlei* populations.

Anhang E4. *K* = 3 als bestmögliche Anzahl von Clustern innerhalb der analysierten *A. rendlei*-Populationen (Methode nach EVANNO et al. 2005).



Supplement E5. Patterns of genetic diversity indices of the 38 studied *Alopecurus rendlei* populations. N_{AP} = number of alleles per population, N_E = number of effective alleles, I = Shannon index, N_P = number of private alleles, uH_E = unbiased expected heterozygosity.

Anhang E5. Muster der genetischen Diversitäts-Indizes der 38 untersuchten *Alopecurus rendlei*-Populationen. N_{AP} = Anzahl an Allelen pro Population, N_E = Anzahl an effektiven Allelen, I = Shannon Index, N_P = Anzahl an privaten Allelen, uH_E = fehlerfreie erwartete Heterozygotie.



Supplement E6. Two-dimensional principal coordinates analysis (PCoA) of the 556 individuals of all populations, national cluster membership is shown as symbol (for abbreviations see supplement E1). Axes 1, 2, and 3 explain 14.76% of the variance.

Anhang E6. Biplot zur PCoA auf Individuenniveau. Abbildung der 556 Individuen aller Populationen, die Zugehörigkeit zu den nationalen Clustern ist als Symbol dargestellt (Abkürzungen siehe Anhang E1). Die Achsen 1, 2 und 3 erklären in Summe 14,76 % der Varianz.