Genetic diversity and implications for conservation strategies of *Drosera rotundifolia* L. (Droseraceae) in northern Germany (Schleswig-Holstein)

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SUMMARY

Drosera rotundifolia L. is a rare carnivorous herbaceous plant which occupies wet, acidic bogs and fens. Today species which are specialists on oligotrophic and acidic habitats like *D. rotundifolia* have declined as a result of land use changes, land reclamation and drainage. The aim of our study was to assess patterns of genetic diversity in twelve fragmented *D. rotundifolia* populations of northern Germany (Schleswig-Holstein) by the use of Inter-simple sequence repeats (ISSR). ISSR is a PCR fingerprint technique, which uses microsatellite sequences as primers to generate multilocus markers. We scored 84 individuals at a total of 120 ISSR markers. We found a wide range of population genetic diversity (H*e* = 0.109–0.247) described by the ISSR data. There was a weak relationship between genetic isolation and distance ($P = 0.010$) and a large (significant) proportion of genetic variation within populations (75 %, $P < 0.001$) and 25 % among populations. STRUCTURE analysis showed that the model with three inferred clusters ($K = 3$) best described the ISSR data. Some dominant clusters at each site corresponded to the results from the principal coordinate analysis (PCoA), which visualises genetic patterns of individual plants. The patterns of genetic diversity by the ISSR data showed, for some local genotypes of the *D. rotundifolia* populations a clear separation, pointing towards conservation strategies for each population of *D. rotundifolia* in northern Germany rather than for the species as a whole.

KEY WORDS: genotype, Inter-simple sequence repeat (ISSR)

INTRODUCTION

Since the last glaciation in Europe, areas have been recolonised by plants following the glacial retreat. However, patterns of plant species distribution do not only reflect habitat preferences and performance in the natural selection process, but also human land use scenarios. A central issue for specialist plants of habitats like bogs and fens is the drainage and land reclamation of these areas, mostly for agricultural purposes. Plant populations affected by such human impacts do not necessarily represent the original diversity existing after the retreat of the glaciers but may represent genetically isolated populations threatened by the fact that their habitats are being affected by anthropogenic constraints. In Germany, bogs and fens have been destroyed on a large scale by changes in land use, such as destruction by peat depletion for horticultural substrates or the use of peat as fossil burning, land reclamation and/or drainage for agriculture (Ullrich & Riecken 2012), followed by a decline of *Drosera rotundifolia* L. and other *Drosera* species (Eschenbrenner *et al.* 2016). As a consequence, native *Drosera* species in Germany (*D. anglica*, *D. intermedia* and *D. rotundifolia*) are on the Red List as endangered or critically endangered, and some are already extinct or lost. For example, the round-leaved sundew (*D. rotundifolia*) in Schleswig-Holstein is one of the most strongly protected species under the Federal Nature Conservation Act and is classified in the Red List of Mierwald & Romahn (2006) as "endangered" (category 3). In addition to the round-leaved sundew, there is the oblong-leaved sundew (*Drosera intermedia* Hayne) in Schleswig-Holstein. This is now considered as "critically endangered" (category 1, Mierwald & Romahn 2006). The third naturally occurring sundew in Germany is the great sundew *Drosera anglica* Huds. which is, according to Mierwald & Romahn (2006), classified as "extinct or lost" (category 0).

D. rotundifolia is native in northern Germany and the main habitats are acidic bogs and poor fens (Mayer 2005). *D. rotundifolia* grows primarily in *Sphagnum*-dominated communities (Baranyai & Joosten 2016). Additionally, the two other native *Drosera*-species, *D. intermedia* and *D. anglica*, can be found on raised bogs, transitional mires and fens (Sebald *et al.* 1992). In northern Germany, bogs and fens are the remains of a once more widespread

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vegetation type that began to grow in the region around 11,000 years ago, after Weichselian glacial period (LLUR-SH 2016).

At the beginning of the 19th century about 10 % of the country's area in Schleswig-Holstein was still covered with bogs, approximately 160,000 ha (LLUR-SH 2016). Today, after LLUR-SH 2016 still 130,000 ha of bog area exist, however, the largest part is under intensive agricultural use. The anthropogenic interventions, such as drainage of the bogs, draining for agricultural use, also the peat digging for the production of solid fuel or as a component of horticultural soils, have contributed to the considerable decline in the area of bog-land in Schleswig-Holstein (LLUR-SH 2016). Intact, unaffected bogs no longer exist in Schleswig-Holstein (Bretschneider 2016). Only 12 % of bogs are considered near-natural, still functioning as carbon sinks (Burbaum & Filipinski 2016, LLUR-SH 2016). According to Schrautzer *et al.* (2016) in Schleswig-Holstein there are about 995 km^2 of fens, of which almost 86 % are in agricultural use and about 285 km^2 are raised bogs of which 62 % are in agricultural use. After Schrautzer *et al.* (2016), the plant communities which occupy bogs are highly specialised. Typically, species like *D. rotundifolia* are morphologically and physiologically adapted to, and able to cope with, various stress situations such as low oxygen content, nutrient deficiencies and temporary dryness. In our study we focussed on this carnivorous plant species, as it is the most abundant native *Drosera*-species in Schleswig-Holstein.

Drosera rotundifolia is a perennial hemicryptophyte, which produces prominent, hairless hibernacula (frost-resistant winter buds) in order to survive the winter (Lowrie *et al*. 2017). According to Sebald *et al.* (1992) leaves are formed as a basal rosette. A typical feature of this plant is its 5–10 mm long and up to 15 mm wide, rounded leaf blade, which turns into the 15–50 mm long, hairy stem (Sebald *et al.* 1992; Eschenbrenner *et al.* 2015/2016). Two types of glands can clearly be distinguished on the leaves of *Drosera*, specialised for different purposes. The stalked glands of the first type secrete glue copiously and perform a primary function of trapping insect prey. The second type, the sessile or digestive glands, are positioned among the stalked glands (tentacles) on the surface of the lamina. These glands fulfil the primary function of secreting enzymes and absorbing nutrients once prey has been caught (McPherson 2010). The round-leaved sundew *D. rotundifolia* possesses the ability to reproduce both asexually (vegetatively) and sexually by producing seeds from its hermaphroditic flowers. Sexual reproduction is the main method of reproduction and is mostly autogamous (Hegi 1961). *D. rotundifolia* forms 1–15 (but up to 25) radially symmetric, short-petioled and androgynous flowers in a one-sided inflorescence (Baranyai & Joosten 2016). At the beginning of the flowering period the plant forms smaller unopened cleistogamic flowers, whereas later, the plants produce well-developed reproductive chasmogamous flowers. Chasmogamous flowers bloom from June to August (Sebald *et al.* 1992) but only for a few hours (Hegi 1961). In the flowering process temperature seems to play a pivotal role. Only at temperatures of 25–30 °C do flowers begin to open, and full blooming occurs only at temperatures of at least 35 °C (Baranyai & Joosten 2016). After pollination, seeds are released in autumn (Baranyai & Joosten 2016). Seeds are small, slender and several times longer than wide (Lowrie *et al.* 2017) with a multi-layered testa and acute ends (Hegi 1961). Dispersal of the *D. rotundifolia* seeds is by wind (anemochorous), animals (zoochorous) and water (hydrochorous) (Hegi 1961, Baranyai & Joosten 2016, Fleischmann *et al.* 2018).

Due to the substantial decline of *Drosera* habitats, the species is protected in Germany. The aim of this study was to obtain information on the status of genetic diversity and variability of several populations of the species *D. rotundifolia* L. in Schleswig-Holstein. We tested the hypothesis that due to the fragmentation of habitats, locally adapted genotypes on the mainland as well as on islands (Amrum, Sylt) were formed with strong population structuring. According to Oelke (2003), genetic diversity within species and their individual populations is the prerequisite for adaptation under variable environmental conditions, the long-term evolution of a species, and high fitness of individuals. Oostermeijer (2000) and Oelke (2003) pointed to reasons for the loss of genetic diversity, e.g. the reduced gene flow resulting from isolation and habitat fragmentation followed by genetic drift. Low genetic diversity can reduce the potential for evolutionary adaptation and lead to further population decline and finally extinction (Oelke 2003).

For the investigation of the genetic diversity of *D. rotundifolia* populations we applied the high-resolution fingerprint method ISSR (Inter-simple sequence repeat). The PCR-based ISSR method makes it possible to compare anonymous amplified DNA segments. To create these segments, genetic primers bind on the targets (identical microsatellite repeat regions scattered throughout the genome) and the DNA fragment between the two identical microsatellite repeat regions (simple sequence repeats, SSR) is amplified by the polymerase chain

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reaction (PCR). This technique allows the comparison of individuals (Reddy *et al.* 2002) differing in the length of the amplified fragments between these microsatellite-based primers. The method uses microsatellites as primers (usually 16–25 bp long) which can be di-, tri- or tetra-nucleotide repeats (Reddy *et al.* 2002). The SSRs occur in plants on average every 6–7 kb (Cardle *et al.* 2000), and can also be used to amplify and analyse the length variation between the target sequences. ISSR markers are highly polymorphic, useful in studies of genetic diversity (Reddy *et al.* 2002), easy to establish and ideal genetic markers for organisms whose genetic information is lacking (Ng & Tan 2015).

METHODS

Study region and sampling

For this study we sampled *D. rotundifolia* on eight sites in raised bogs and fens in Schleswig-Holstein, Germany (Figure 1). Plants were collected in the field, potted into substrate from the growth locality and then cultivated in the botanical garden of the Justus-Liebig-University at Giessen. The sampling took place in June 2016 and the regulatory approvals were granted for all of the sampling by permission of the Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein and the nature conservation authorities for the Kreis Nordfriesland and Schleswig-Flensburg. Four *D. rotundifolia* populations from a former study by Eschenbrenner *et al.* (2016) were included; the sampling of these populations was in 2013. The collection sites in 2016 on the mainland were located in three nature reserves (NSG) and three Flora-Fauna Habitats (FFH). Two other collection sites were on the island of Amrum, in the nature reserves Amrum Wittdün and Amrumer Dünen. One collection site was in a nature reserve on the neighbouring island of Sylt. In 2013 the sampling took place in three FFH areas on the mainland Schleswig-Holstein and one island population from Amrum (Table 1). The distance between the different studied populations ranged from 5.6 km (e.g. EN–LH) to 129.5 km (e.g. SY–NH). For the population genetic analyses, in summary, a total of 84 individuals from 12 populations in Schleswig-Holstein were investigated.

DNA extraction

The cultivated plants were transferred from the botanical garden into the laboratory and for DNA extraction 100 mg fresh leaf material was taken and was immediately homogenised with a pestle and mortar in liquid nitrogen. For the DNA extraction we followed the instructions of Biteau *et al.* (2011) with some changes: elution was after five minutes incubation with AE-buffer. This method based on a combination of a borate extraction buffer with the use of a Qiagen Plant Mini extraction kit, and a proteinase K treatment during extraction. It is an efficient method for isolating DNA specifically developed for *Drosera* species. The gained DNA was the template for our ISSR-PCR. DNA quantity and quality were checked on a NanoPhotometer™ (Implen GmbH, München, Germany).

ISSR analysis

After an initial primer screening with seven primers from Dogan *et al.* (2010) and two from Bradeen *et al.* (2002), we chose five primers. These final primers proved to be informative and provided clear bands which were sufficiently polymorphic to show variation within and among populations, as shown in Table 2 with the optimised annealing temperature and PCR-additive. The PCR reaction in 25 µl contained for the Primers F2 and ISSR5 18.10 µl ddH_2O (Rotipuran®, Ultra Roth®), 2.5 µl 10x Dream Taq. Buffer incl. $MgCl_2$ (Fermentas, Thermo Scientific), 2 µl dNTPs (2mM) (Fermentas, Thermo Scientific), 0.5 µl Primer (10 pm/µl) (Metabion), 0.5 µl Dimethylsulfoxid (5 %) (DMSO, Roth), 0.4 µl Dream Taq Polymerase (5 units/µl) (Fermentas, Thermo Scientific) and 1 µl DNA Template (ca. 10 ng/µl).

For the Primers ISSR4, F5 and F6 the PCR reaction in 25µl contained 13.10 µl ddH_2O (Rotipuran® Ultra Roth®), 2.5 µl 10x Dream Taq. Buffer incl. $MgCl_2$ (Fermentas, Thermo Scientific), 2 µl dNTPs (2mM) (Fermentas, Thermo Scientific), 0.5 µl Primer (10 pm/µl) (Metabion), 5.0 µl Betaine monohydrate (5 M) (Sigma-Aldrich) and 0.5 µl Bovine Serum Albumin (10 ng/µl) (BSA; Fermentas, Thermo Scientific) 0.4 µl Dream Taq Polymerase (5 units/µl) (Fermentas, Thermo Scientific) and 1 µl DNA Template (ca. 10 ng/µl). After an initial denaturation step of 2 min at 94 °C, amplification reactions were cycled 30 times at 94 °C for 30 seconds. The primer annealing was for 45 seconds at a temperature specific for each primer see Table 2, the elongation was for 1 min at 72 °C. A final extension was performed for 10 min at 72 °C. Upon completion of the reaction 12 µl aliquots of the PCR products were mixed with 4 µl SYBR® Gold (Invitrogen™) and loaded onto a 2 % agarose-gel. As standard 7 µl GeneRuler TM 100 bp Plus DNA Ladder (Fermentas, Life Science) were loaded onto the gel (75–90 V for 60–80 min). Amplified fragments were visualised under a UV transilluminator and photographed using a gel

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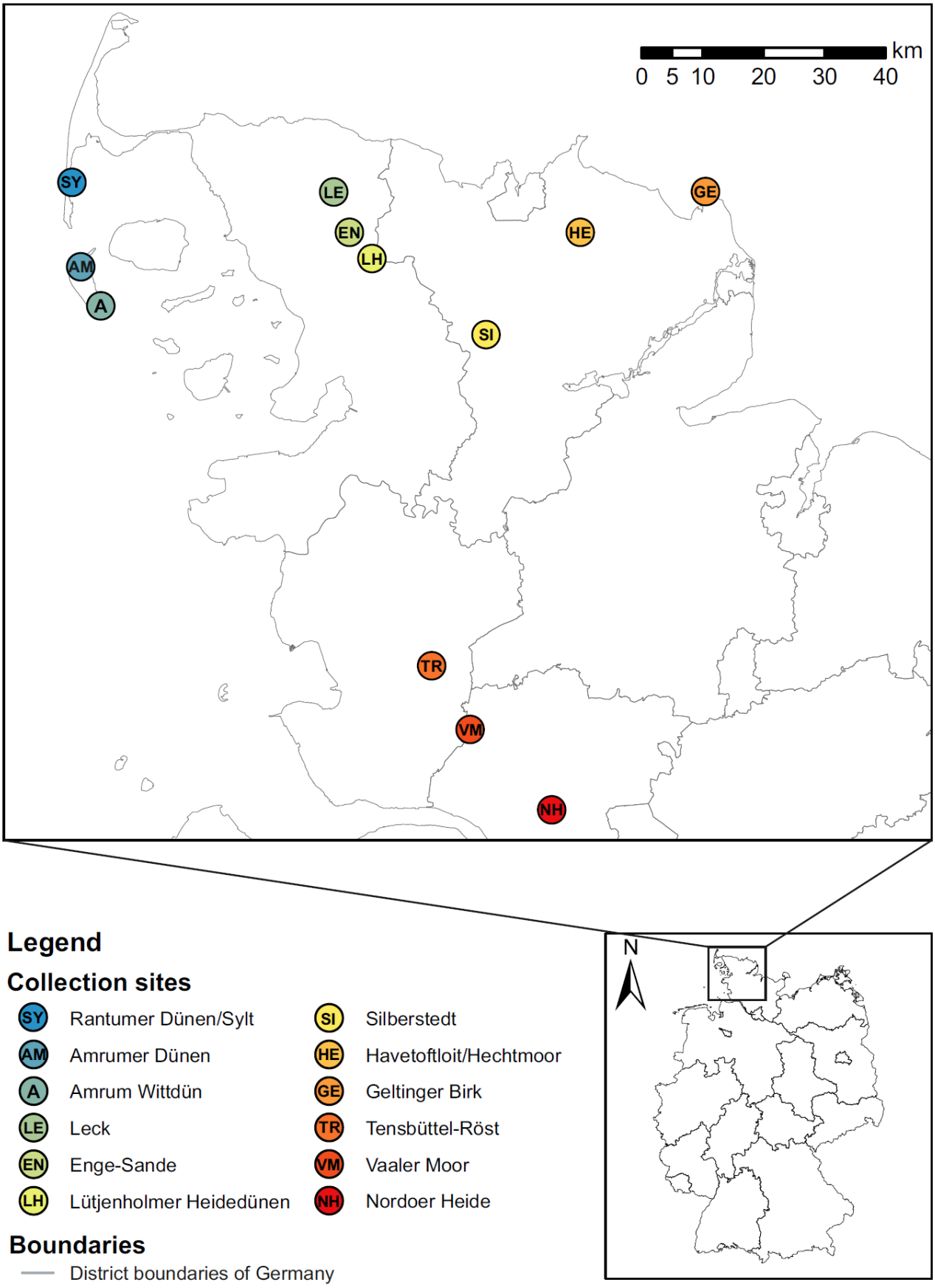

Figure 1. Map and collection sites of the study region in Schleswig-Holstein, Germany. Map was prepared with ArcGIS Desktop (ArcGIS Desktop 10.2.2., Esri).

Table 1. Overview of sampled *Drosera rotundifolia* populations in Schleswig-Holstein, protection category: nature reserve (NSG) Flora-Fauna-Habitat (FFH), Gauss-Krueger coordinates (GK_R; GK_H), estimated population size (- no data available), number of analysed individuals (n), sum of loci (#loc.), percentage of polymorphic loci (PLP) and Nei´s gene Diversity (H*e*) after Lynch & Milligan (1994).

Population-ID	Locations	Commune	Protection category	GK_R	GK_H	Population size	n	#loc.	PLP (%)	H*e*
SY	*Rantumer Dünen/Sylt*	Nordfriesland	NSG	3454500	6075332	> 100	5	120	37.5	0.109
AM	*Amrum Dünen*	Nordfriesland	NSG	3456410	6061782	–	8	120	47.5	0.174
A	*Amrum Wittdün*	Nordfriesland	NSG	3459685	6055397	26–50	8	120	70.8	0.247
LE	*Leck*	Nordfriesland	NSG	3497881	6074052	> 100	5	120	39.2	0.173
EN	*Enge-Sande*	Nordfriesland	FFH	3500451	6067436	26–50	7	120	68.3	0.196
LH	*Lütjenholmer Heidedünen*	Nordfriesland	NSG	3504151	6063144	> 100	8	120	55.8	0.223
SI	*Silberstedt*	Schleswig-Flensburg	FFH	3522854	6050685	26–50	7	120	44.2	0.166
HE	*Havetoftloit/Hechtmoor*	Schleswig-Flensburg	FFH	3538309	6059389	51–100	8	120	52.5	0.136
GE	*Geltinger Birk*	Schleswig-Flensburg	NSG	3558838	6074129	> 1000	7	120	56.7	0.161
TR	*Tensbüttel-Röst*	Dithmarschen	FFH	3513951	5996376	> 100	8	120	50.8	0.218
VM	*Vaaler Moor*	Steinburg	FFH	3520269	5985937	51–100	5	120	47.5	0.197
NH	*Nordoer Heide*	Steinburg	FFH	3533659	5972781	–	8	120	52.5	0.218

Table 2. Used ISSR-Primer after Dogan *et al.* (2010) and Bradeen *et al.* (2002) †.

Primer	Primer sequence	TAnnealing in °C	PCR-Additive
ISSR F2	CTCGTGTGTGTGTGTGTGT	55.0	10% DMSO
ISSR F5	AGAGAGAGAGAGAGAG	47.0	Betain/BSA
ISSR F6	CCACCACCACCACCA	49.1	Betain/BSA
ISSR 4†	GACAGACAGACA	37.0	Betain/BSA
ISSR 5†	VHVCTCTCTCTCTCTCTCT	55.0	10% DMSO

documentation system (Vilbert Lourmat, Infinity model). Fragments in the range of 200 to 2000 bp were evaluated and transferred manually into a 0/1-matrix. Whereas a character ("locus") is shown as a band position which is either present (1) or absent (0) in a particular line.

Data analysis

AFLPsurv version 1.0 (Vekemans 2002) was used to calculate the percentage of polymorphic loci and genetic diversity (H*e*) after Lynch & Milligan (1994); and the F_{ST}-values between populations (Wright´s fixation index). To explore and visualise individual genetic patterns a principal coordinate analysis (PCoA) was conducted with GenAIex 6.5 (Genetic Analysis in Excel; Peakall & Smouse 2012). An analysis of molecular variance (AMOVA) was performed in GenAIex 6.5 to evaluate the genetic variation within and among populations and significance tests were performed using 999 permutations. A Mantel test was also performed with GenAIex 6.5, to correlate the genetic distance with geographic distance. STRUCTURE, version 2.3.3 (Pritchard *et al.* 2000) was applied to explore genetic affiliation of individuals to genetic clusters by applying the admixture model; PLOIDY=2 and; RECESSIVEALLELES=0. For all other settings the default options were used. Calculations were conducted with 100,000 Markov Chain Monte Carlo (MCMC) replicates with a burn-in period of 50,000 and ten repeats per run for each cluster, K = 1 to K = 12. To identify the most likely K modal distribution, delta K (Evanno *et al.* 2005) was determined using STRUCTURE HARVESTER (Earl & von Holdt 2012). To verify the most probable cluster membership coefficient among the ten runs of STRUCTURE and STRUCTURE HARVESTER we used CLUMPP v.1.1.2 for the analyses (Jakobsson & Rosenberg 2007). Corresponding graphs were constructed with the software DISTRUCT (Rosenberg 2004).

RESULTS

Genetic diversity

For the 84 investigated individuals we detected 120 loci with a variation of polymorphic loci between 37.5 % and 70.8 % (Table 1). The genetic diversity (H*e*) has a range between 0.109 and 0.247 for the different *D. rotundifolia* populations.

The analysis of the molecular variance (AMOVA) for the 12 populations of *D. rotundifolia* showed that the greatest variation was found within populations (75 %). 25 % of the variation occurred among populations (Table 3). The F_{ST}-value of 0.25 (P = 0.001) is indicative of a great genetic differentiation between the different populations.

In a principal coordinates analysis (PCoA) of the 12 *D. rotundifolia* populations (Figure 2) the first two axes explain 6.73 % and 12.88 % of the total variation. Some of the individuals cluster according to their population affiliation like Lütjenholmer Heidedünen (LH) or Nordoer Heide (NH), and some contribute to larger clusters. We can identify three of the larger clusters: the first one comprises the populations from Amrumer Dünen (AM), Tensbüttel-Röst (TR) Nordoer Heide (NH) and Vaaler Moor (VM), the second the populations of Lütjenholmer Heidedünen (LH) and Amrumer

Table 3. Analysis of molecular variance (AMOVA) performed by grouping the 12 *Drosera rotundifolia* populations; variance (V); percentage of total variance (total %); degree of freedom (df).

Source of variation	df	V	Total (%)	F_{st}	*P*-value
Among Populations	11	4.351	25	0.25	0.001
Within Populations	72	13.119	75		

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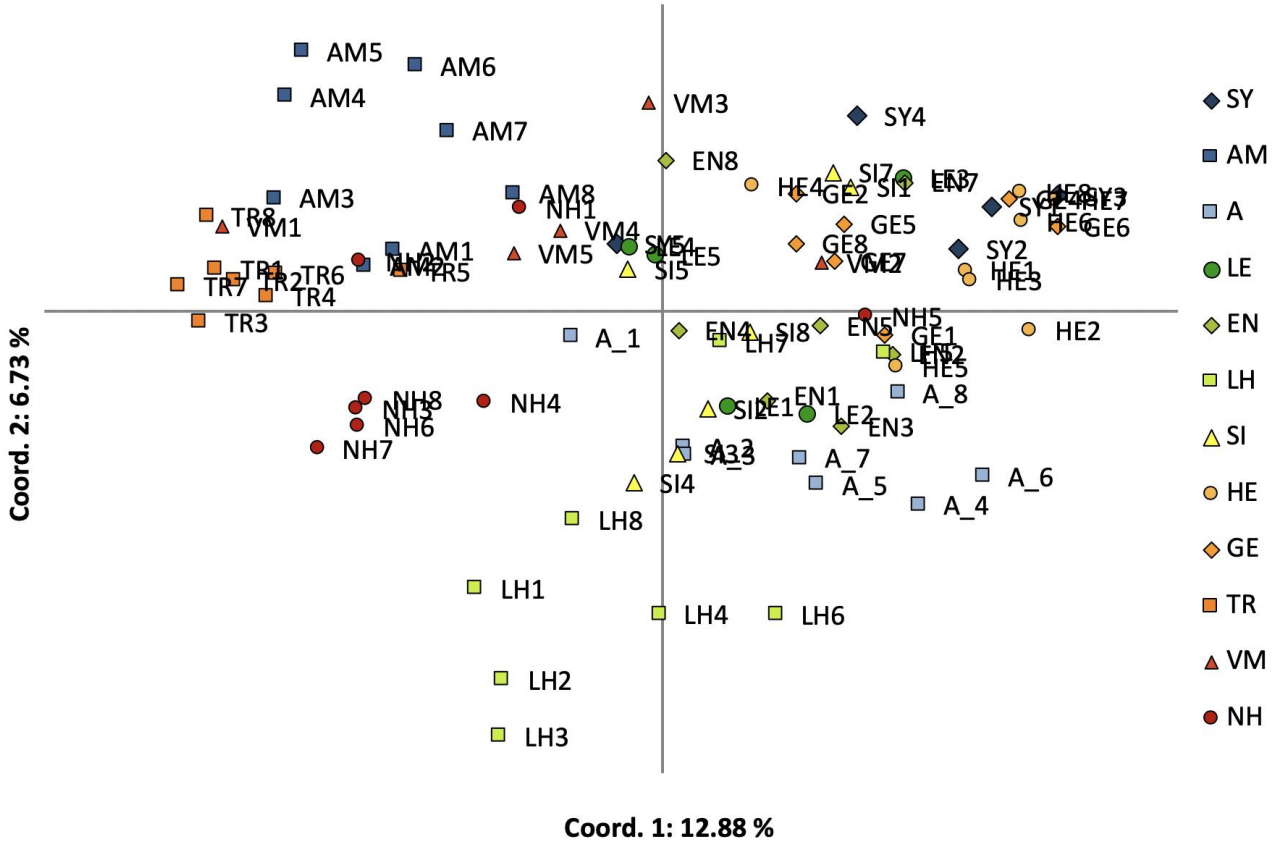

Figure 2. Principal coordinate analysis (PCoA) for genetic distances between individuals of *Drosera rotundifolia* in Schleswig-Holstein. Sampling regions indicated by colour and aberrations according to Table 1.

Wittdün (A) and the last one is an admixture of the residual mainland populations and the population from Sylt. Due to the scattering of individuals, a clear assignment of the population affiliation is not readily possible yet.

The STRUCTURE analysis revealed three clusters to best represent the population genetic pattern within the dataset (Figure 3). As shown in Figure 3 one of the clusters presented in orange is dominant in the populations of Sylt (SY), Leck (LE); Enge-Sande (EN), Hechtmoor (HE) and Geltinger Birk (GE). The second cluster in blue is mainly represented in the locations of Amrum Wittdün (A), Lütjenholmer Heidedünen (LH) and Silberstedt (SI). The third cluster presented in yellow is dominant in the populations of Tensbüttel-Röst (TR), Vaaler Moor (VM), Nordoer Heide (NH) and Amrumer Dünen (AM). The Mantel test showed a significant (P = 0.010) but weak (Rxy = 0.190) correlation between genetic and geographic distance for the 12 *D. rotundifolia* populations (Figure 4).

DISCUSSION

The main goal of our study was to assess patterns of genetic diversity in twelve *D. rotundifolia* populations of northern Germany (Schleswig-Holstein) by using Inter-simple sequence repeat (ISSR). The first part in our hypothesis states that due to the fragmentation of the habitats, locally defined genotypes on the mainland exist, with strong population structuring. We partly agree with this since we detected high levels of population genetic diversity by the ISSR data, in comparison to Chung *et al.* (2013), who found very weak levels of genetic diversity in Korean *Drosera rotundifolia* (H*e* = 0.005, with allozyme electrophoresis). Wang *et al*. (2004) observed high genetic diversity in the carnivorous plant *Sarracenia leucophylla* (H*e* = 0.224, with enzyme electrophoresis). However, there was a weak relationship between genetic isolation and distance and a large (significant) proportion of genetic variation was partitioned within populations

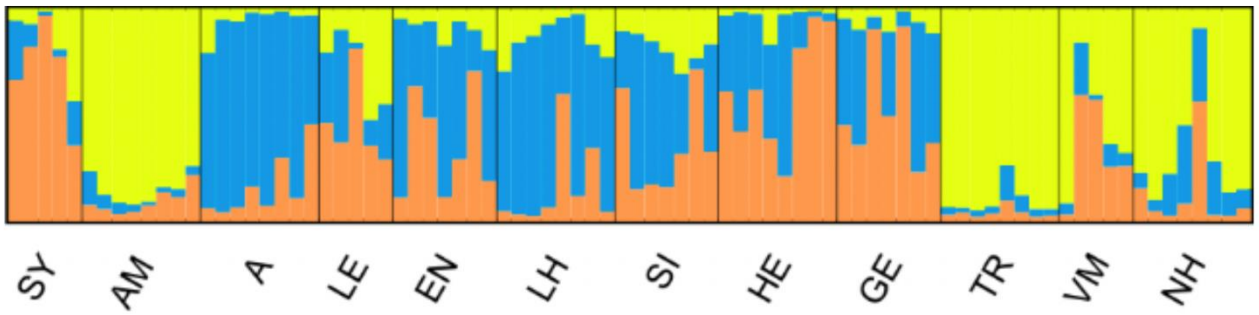

Figure 3. Population genetic structure of 12 *Drosera rotundifolia* populations as revealed by STRUCTURE analysis using the admixture model for K = 3. Each individual is represented by a vertical bar, and fractional membership in each of the clusters is indicated by colours. Population-IDs according to Table 1.

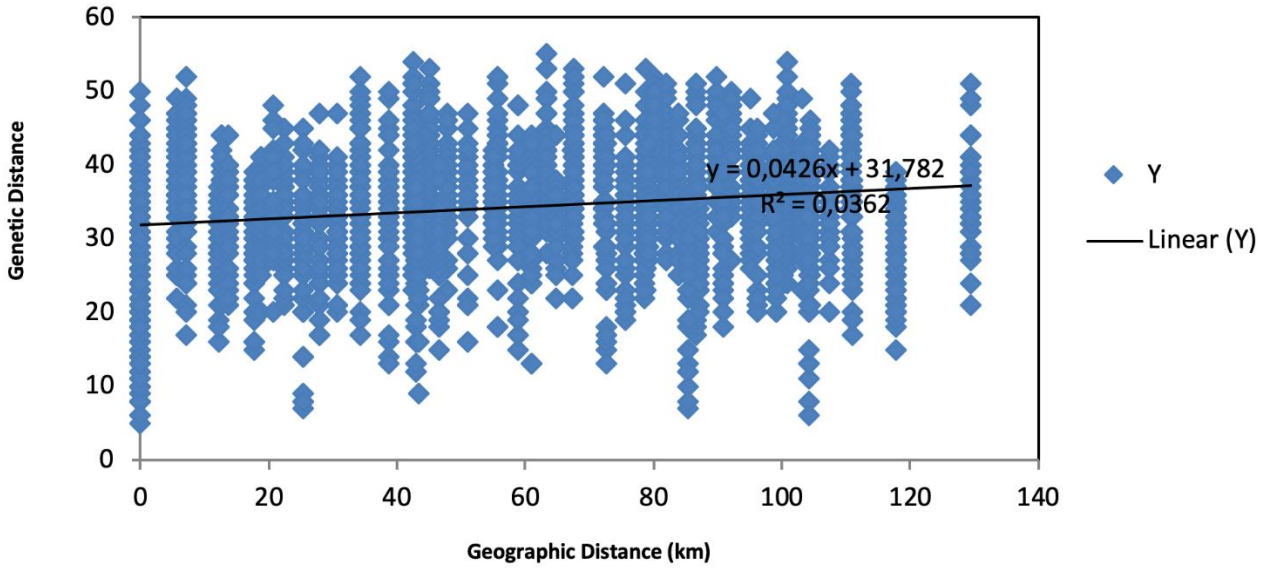

Figure 4. A Mantel test to correlate the genetic distance with geographic distance for the 12 *D. rotundifolia* populations.

(75 %) and 25 % among populations (AMOVA). The patterns of genetic diversity revealed by the ISSR data showed, for some local genotypes of the *D. rotundifolia* populations, a clear separation and corresponded to the results from PCoA and the STRUCTURE analysis. For example, cluster 1: TR, VM, NH and AM shown in Figure 2 (PCoA) and presented in yellow in Figure 3 (STRUCTURE analysis) or cluster 2 comprises the populations LH, SI and A shown in Figure 2 (PCoA) and presented in blue in Figure 3 (STRUCTURE analysis). The relatively clear separation of the *D. rotundifolia* populations is on one hand related to the reproduction and dispersal strategies of the genus *Drosera* and on the other hand due to fragmentation of the habitats. Sexual reproduction of *D. rotundifolia* is mostly autogamous or cleistogamous (Seeholzer 1993). A high proportion of cleistogamic flowers in a population may lead to inbreeding due to the loss or non-existence of genetic exchange (Baranyai & Joosten 2016). Chasmogamous flowers open only for a few hours on a single day (Hegi 1961). Due to this, pollination by e.g. wasps, beetles, bees and hover flies (McPherson 2010) is limited. Furthermore, the genetic variation of *D. rotundifolia* populations are not only influenced by pollen from outside populations, but also by different seed dispersal strategies like zoochory or anemochory (Hegi 1961, Fleischmann *et al*. 2018). Specific long distance dispersal mechanisms are not present in *D. rotundifolia*, but seeds are known to be distributed by wind, water and animals (Crowder *et al*. 1990). An example for the zoochorous dispersal by bird is the Eurasian Golden Plover (*Pluvialis*

apricaria) which nests in northern Germany. According to Hötker (2004) +/- 89000 birds (*Pluvialis apricaria*) were counted in Schleswig-Holstein in autumn 2003, showing a distribution of the Eurasian Golden Plover on collection sites such as the Island Sylt and on the mainland (GE). Wind is also one of the dispersal strategies of the *D. rotundifolia* seeds (Baranyai & Joosten 2016) and the main wind direction in North Germany (Schleswig-Holstein) is West-East (Bürger 2003). However, estimation of dispersal suggests that most of the seeds can only reach a few centimetres distance from the parent plant (Wolf *et al.* 2006). Because of a lack of experimental data, Chung *et al.* (2013) compared *D. rotundifolia* with *Sarracenia purpurea*. Both species exhibit similar genetic population structure, and for *Sarracenia purpurea* dispersal is experimentally proven to reach a distance of 12.8 cm (Ellison & Parker 2002), although long distance dispersal cannot be ruled out. With respect to habitat fragmentation, *D. rotundifolia* and other *Drosera* species of the northern hemisphere are today relicts of a pre-anthropogenic but postglacial period, with a high degree of specialisation which survives in raised bogs (Seeholzer 1993). The species *D. rotundifolia* is specialised and adapted to the microclimate conditions of peatlands, for example, the higher air humidity compared to mineral soils or lower topsoil temperatures in the summer period (Seeholzer 1993, Baranyai & Joosten 2016), thus, colonisation outside its bog habitats is impossible. The destruction of *Drosera* habitats (especially bogs and fens), as well as their eutrophication, leads to the reduction of natural *Drosera* populations. By, for example, drainage of peats and conversion to agricultural land use, these fragmented habitats are no longer connected and thus conditions develop which today form barriers between populations.

The second part of our hypothesis states that due to the fragmentation of habitats, locally adapted genotypes on the islands Amrum and Sylt were formed. If so, we should detect structured populations that differ between populations but are more or less homogenous within populations. We partly agree with this proposition too. The patterns of genetic diversity showed a clear separation between the local genotypes of the *D. rotundifolia* populations on the islands Amrum and Sylt (shown in Table 1 (H*e*) and Figure 3). In addition, the Nei´s gene diversity index (H*e*, Table 1) and the STRUCTURE analysis (Figure 3) showed a clear separation between both *D. rotundifolia* populations on the island Amrum. The relatively clear separation of the fragmented *D. rotundifolia* populations Amrumer Dünen (H*e* = 0.174) and Amrum Wittdün (H*e* = 0.247) is probably related to the ecological conditions of the habitats in the investigation area. According to the view of Baranyai & Joosten (2016), that *D. rotundifolia* grows in the highest areas of bog micro relief where it is able to grow fast enough to evade competition with the *Sphagnum* mosses, we observed that the northern *D. rotundifolia* population on the island Amrum (Amrumer Dünen) indeed grows on rather high *Sphagnum* hummocks. In contrast, the southern *D. rotundifolia* population on the island Amrum (Amrum Wittdün) grows on moist sand.

In summary, the patterns of genetic differentiation and diversity in the ISSR data showed a clear separation for some local genotypes of the *D. rotundifolia* populations in Schleswig-Holstein. The relatively clear separation of the fragmented *D. rotundifolia* populations is caused by the reproduction and different dispersal strategies of *D. rotundifolia*. According to Tata *et al.* (2018) the genetic diversity of a species is related to its effective population size, pollination type, dispersal system or selection pressure. Destruction of *Drosera* habitats (especially bogs and fens), as well as their eutrophication, leads to the reduction of natural *Drosera* populations and raises the importance of reproduction or pollination processes as a barrier between the populations. As a consequence, efforts to preserve *D. rotundifolia* must include a population-based concept, rather than a species-based concept, since each population represents an observable genotype. To support the species as a whole at present, the ecological conditions need to be preserved, as *Drosera* is a sensitive indicator of global climate change and environmental impacts such as enhanced nitrogen deposition (Nordbakken *et al* 2004). To restore the declining populations it would be necessary to focus on population based seed collecting, followed by ex-situ cultures and replanting with plants originating from the specific population, to preserve the singularity of the genotype.

Chung *et al.* (2013) already concluded that conservation strategies for *D. rotundifolia* should focus on ex-situ germplasm collections to support in situ-populations. Our recommendations for *D. rotundifolia* in the investigated area follow this approach. It will, on one hand, be necessary to protect and manage the remaining populations and habitat. Surface management to create open space supports the seed bank and germination positively. On the other hand, we recommend site specific ex-situ cultivation and replanting or sowing. Due to the existing genotypic diversification we would only recommend the use of individuals from the local

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genotypes, especially from the nearest neighbours to boost the local populations.

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