

Research

Population structure of *Rosa spinosissima* L. on the Frisian Islands and introgression from cultivated material

Eike Mayland-Quellhorst and Volker Wissemann

E. Mayland-Quellhorst (<https://orcid.org/0000-0003-0688-7928>) ✉ (eike.mayland.quellhorst@uni-oldenburg.de), Carl-von-Ossietzky Univ. Oldenburg, Oldenburg, Germany. – V. Wissemann (<https://orcid.org/0000-0003-0345-2603>), Justus-Liebig-Univ., Giessen, Germany.

Nordic Journal of Botany

2021: e02941

doi: 10.1111/njb.02941

Subject Editor:

Alicia Montesinos Navarro

Editor-in-Chief: Sara Cousins

Accepted 14 December 2020

Published 17 February 2021



Rosa spinosissima L. is part of the highly dynamic coastal habitat of the Wadden Sea, between the coast of northwestern continental Europe and the Frisian Islands. Plants of this species grow clonally via root suckers to form large patches, which stabilize dunes and help to protect the islands and used for this purpose locally on the island Sylt. *Rosa spinosissima* plants are affected by both water and wind in their harsh environment, and are threatened by human impact via habitat destruction and introduction of foreign species. In these northern parts of its range *R. spinosissima* is categorized as endangered.

The population structure of *R. spinosissima* was investigated using ten microsatellite loci and 195 individuals of Frisian Island populations and European coastal continental samples. DAPC, Kmeans and Structure analyses were conducted to study the genetic composition and integrity of these natural populations relative to cultivated and nursery material mostly consisting of central continental genotypes.

The DAPC and Structure analyses differentiated populations of the Frisian Islands between each other and separated them from nursery samples, but some natural populations showing an impact of nursery and cultivated genotypes. In addition, some clonal patches of *R. spinosissima* were detected. Our results show that the natural Frisian Island populations of *R. spinosissima* are threatened by the introduction of non-adaptive genotypes into the gene pool.

Keywords: invasive biology, microsatellite, population structure, *Rosa spinosissima*, Wadden Sea

Introduction

The coastal habitats of the Wadden Sea, a UNESCO World Heritage Site, are highly dynamic environments due to influences of water and wind combined with high habitat and species diversity. The tidal flow in particular has a strong influence on the Wadden Sea area including its islands. Whereas the German East Frisian Islands (in our study represented by Norderney) were formed by water, wind and the tides via alluvial accumulation of sand, the North Frisian Islands (Sylt and Amrum) – although strongly shaped by these elements – are by contrast remnants of the mainland (Hartung 1958). The island Helgoland is with its distance of around 50 km from the mainland and its



www.nordicbotany.org

© 2021 The Authors. Nordic Journal of Botany published by John Wiley & Sons Ltd on behalf of Nordic Society Oikos

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

composition of sedimentary rock quite distinct from both the East Frisian and North Frisian islands.

On the Frisian Islands and the coast of the North Sea, natural habitats which harbour coastal plants are susceptible to direct physical human impacts and invasive introduced plants. These habitats are a challenging environment for native coastal plants which grow in the alluvial and nutrient-poor soil (Berendse et al. 1998) and must endure high salt content, high water content in the soil, salt spray in the air (Dijkema and Wolff 1983), grazing, burial by sand and being covered up by other shrubs. *Rosa spinosissima* L. is found naturally in these habitats and is particularly susceptible to competition and replacement by foreign plants such as *R. rugosa* Thunb. (Isermann 2008). The original habitat of *R. rugosa* are the coastal areas of Kamtschatka (Russia) and Hokkaido (Japan) and the Manchurian region to north-east China (Yang et al. 2009), and like *R. spinosissima* can form large patches of clonal individuals with root suckers (Bruun 2005, Mayland-Quellhorst et al. 2012). Such growth and dispersal characteristics are very beneficial for the life in the alluvial substrate and help stabilize the sand dunes. Occasionally these two *Rosa* species are deliberately planted for coastal management purposes. *R. rugosa* is used more frequently for this, as its habit is much stronger and the plants more vigorous than the smaller *R. spinosissima*, and its larger red or white flowers and large red hips also make it more attractive from an ornamental perspective and usage of the hips for human consumption.

Rosa spinosissima is an allopolyploid ($2n=4x=28$, Fougère-Danezan et al. 2014) whose natural distribution encompasses the area from Europe to the central parts of Asia (Mayland-Quellhorst et al. 2012). Northern populations in Europe have small plants (< 60 cm tall), whereas southern populations in France, central Germany to Asia comprise larger plants (> 80 cm, up to 2 m tall). Plants with this large habit are also the dominant form available in nurseries. Both morphotypes grow on poorer habitats like sandy soil, rocky and coarse-textured material (Mayland-Quellhorst et al. 2012). Up to now it is not clear if these two types are remnants of different last glacial maximum refugia (Abbott and Brochmann 2003, Brochmann et al. 2004, Hewitt 2004) where the small habit individuals survived in separated habitats or if they have developed their different growth forms due to ecological conditions.

A particular threat to the isolated gene pool of Frisian Island populations of *R. spinosissima* appears to be the planting of nursery material in gardens and urban green spaces. As nurseries seldom sell locally sourced plants, such practice might introduce nonadaptive characteristics into the natural Frisian Island populations. A second threat to the gene pool itself or the species reproductive success can be the very abundant *R. rugosa*. For example, Andersen et al. (2016) showed for *R. × sabinii*, a cross of *R. spinosissima* and *R. mollis*, that the reproductive success of *R. spinosissima* could be reduced by reciprocal hybridisation events and pollen competition.

The genetic structure of several other *Rosa* species were analysed in the past with a well established co-dominant SSR marker system (Esselink et al. 2003, Nybom et al. 2005,

Babaei et al. 2007). This system will be used for *R. spinosissima* and will make it possible to get detailed information of the four chromosome sets and with this a detailed picture of the population history and gene flow.

To assist with conserving the populations of *R. spinosissima* on the Frisian Islands, we aim here to determine: 1) the population structure, genetic diversity and relationship among populations and among islands, that is to see if the populations of the different islands are connected with geneflow or this geneflow is restricted due to clonal reproduction only, 2) the relationship of the Wadden Sea island populations to the mainland population Bloemendaal and cultivated/nursery material and 3) whether there is any evidence for admixture between these different gene pools of *R. spinosissima*.

Material and methods

The material for this study (Table 1, Fig. 1) was sampled from natural populations and cultivated populations of *Rosa spinosissima* from urban green spaces as well as cultivated material from several different nurseries. The natural populations were collected from the Frisian Islands of the German Wadden Sea (Sylt, Norderney, Amrum, Helgoland) and from continental locations on the Dutch Wadden Sea coast in Bloemendaal an Zee. The cultivated material was collected from public parks in central Germany (Marburg, Giessen, Staufenberg) and also purchased from nurseries (five samples each from two nurseries, and 10 samples from another 10 nurseries, which were combined in an artificial group). The samples of the nursery Raa-Besenbeck are offsprings of seeds collected by the nursery owner on the island Sylt from several populations.

DNA from a total of 195 individuals of *R. spinosissima* was extracted from 20 mg silica-dried leaf material with the Qiagen Plant Mini kit following the manufacturer's instructions. Some of these individuals were extracted in a semi-automated way using a QiaCube but with the same kit and protocol.

Genotyping of all individuals was done with 10 simple sequence repeat (SSR) loci (Esselink et al. 2003 and personal communication by G. Werlemark, Table 2), which are known to belong to different linkage groups in the congeneric *Rosa hybrida*. These loci were amplified in four multiplex PCRs which were subsequently genotyped as three multiplexed fragment length analyses. The multiplex sets were arranged with two two-primer (P518 and D221; P50 and E2b) and two three-primer multiplex PCRs (D201, AB40 and AB73; O506, B303 and O517). For all PCRs 30 ng DNA were used. The reaction mastermix had a total volume of 12.5 µl and contained 1× polymerase buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.4 pmol µl⁻¹ of each primer (Table 2), 0.5 U Fermentas recombinant Taq (St. Leon-Rot, Germany) and 5% DMSO. The reactions were run on an Eppendorf MasterCycler (Hamburg, Germany) with an initial denaturation step of two minutes at 94°C followed by 35 cycles denaturation for 30 s at 94°C, annealing for 30 s at

Table 1. Natural, cultivated and nursery populations of *R. spinosissima* with sample size per population and geographic coordinates in decimal degrees.

Population	Size	Coordinates
Natural		
Helgoland	10	7°88'42.14"E, 54°18'73.22"N
Sylt1	10	8°44'88.9"0E, 55°04'44.40"N
Sylt2	10	8°35'38.90"E, 54°95'11.10"N
Sylt3	10	8°35'63.90"E, 54°94'63.90"N
Sylt4	10	8°40'52.80"E, 55°01'02.80"N
Sylt5	10	8°40'16.70"E, 55°02'13.90"N
Sylt6	10	8°38'00.00"E, 55°01'69.40"N
Sylt7	9	8°41'30.60"E, 55°04'83.30"N
Sylt8	10	8°37'47.20"E, 54°99'69.40"N
Norderney1	10	7°18'37.50"E, 53°71'84.70"N
Norderney2	10	7°19'44.20"E, 53°71'90.80"N
Norderney3	10	7°17'33.90"E, 53°71'66.90"N
Amrum1	10	8°32'70.80"E, 54°64'75.30"N
Amrum2	10	8°32'70.80"E, 54°64'75.30"N
Amrum	2	8°35'92.39"E, 54°64'66.53"N
Bloemendaal	5	4°55'42.70"E, 52°40'84.20"N
Civic greens		
Freyburg	2	11°78'11.74699"E, 51°20'04.2067"N
Marburg	10	8°76'90.4048"E, 50°78'73.12705"N
Staufenberg	3	8°71'88.24079"E, 50°66'74.96836"N
Giessen	10	8°68'35.68621"E, 50°59'70.11484"N
Ebsdorf	5	8°80'77.3"E, 50°73'21.81"N
Nurseries		
Ten different nurseries	10	na
Elmshorn	5	na
Raa-Besenbeck (material collected at Sylt)	5	na

50°C, elongation for 45 s at 72°C and a final elongation of 72°C for 5 min.

Fragment length analysis was done on an ABI 3170 XL at LGC Genomics. Quality assessment and raw data analysis were conducted with Peak Scanner™ Software ver. 1.0.

Due to the hybrid origin of the allopolyploid *R. spinosissima*, the inheritance mode of the chromosomes is not clear and possibly could be functional diploid (disomic inheritance), so the SSR alleles could not be treated as independent (Clark and Jasieniuk 2011). Furthermore the retaining of the true allelic state is often difficult in polyploids due to dosage effects in the analysis. For seven of the 10 loci it was possible to infer the allelic configuration of each individual's locus using the dosage dependent allele identification MAC-PR (Esselink et al. 2004) whereby the height and the area of each fragment's peak was compared and missing alleles inferred. The other three loci were treated as genotypic phenotype. We tried none the less our best to retain the 'true' allelic state to get the best genotypic phenotype as possible. We therefore based our analyses on genetic distances which were conducted in R (<www.r-project.org>) with packages polysat (Clark and Jasieniuk 2011) and adegenet (Jombart 2008, Jombart and Ahmed 2011). The population structure was also analysed using Bayesian inference with Structure (Pritchard et al. 2000) summaries and analyses with the Structure results were conducted with the R package pophelper (Francis 2017).

A discriminant analysis of principal components (DAPC) was done with the above mentioned R package adegenet.

In short: a dimension reduction of a principal component analysis (PCA) of the SSR data is done by looking for linear combinations of the PCs by linear discrimination. We did a cross validation of the DAPC with the respective function `adegenet::xvalDAPC` to see which amount of retained principal components show the highest outcome of prediction with the smallest deviation. This value of numbers of retained principal components was further used in a Kmeans cluster analysis and the following DAPC analyses. Different runs of the Kmeans cluster analysis with the `adegenet::find.clusters` function was run for K=2, K=3, K=6 and K=8 with 1000 randomly starting centroids (`n.start=1000`) to get convergence in each Kmeans iteration. A total of 140 principal components (`n.pca=140`) were retained. This different Ks resembles the 'edges' in the banana shaped BIC analysis plot, which is characteristic for a stepping stone model of genetic differentiation as Jombart et al. (2010) describes it in the `adegenet` help. Different grouping information was added to the genotypic dataset in the DAPC to be used as priors. A run without grouping prior was followed by the prior of the Kmeans cluster analysis groups. Grouping information of the Structure analyses for Ks 2, 3 and 6 was also used in the DAPC as prior.

The population analysis with Structure was done with 20 000 MCMC iterations of which 10 000 were discarded as burnin. We ran 100 replications of a range of Ks from 1 to 12. The runs were collected, sorted and merged by functions in the package pophelper to produce assignment barplots.

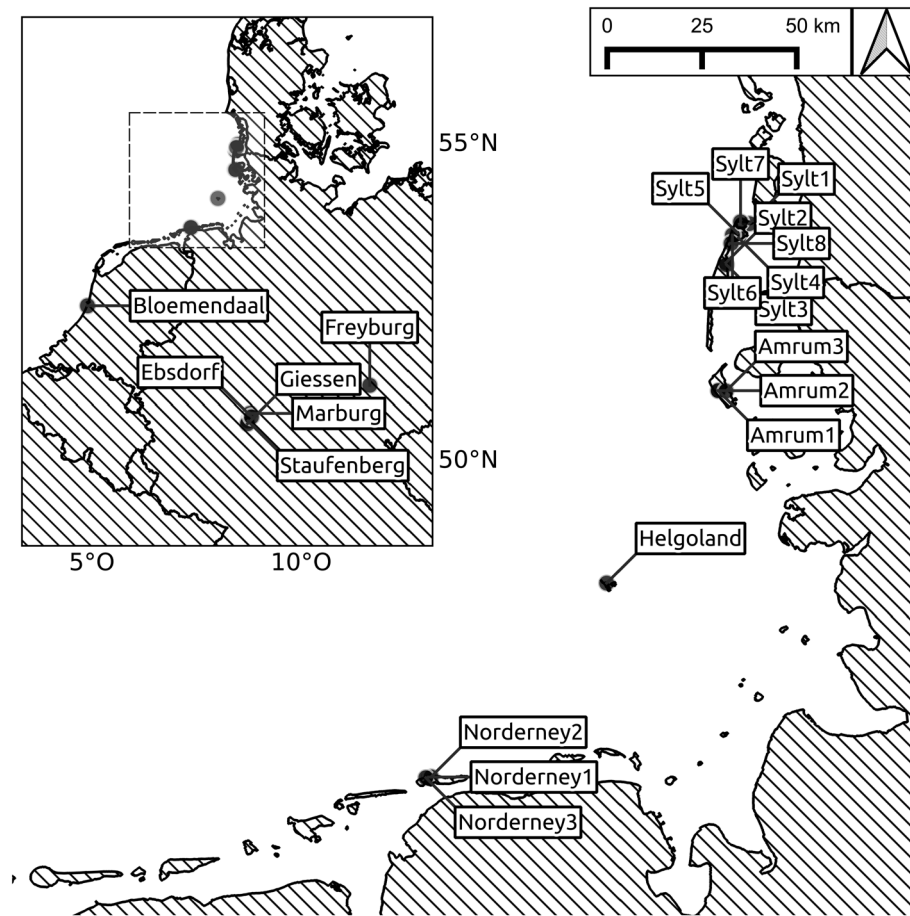


Figure 1. Sampled populations of *Rosa spinosissima* L. in Germany and the Netherlands. Samples collected from nurseries are not shown, the Nursery in Raa-Beesenbeck collected seed material from several populations on Sylt.

An estimation of an optimal K was done with Evannos deltaK (Evanno et al. 2005) in the R::pophelper package (Francis 2017).

We tested for clonality or limited genetic diversity with the polysat::assignClones function with a threshold of zero distance (Clark and Jasieniuk 2011). This method uses a similar approach as GenoType (Meirmans and Van Tienderen 2004) does and is described by Clark and Jasieniuk (2011) as comparable.

Results

A total of 160 alleles for the 10 SSR were found in 195 individuals (Table 2). The loci RhD201, RhD221 and RhE2b have the lowest number of different alleles with 13, 9 and 11 alleles, respectively (Table 2). By contrast, the loci RhAB40, RhAB73 and P50 had the most alleles with 28, 25 and 35, respectively. All loci showed variable alleles, but some alleles seem to occur in all individuals. For example, in locus RhP518 alleles 119 and 130 were found in nearly all sampled individuals, and locus in RhD221 the allele 215/216 occurred in all sampled individuals. The allelic configuration of the MAC-PR approach could not be determined for the

following loci: RhAB40, RhB303 and RhD221, here were only the occurring alleles recorded and not all four possible alleles.

DAPC and population structure

The analysis of structure in the genetic diversity with the discriminant analysis did not show a clear pattern of a best K grouping in the BIC plot (Supporting information). In the STRUCTURE results the Evanno deltaK showed a modal at the K of 2 (Supporting information). We used further in the DAPC analysis as grouping prior the K s of the Kmeans analysis where the steepness of the curve changed, which was the case at K s 2, 3, 6, 8 and 15 ($K=15$ not shown) and the group assignments of K s 2, 3, 6 and 8 of the STRUCTURE analysis. The higher values of K were used to find possible substructure between the island populations.

The PCA scatter plot of the DAPC analysis shows a separation of the samples of *R. spinosissima* from Sylt3, Sylt2, Amrum1 and Amrum2 from the rest at the lower left section, with one sample of the population Amrum close to them (Fig. 2). The other populations from Sylt form with the samples of Raa-Besenbeck an own cluster at the right hand side of the plot. Next to it to the left are populations from Norderney and

Table 2. Primer information for the SSR loci with repeats, expected allele size and total allele count for *Rosa spinosissima* L.

Locus	Primer forward	Primer reverse	Repeats	Expected allele size	Allele count
RhD201	GGTATGCAATAAGAGATACAGT	GTTCTCTCAACAAACCCATTTTGAAGGG	3	164–229	13
RhAB40	AATTGTGTCAATGCCAACAC	GTTCTTGTCTCAACCCATCGAGGTTTG	2	158–259	28
RhAB73	GGTTAGACGGGTGGAAGAAG	GTTTACTGCCGATAGAAAGTATTTTCATCA	2	144–191	25
RhO506	GAAGCCTCAGCAGCATCCTCAAT	GTTCTTCACTGCCAACAAAGCCCATTTGG	3	189–235	15
RhB303	CACCTGCAACAACCCCAATAGC	GTTCTTGTCTTCACTGTTAGCTGTGCTG	2	109–170	8
RhO517	CGGCGACGAACAATCAGCATATC	GTTCTTGTGAAGAACGAGCGCGACGCTAA	3	245–270	10
RhP518	TTCGATCTCCATCTGCAAGA	GTTCTTCTTATAATCTATTACGAAGGCTGG	3	119–150	10
P50	TGATGAAATCATCCGAGTGTGAG	GTTTCACTTTCATTGGAATGCCAGAAAT	3	189–349	35
RhD221	CGTAATTGCTGTGTGACTGCTTT	GTTCTTGGCGCTACGAGGAAATCAA	3	198–232	9
RhE2b	CTTTGCATCAGAAATCTGCTGCATT	GTTCTTGCAGACACAGTTCATTAAAGCAG	3	155–175	11
Total					164

the coastal mainland population Bloemendaal, of which some individuals overlap with the cluster of the remaining samples of nurseries and cultivated origin at the top left section (Fig. 2). The Kmeans cluster analysis showed in the BIC analysis only a maximum of 15 groups (Supporting information). As the group assignments are not absolute, but probabilistic the relative assignments are plotted in each of the K-group colors leading to mixed membership probabilities. Based on an analysis with the Kmeans values ($K=2$) used as prior, the DAPC barplot in Fig. 3 shows that there are two groups; one with the natural populations, and one with the cultivated genotypes and the populations of Helgoland, Amrum, and from Sylt3, showing that these natural populations are either cultivated plants or an admixture. Thus, the two groups can be summarized as a ‘natural group’ and a ‘cultivated group’. One sample of the small population Amrum, which was collected near buildings is also assigned to the ‘cultivated group’. With growing K s of $K3$, $K6$ and $K8$ populations on the islands Sylt, Norderney and Amrum get more separated. The population Raa-Besenbeck, being sold as samples of *R. spinosissima* grown of seeds collected on Sylt, revealed at $K8$ a mixture of samples belonging to two Sylt clusters. The coastal population Bloemendaal showed at $K8$ a mixture of samples belonging to the ‘mainland’ cluster and to the nearest East Frisian island Norderney.

Two partitions also were observable in the Structure analyses (Fig. 4), where Evanno’s delta K method, as indicator for an optimal K , showed the highest value at $K=2$ (Supporting information). The is the same differentiation, as in the DAPC result (Fig. 2). The natural island and mainland populations can be observed on the one hand, with the nursery and urban green samples on the other, but this Structure analysis based on Bayesian model analysis revealing more admixture details in the samples, namely in the Sylt3 and Bloemendaal populations (Fig. 4) starting from $K=2$.

In addition to Structure $K=2$ which had the highest delta K (Supporting information), partitions of $K=3$, $K=6$ and $K=8$ were analyzed following the Kmeans grouping. Again the $K=3$ partition resolved more fully the Frisian Island populations by dividing the Sylt and the Norderney populations into two parts (Fig. 4). Using the assigned cluster information from this Structure analysis in a DAPC as grouping prior revealed the same sample assignment as the Kmeans cluster information (Supporting information). The admixture signal of Sylt and Bloemendaal becomes stronger in $K=3$, $K=6$ and $K=8$ and leads to a separation of Sylt3 in $K=8$ into an own cluster. Also the population Raa-Besenbeck shows a high level of admixture in $K=6$ and $K=8$. Please be aware of switched colors for the cultivated populations from red to orange in $K=6$ and from red to green for Amrum1 and Amrum2 in $K=8$ due to the pophelper::alignK procedure.

Population clonality

With our very strict threshold we found 32 clones of which the most belongs to the Frisian island populations. Most prominently Sylt8 had 7 out of 10 clones, Norderney1 and Sylt7 6 out of 10 and Amrum2 5 out of 10.

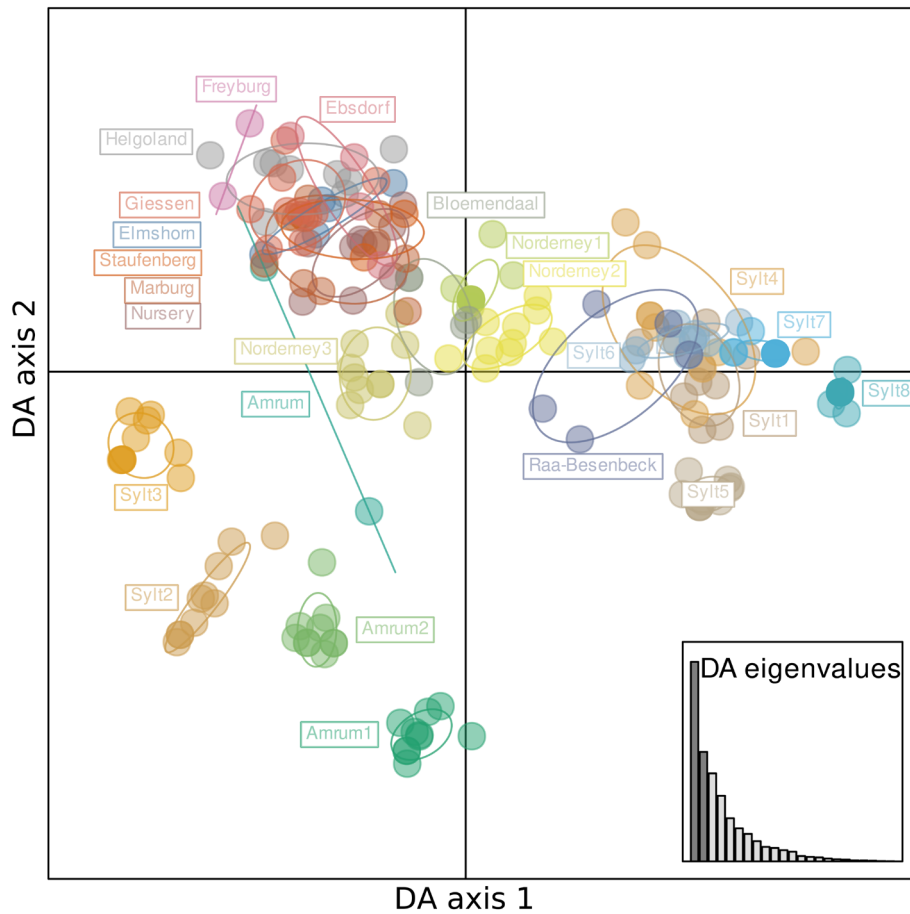


Figure 2. DAPC plot of a crossvalidation analysis (adeigenet::xvalDAPC, (Jombart 2008)) of genotypes from samples of *Rosa spinosissima*. The discriminant analysis of principal components reduces the dimensions of a principal components analysis by linear discrimination of the principal components. With the crossvalidation several numbers of retained principal components are tested for validity to objectify the decision of used data in the DAPC. Colors represent the sampled populations, with the name in the boxes and the distribution of the sample points given by the ellipse. North Frisian Islands of Sylt and Amrum separate from the rest, the East Frisian Island Norderney separate also with a central position in the plot and populations with cultivated material build a single cluster at the top with some samples of Amrum, Norderney and Bloemendaal close to it or member of that cluster. The inset barplot show the eigenvalues of the discriminant analysis and in dark grey the scatter plot axes.

Discussion

The Wadden Sea coast is a unique environment which presents challenging habitats for coastal plants due to their abiotic and biotic conditions and isolated location. Our analyses of *Rosa spinosissima* show that the genotypes of the Frisian Island populations can be identified in our analyses as mostly separate identities and can be separated from the cultivated material, as can be observed as the bipartition in the DAPC and Structure analyses of the SSR data (Fig. 2, 3 and 4). The nearest mainland population Bloemendaal shows similar but more diverse allelic combinations in the sampled individuals.

Genetic differences also exist among Frisian Island populations, e.g. between the populations at Sylt and between the other islands, as visible in the Structure partition of $K=3$ and $K=6$ (Fig. 4). On the other hand, the clonal growth of large patches can be observed with our data as some populations were put into unique clusters even with large K s and

our clonal assignment analysis. Most of the populations on the Frisian Islands on the German North Sea coast seem to be natural populations with only little gene flow between them, as most samples from natural populations group with other samples from the same population in unique clusters. One exception was the population Sylt3 whose individuals displayed high levels of admixture including the influence of nursery material, and could not be unambiguously assigned to a cluster of the other Sylt populations nor to the mainland cluster (Fig. 3, 4). Population Sylt3 is located in the central part of the island of Sylt which has a much higher human population density than the northern part, where most of the other sampled Sylt populations are located. In addition to Sylt3, the Helgoland population is the only other island population which seems to be of pure cultivated origin (Fig. 3, 4). The population of Bloemendaal, located on the Netherlands coast, is the only natural population in which most individuals contain admixed profiles of all three Structure groups with

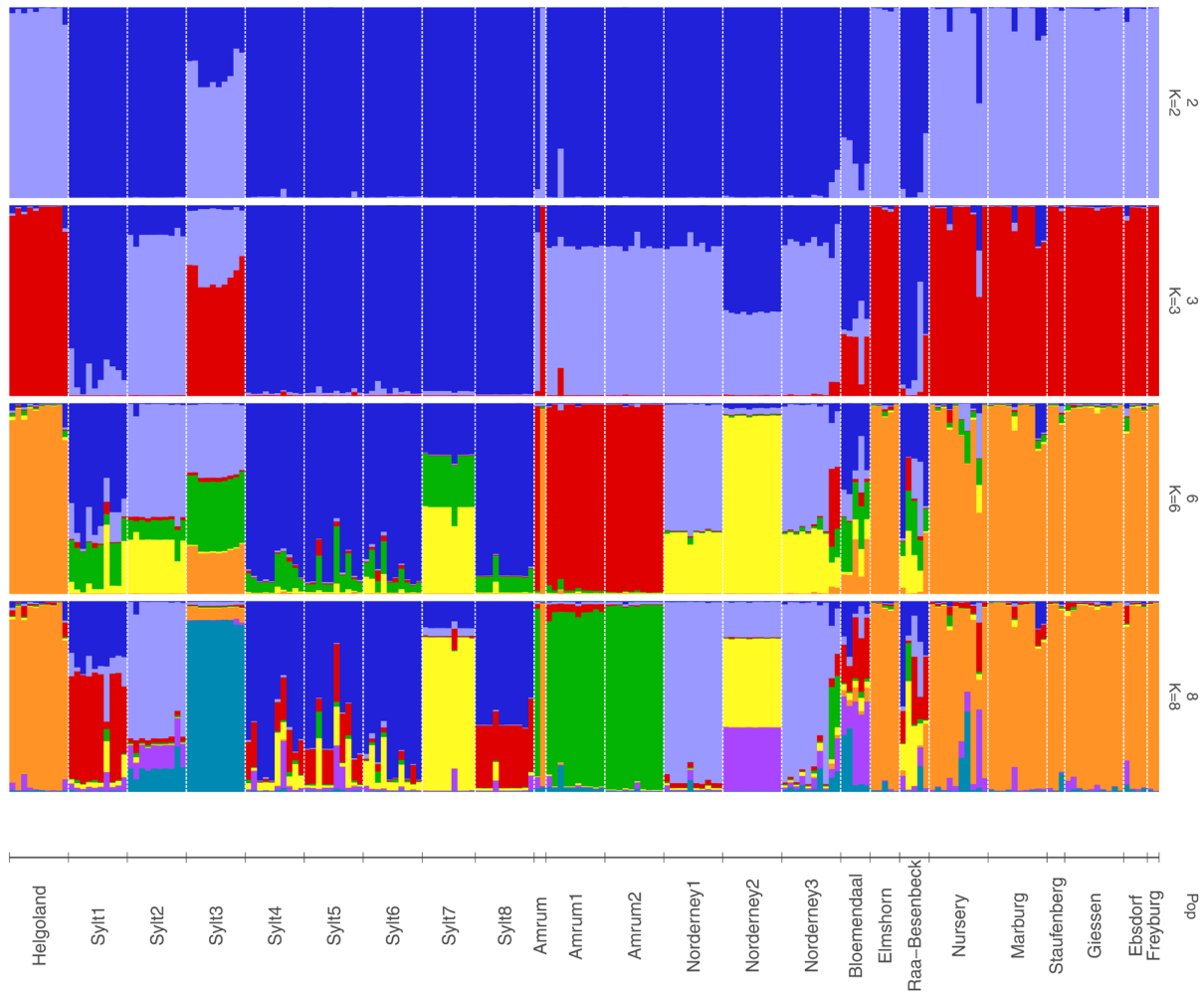


Figure 4. A barplot representation of Structure (Pritchard et al. 2000) sample assignment probabilities as mean assignment values aggregated in R with pophelper::sortQ, alignK, mergeQ (Francis 2017). Assignment probabilities to $K=2$, $K=3$, $K=6$ and $K=8$ groups are shown. Evanno's optimal number of K (pophelper::evannoMethodStructure) reported $K=2$, but higher numbers of K made biologically and geographically sense, so they are reported here.

population. So finding single or few populations in a region needs special consideration and investigation of the population diversity for conservation treatments regarding the genotype diversity.

It is likely that character states beneficial for life in salt-influenced areas are differentially fixed in the two habit morphotypes of *R. spinosissima*, although data addressing this question are currently not available. This different adaptation might be based on a disjunct distribution during the last ice age where the northern ones could have been permanently growing in similar habitats on the continental shelf at the coast line. If indeed that is the case, introduction of nonadaptive seed material and plants to the Frisian Islands and other coastal populations may reduce the fitness of *R. spinosissima* in the Wadden Sea habitats. It is therefore strongly recommended that only autochthonous plant and seed material originating from this coastal habitats are planted in this areas. If plant material is chosen, then a main focus has to be that this is not grown clonally of only one or few source plants.

Fougère-Danezan et al. (2014) proposed with a network analysis of GAPDH sequences potential parent species of *R. spinosissima* belonging to groups of *Pimpinellifolia* and *Cinnamomae*. With this chromosome configuration the alleles might not be equally segregated during meiosis (Clark and Jasieniuk 2011) which make the analyses of population structure and hybridisation difficult.

Beside the already mentioned thread of the genetic integrity through non-autochthonous *R. spinosissima* seed material this might also hold true for *R. rugosa* seeds and plants. The location of one GAPDH allele of *R. spinosissima* in the network analysis of Andersen et al. (2016) as direct sister to *R. rugosa* indicate that there might no huge barrier against hybridisation as also shown for *R. mollis* with *R. rugosa* and its hybrid *R. × mangii* (Kellner et al. 2012) or *R. mollis* with *R. spinosissima* *R. × sabinii* (Andersen et al. 2016). So the genetic integrity of the island populations might be directly influenced by *R. rugosa* through introgression or with pollen competition like also Andersen et al. (2016) showed in

a similar constellation of *R. mollis* and *R. spinosissima* on a Norwegian island.

To conclude our findings. The populations of *R. spinosissima* at the islands of the German Wadden Sea are different in morphology from large growing main continental populations. They also differ genetically from cultivated genotypes, of which the exact origin is unknown, but can have their origin in southern German or Austrian populations, as they show the same morphology. If this is just of the very narrow genotype diversity bourne in the selection and cultivation by nurseries needs future investigation. The population structure is very homogeneous in the single island populations, which can be a result of mostly clonal populations with limited gene flow. On the islands is strong influence of cultivated material, which also pops up in populations not directly connected to human settlements. Whether this genetical differentiation between the island and coastal populations of the northern morphotype and the southern central continental morphotype is based on historical partitions like in *Rosa mollis/villosa* (Kellner et al. 2013) or ecological traits is not distinguishable in this study and remains for future common garden experiments and future analysis of historical genetic data of ancient pollen derived from sediments. To date the genetic integrity of the Wadden Sea populations of *R. spinosissima* has to be preserved by using only autochthonous seed material for new plantings.

Data availability statement

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.vx0k6djqq>> (Mayland-Quellhorst and Wissemann 2020).

Acknowledgements – We would like to thank all people sending us samples of *R. spinosissima*. Thanks also to colleagues Maike Isermann, F. Schluetz, Karsten Wesche and Petra Gebauer. We would also like to thank Heidi Meudt for critical revision of the manuscript.

Author contributions

Eike Mayland-Quellhorst: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Software (lead); Visualization (lead); Writing – original draft (lead).
Volker Wissemann: Conceptualization (supporting); Funding acquisition (lead); Project administration (supporting); Resources (lead); Writing – review and editing (supporting).

References

Abbott, R. J. and Brochmann, C. 2003. History and evolution of the arctic flora: in the footsteps of Eric Hultén. – *Mol. Ecol.* 12: 299–313.

- Andersen, H. L. et al. 2016. Hybridization between the locally endangered *Rosa spinosissima* and *Rosa mollis* results in the pentaploid *Rosa* × *sabini* in western Norway. – *Nord. J. Bot.* 34: 645–646.
- Babaei, A. et al. 2007. Microsatellite analysis of Damask rose (*Rosa damascena* Mill.) accessions from various regions in Iran reveals multiple genotypes. – *BMC Plant Biol.* 7: 12.
- Berendse, F. et al. 1998. Soil organic matter accumulation and its implications for nitrogen mineralization and plant species composition during succession in coastal dune slacks. – *Plant Ecol.* 137: 71–78.
- Brochmann, C. et al. 2004. Polyploidy in arctic plants. – *Biol. J. Linn. Soc.* 82: 521–536.
- Bruun, H. H. 2005. *Rosa rugosa* Thunb. ex Murray. – *J. Ecol.* 93: 441–470.
- Clark, L. V. and Jasieniuk, M. 2011. polysat: an R package for polyploid microsatellite analysis. – *Mol. Ecol. Resour.* 11: 562–566.
- Dijkema, K. S. and Wolff, W. J. 1983. Flora and vegetation of the Wadden Sea islands and coastal areas. – AA Balkema.
- Esselink, G. D. et al. 2003. Identification of cut rose *Rosa hybrida* and rootstock varieties using robust sequence tagged microsatellite site markers. – *Theor. Appl. Genet.* 106: 277–286.
- Esselink, G. D. et al. 2004. Assignment of allelic configuration in polyploids using the MAC-PR (microsatellite DNA allele counting-peak ratios) method. – *Theor. Appl. Genet.* 109: 402–408.
- Evanno, G. et al. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. – *Mol. Ecol.* 14: 2611–2620.
- Fougère-Danezan, M. et al. 2014. Phylogeny and biogeography of wild roses with specific attention to polyploids. – *Ann. Bot.* 115: 275–291.
- Francis, R. M. 2017. pophelper: an R package and web app to analyse and visualize population structure. – *Mol. Ecol. Resour.* 17: 27–32.
- Hartung, W. 1958. Entstehung und Probleme der Nordseeküste mit den Ostfriesischen Inseln. – *Z. Dtsch. Geol. Ges.* 110: 592–636.
- Hewitt, G. 2004. Genetic consequences of climatic oscillations in the Quaternary. – *Phil. Trans. R. Soc. B* 359: 183–195.
- Isermann, M. 2008. Expansion of *Rosa rugosa* and *Hippophaë rhamnoides* in coastal grey dunes: effects at different spatial scales. – *Flora-Morphol. Distrib. Funct. Ecol. Plants* 203: 273–280.
- Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. – *Bioinformatics* 24: 1403–1405.
- Jombart, T. and Ahmed, I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. – *Bioinformatics* 27: 3070–3071.
- Jombart, T. et al. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. – *BMC Genet.* 11: 94.
- Kellner, A. et al. 2012. Hybridization with invasive *Rosa rugosa* threatens the genetic integrity of native *Rosa mollis*. – *Bot. J. Linn. Soc.* 170: 472–484.
- Kellner, A. et al. 2013. Low genetic and morphological differentiation in the European species complex of *Rosa sherardii*, *R. mollis* and *R. villosa* (Rosa section Caninae subsection Vestitae). – *Bot. J. Linn. Soc.* 174: 240–256.
- Mayland-Quellhorst, E. and Wissemann, V. 2020. Data from: Population structure of *Rosa spinosissima* L. on the Frisian Islands and introgression from cultivated material. – Dryad

- Digital Repository, <<http://dx.doi.org/10.5061/dryad.vx0k6djqq>.
- Mayland-Quellhorst, E. et al. 2012. Biological Flora of the British Isles: *Rosa spinosissima* L. – J. Ecol. 100: 561–576.
- Meirmans, P. G. and Van Tienderen, P. H. 2004. Genotype and Genodive: two programs for the analysis of genetic diversity of asexual organisms. – Mol. Ecol. Notes 4: 792–794.
- Nybom, H. et al. 2005. Sexual preferences linked to rose taxonomy and cytology. – Acta Horticulturae 690: 21–28.
- Pritchard, J. K. et al. 2000. Inference of population structure using multilocus genotype data. – Genetics 155: 945–959.
- Yang, J.-H. et al. 2009. Genetic diversity of the endangered species *Rosa rugosa* Thunb. in China and implications for conservation strategies. – J. Syst. Evol. 47: 515–524.