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GENETIC DIVERSITY OF THE GREEN MACROALGA *ULVA LINZA* IN KATTEGAT AND THE BALTIC SEA WITH RECOMMENDATIONS FOR AQUACULTURE



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1 Abstract

Genetic diversity is essential for the establishment of a sustainable seaweed aquaculture, as it serves as the foundation for population health and resilience to environmental stressors. The interest in green algal species cultivation has grown worldwide, particularly of the genus *Ulva*, commonly known as sea lettuces. *Ulva* is characterized by a haplodiplontic life cycle, with partially clonal reproduction. The mode of reproduction, sexual versus asexual, affects the genetic composition of a population and thereby shaping its ecological success and adaptive capacity. In this MSc thesis, the genetic diversity of *U. linza* was assessed and clonal lineages were characterized along the salinity gradient of Skagerrak, Kattegat, Öresund, and the Baltic Sea. To assess the genetic variation, 67 samples of *U. linza* were genotyped with 21,205 2b-RAD derived single nucleotide polymorphisms (SNPs). Overall, clonality was high. Among the 67 samples, 31 multilocus genotypes (MLG) were identified, two of which comprised 10 ramets each. Individuals in the Baltic Sea are predominantly clonal and genetic diversity in this region is low. In contrast, the highest genetic diversity was identified in Kattegat and Öresund with several smaller clonal lineages. In the Baltic Sea, individuals belonging to the same clonal lineage showed a broad distribution. This is the first study outlining the genetic diversity of *U. linza* in the Nordics. The here identified clusters of clonality and genetic variation can be used for prioritizing genetic resources of populations for the recruitment of individuals for aquaculture practices.

Keywords: Clonality, *Ulva linza*, Genetic diversity, Aquaculture, 2b-RAD

2 Introduction

2.1 Seaweed aquaculture

The world's population is growing, leading to an increased food demand. The global food demand projections indicate a potential 60 % rise when the world population reaches 9.3 billion. At the same time, the impacts of climate change threatens the agriculture stability (FAO, 2023). To secure the future human food supply, the cultivation of macroalgae emerges as a promising alternative, offering a sustainable marine resource to meet the food demands. The seaweed aquaculture, characterized by fast growth and environmental sustainability, has gained momentum in the last decade. In 2016, the seaweed production peaked at 30.2 million tons (Alemañ et al., 2019) and in 2019, the seaweed industry made up 5.4 % of the USD 275 billion global aquaculture production value (FAO, 2021). Despite its prominence in Eastern Asia, where seaweeds are widely consumed, its utilization remains relatively underexplored in other regions of the planet (FAO, 2021).

Expanding seaweed biomass production not only addresses food supply concerns but also offers utility for diverse applications, including feed production, pharmaceuticals and biotechnological applications. Moreover, seaweed cultivation fosters economic growth in coastal communities while enhancing food security (Brakel et al., 2021). In addition, seaweed aquaculture provides vital ecosystem services such as habitats provision, shoreline protection, and mitigation of ocean acidification and deoxygenation, thus contribution positively to climate change adaptation (Fernández et al., 2019).

2.2 *Ulva*

The interest in green algal species cultivation has grown worldwide (Charrier et al., 2017; FAO, 2020), particularly of the genus *Ulva*, commonly known as sea lettuces. *Ulva* is known for its ability to rapidly grow under various conditions (Simon et al., 2022) and their nutritional value such as a high protein content (Cardoso et al., 2023).

However, accurate identification of *Ulva* species worldwide remains challenging due to extreme morphological plasticity and cryptic speciation, influenced by changing environments (Steinhagen et al., 2019).

As the sea-based cultivation continues to expand, the accurate species identification of taxa becomes increasingly important and while traditional taxonomic methods rely on morphological traits to identify *Ulva* species, molecular techniques such as DNA barcoding may offer a more reliable alternative, particularly in geographic regions where algal diversity is understudied (Lagourgue et al., 2022; Wichard et al., 2015; Steinhagen et al., 2023).

Moreover, assessing genetic diversity within *Ulva* is crucial for the implementation of breeding strategies, population health, and resilience to environmental stressors (Salgotra & Chauhan, 2023).

2.3 Life cycle

Ulva species are characterized by a haplodiplontic life cycle, alternating between free-living haploid gametophyte and diploid sporophyte stages, both of which are isomorphic and therefore indistinguishable (Mantri et al., 2020). During the sexual cycle, the diploid sporophyte undergoes meiosis and produces haploid spores which give rise to the haploid gametophytes. The gametophytes, in turn, produce haploid gametes through mitosis. The diploid sporophyte originates from the fusion of gametes of the opposite mating type (Wichard et al., 2015). The sexual processes of segregation and recombination (meiosis) and fusion (fertilization) cause a spatial and temporal separation of the two life stages (Stoeckel et al., 2021).

Gametes, originating from gametophytes and zoospores, originating from sporophytes, can be morphologically distinguished by the number of flagella, with biflagellate gametes and quadriflagellate zoospores (Løvlie & Bryhni, 1978; Steinhagen et al., 2022).

Additional to the sexual cycling of the ploidy stages, both stages are also capable of clonal reproduction (Steinhagen et al., 2022; Wichard et al., 2015).

This alternation of sexual and clonal reproduction has shown to significantly affect evolutionary trajectories of populations in diploid taxa (Reichel et al., 2016; Rouger et al., 2016). However, genetic consequences of taxa that are both haplodiplontic and partially clonal remain understudied (Krueger-Hadfield et al., 2021; Stoeckel et al., 2021).

The reproductive mode influences within species genetic variation, thereby shaping their ecological success and adaptive capacity (Krueger-Hadfield et al., 2021). As intraspecific genetic diversity reinforces species survival, and their ability to withstand environmental stressors, it serves as the foundation for sustainable cultivation (Brakel et al., 2021). Therefore, elucidating the species genetic structure and distribution of genetic diversity is imperative for sustainable aquaculture practice (Brakel et al., 2021; Guillemin et al., 2008; Krueger-Hadfield et al., 2016)

3 Aim and Hypothesis

This study focuses on assessing the genetic diversity of *Ulva linza* along the salinity gradient of Skagerrak, Kattegat, Öresund and the Baltic Sea, with a particular emphasis on identifying and characterizing clonal lineages. I hypothesize that genetic diversity within *U. linza* will exhibit spatial heterogeneity, with distinct clonal lineages across geographical locations.

4 Material and Methods

4.1 Library preparation

Extracted DNA from 148 *Ulva linza* tissue samples was provided by Steinhagen et al. (2023). The samples were collected across 4 countries, along the Skagerrak, Kattegat, Öresund, and Baltic Sea (Sweden, Denmark, Germany, and

Finland) over the period from 2014 to 2021. The samples were collected from different habitats, including rock pools, national parks, estuaries, harbours, exposed- and sheltered areas. Tissue samples were collected wading or snorkeling. The samples were immediately stored at -20°C at the sampling site and then transferred to -80°C in the laboratory. Previous to DNA extraction, 10-15 mg of tissue were freeze-dried and homogenized with beads in a 1.5 ml Eppendorf tube using a mixer- mill for 5 min at 30 Hz. DNA was extracted from the resulting powder following the NucleoSpinn® Plant II protocol (Macherey-Nagel), applying the Buffer PL1 treatment for cell lysis and DNA was eluted in 200 μl ddH₂O. Afterwards, extracted DNA was purified following the DNA Clean & Concentrator®-25 protocol (Zymo Research) and eluted in 22 μl dd H₂O.

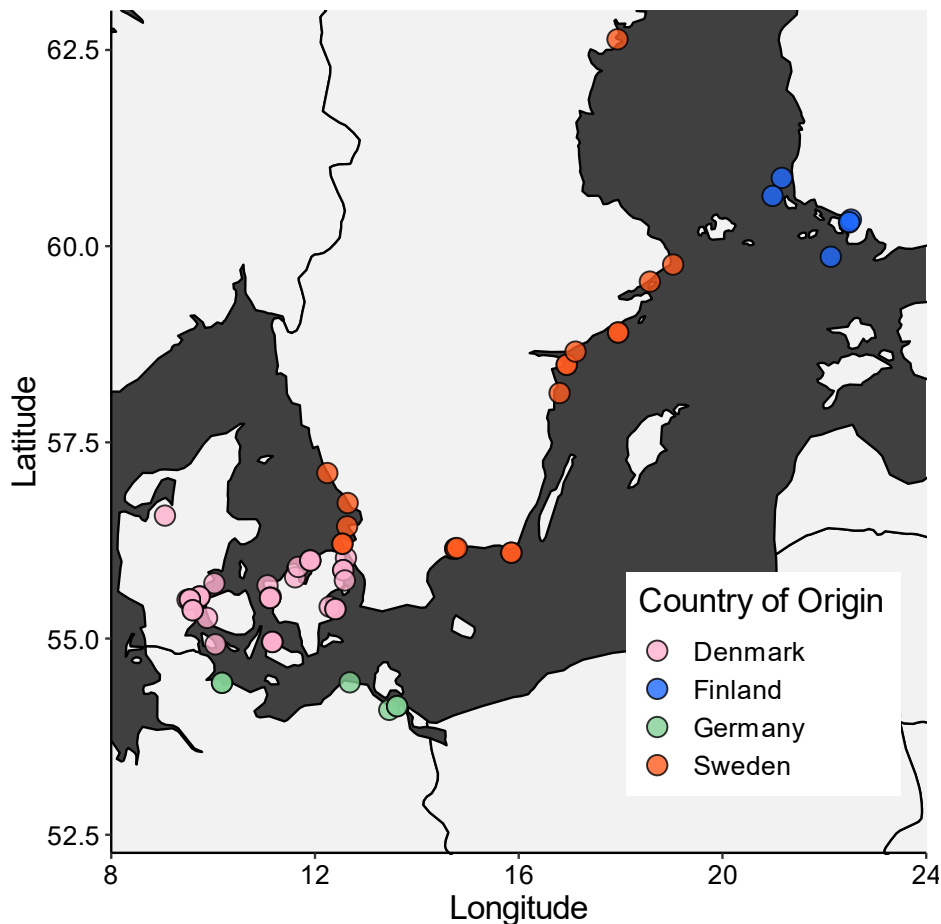


Figure 1. Map of the study region showing 67 sampling site of *Ulva linza*. The colors represent the country of origin of each individual. Coordinate reference system: EPSG:4326.

DNA concentrations were assessed with a Qubit fluorometer (Thermo Fisher Scientific) using the Qubit™ dsDNA-HS (High Sensitivity)-Assay-Kit. Samples with low DNA concentrations were concentrated by evaporation during a heat incubation at 40°C for ~2 hours.

Good quantity DNA samples were selected to generate libraries using the 2b-RAD (restriction-site-association DNA) sequencing method. Additionally, 15 technical replicates were prepared, i.e., samples, for which library preparations were conducted twice. For digestion, ligation, and amplification, a modified protocol of the one described in Wang et al. (2012) was followed. For digestion, the Bcgl restriction

enzyme was used to generate fragments. Adapters were then ligated to the restriction fragments and the constructs were amplified. The 170 bp fragments were cut out- and extracted from a 2 % agarose gel using the MinElute Gel Extraction Kit (Qiagen). Finally, the library concentrations were measured with the Qubit fluorometer and pooled to equal molarities. The libraries were sent to the Science for Life Laboratory ([SciLifeLab](http://www.sciencelife.se)) for Illumina sequencing.

The final dataset comprised 79 sequenced individuals (**Figure 1**; including 12 technical replicates, i.e., 15 % of all samples) and genotyped at 21,205 polymorphic sites (single nucleotide polymorphisms, SNPs). The data set was filtered for loci and individuals with missing data of >5 %.

4.2 Clonality

4.2.1 Clonal lineage identification and genetic distance matrix

In partially clonal reproducing species, the first step in genomic analyses, is to distinguish between clones (ramets = genotype copies) and unique genotypes (genets = multilocus genotype (MLG)). To identify MLGs, the pairwise genetic distances between individuals were calculated using the function “prevosti.dist” from the R package “poppr 2.9.5”, which utilizes Prevosti’s genetic distance to assess the number of different SNPs between samples. In the next step, the maximum genetic distance as cut-off value was calculated to assign ramets to MLGs. The “cutoff_predictor” function in the R package “poppr 2.9.5” utilizes three different clustering algorithms (Farthest Neighbor, Nearest Neighbor, and Unweighted Pair Group Method with Arithmetic Mean (UPGMA)), that act on the distance matrix, to filter for the top fraction of threshold differences and find the largest difference. The average between the genetic distances spanning the largest difference is used to define the genetic cut-off threshold (Supplementary **Figure S1**; Kamvar et al., 2015). To validate the cut-off threshold, the largest genetic distance between a pair of technical replicates, generated through sequencing errors, was calculated, aligning with the value obtained from UPGMA clustering. Individuals with a genetic distance below this threshold were considered to cluster into the same MLG. The cut-off value was applied to obtain individuals assigned to each MLG using the “mlg.filter” function in “poppr 2.9.5”.

The genetic distance matrix was used to create a hierarchical clustering tree to corroborate the accuracy of detecting MLGs, using the “hclust” function of the R package “stats 4.3.2” based on the WPGMA (weighted pair group method with arithmetic mean) agglomeration method (Kamvar et al., 2015). In addition, to better visualize the similarities between genotypes and clonal clusters, the function “pheatmap” of the R package “pheatmap 1.2.12” was utilized to create a clustered heatmap with dendrograms based on the pairwise genetic distance matrix. Further, a Minimum Spanning Network (MSN) was plotted using the distance matrix, to examine interclonal reticulated affinities and reveal if clonal lineages evolved independently. To create the MSN the “poppr.msn” function in the R package “poppr 2.9.5” was used.

4.2.2 Linkage disequilibrium and inbreeding coefficient

To further validate the presence of clonality, each population was tested for Linkage Disequilibrium (LD) by calculating the standardized index association r_d . LD was calculated using the R package “poppr 2.9.5”. The index of association estimates the

non-random association between alleles at different loci (LD) due to clonality (Agapow & Burt, 2001). LD is supposed to break down with time depending on recombination rates.

Another estimate that may indicate the presence of clones, is the inbreeding coefficient (F_{IS}). F_{IS} estimates the deviation of the observed proportion of heterozygotes from expectations under Hardy-Weinberg Equilibrium (HWE). Clonality is expected to lead to an excess of heterozygotes which is indicated by a negative F_{IS} value (Allendorf et al., 2022). F_{IS} was calculated per population as:

$$F_{IS} = 1 - (\text{mean } (H_o) / \text{mean } (uH_e)),$$

where uH_e is the unbiased expected heterozygosity, i.e., corrected for sample size. The function “gl.report.heterozygosity” of the package “dartR 2.9.7” was used (Mijangos et al., 2022).

4.2.3 Genotypic Richness (R)

After establishing MLGs, the genotypic richness (R) was calculated. R characterizes the distribution of ramets into MLGs and defines the clonal sizes by the number of individuals belonging to that MLG. It was calculated according to the equation:

$$R = (\text{number of MLGs} - 1) / (\text{number of Individuals} - 1).$$

R was calculated for each country of origin and ranges from 0, when all individuals belong to the same MLG (all clones), to 1, when all individuals possess a unique genotype (Dorken & Eckert, 2001).

4.3 Genetic differentiation

To investigate the differentiation among MLGs, a commonly used method, the principal component analysis (PCA) of individual genotypes, was carried out using the R package “ade4 1.7-22” (Chessel et al., 2004). For the PCA, a dataset was used that only contained one individual per MLG and per country, and the clones were eliminated.

4.4 Genetic diversity

Heterozygosity is an estimate of genetic diversity and the genome wide individual's mean observed heterozygosity was calculated applying the “detect_mixed_genomes” function found in the “radiator 1.2.9” R package.

4.5 Sampling- and genotyping effort

Finally, the sampling- and genotyping power to capture the genetic diversity and detect clonality were examined using two different approaches, the allele-accumulation curve and the genotype-accumulation curve, respectively. To determine the minimum number of loci necessary to detect differences among individuals and to capture accurate diversity, the genotype accumulation curve was created with the “genotype_curve” function of the “poppr 2.9.5” package. The function “sample_units”

of the R package “Rclone 1.0.3” was used to plot the allele accumulation curves as an indicator of diversity for an increasing number of sampled individuals.

5 Results

5.1 Clonality

The final dataset comprised 67 genotyped individuals and 12 technical replicates, with 21,205 SNP loci. Calculations of Prevosti’s genetic distance resulted in a cut-off value of 0.042, leading to the detection of 31 MLGs. Out of these MLGs, six spread over multiple countries and MLGs with ramets comprised between two to ten individuals (**Figure 2**).

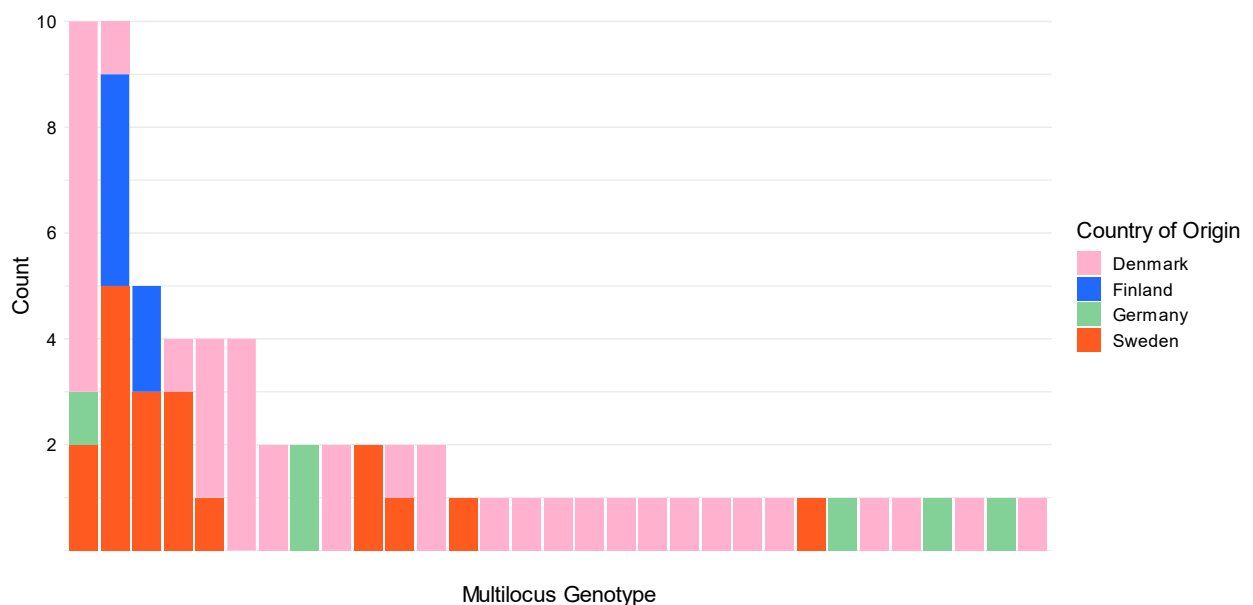


Figure 2. Bar graphs representing unique multilocus genotypes (MLG) and number of ramets per MLG. 67 genotyped individuals with 21,205 SNPs distributed over 31 MLGs. Colors represent the country of origin of each individual.

The dendrogram (including technical replicates), based on Prevosti’s genetic distance matrix, is shown in **Figure 3**. The red horizontal line marks the genetic cut-off value. This means, individuals with nodes below this threshold belong to the same MLG. The branch length represents the degree of differentiation between different individuals. Pairs of technical replicates clustered together.

The dark blue clusters along the diagonal axis in **Figure 4** display the identified clonal lineages. Additionally, there are three bigger light blue clusters around the diagonal, indicating the genetic similarity of individuals within each cluster and a high differentiation between the clusters.

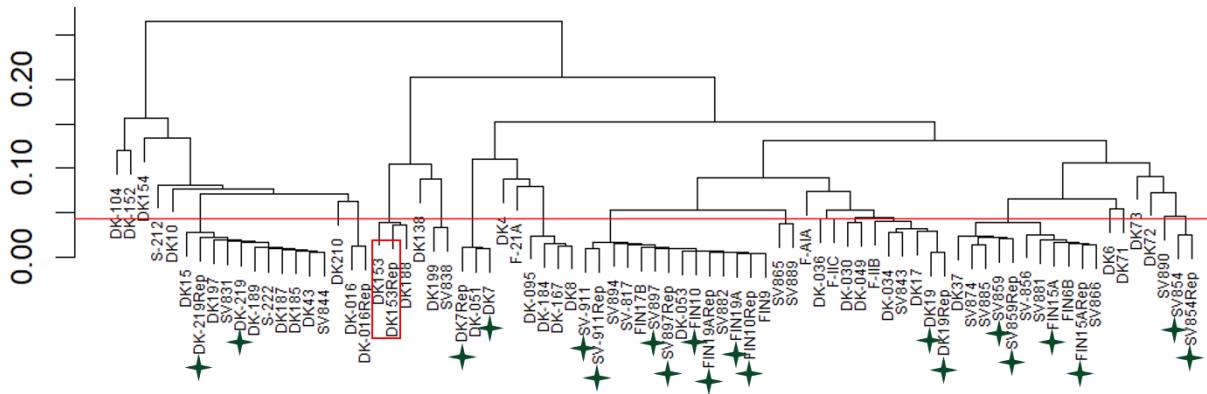


Figure 3. WPGMA (weighted pair group method with arithmetic mean) dendrogram based on Prevosti's genetic distance between 79 genotyped *Ulva linza* samples (including 12 technical replicates). The red line marks the genetic distance cut-off threshold to distinguish between unique genotypes and clones. Individuals belong to the same clonal lineage when the nodes are below the threshold. The red box marks the pair of technical replicates with the largest genetic distance and the green crosses mark the remaining pairs of technical replicates.

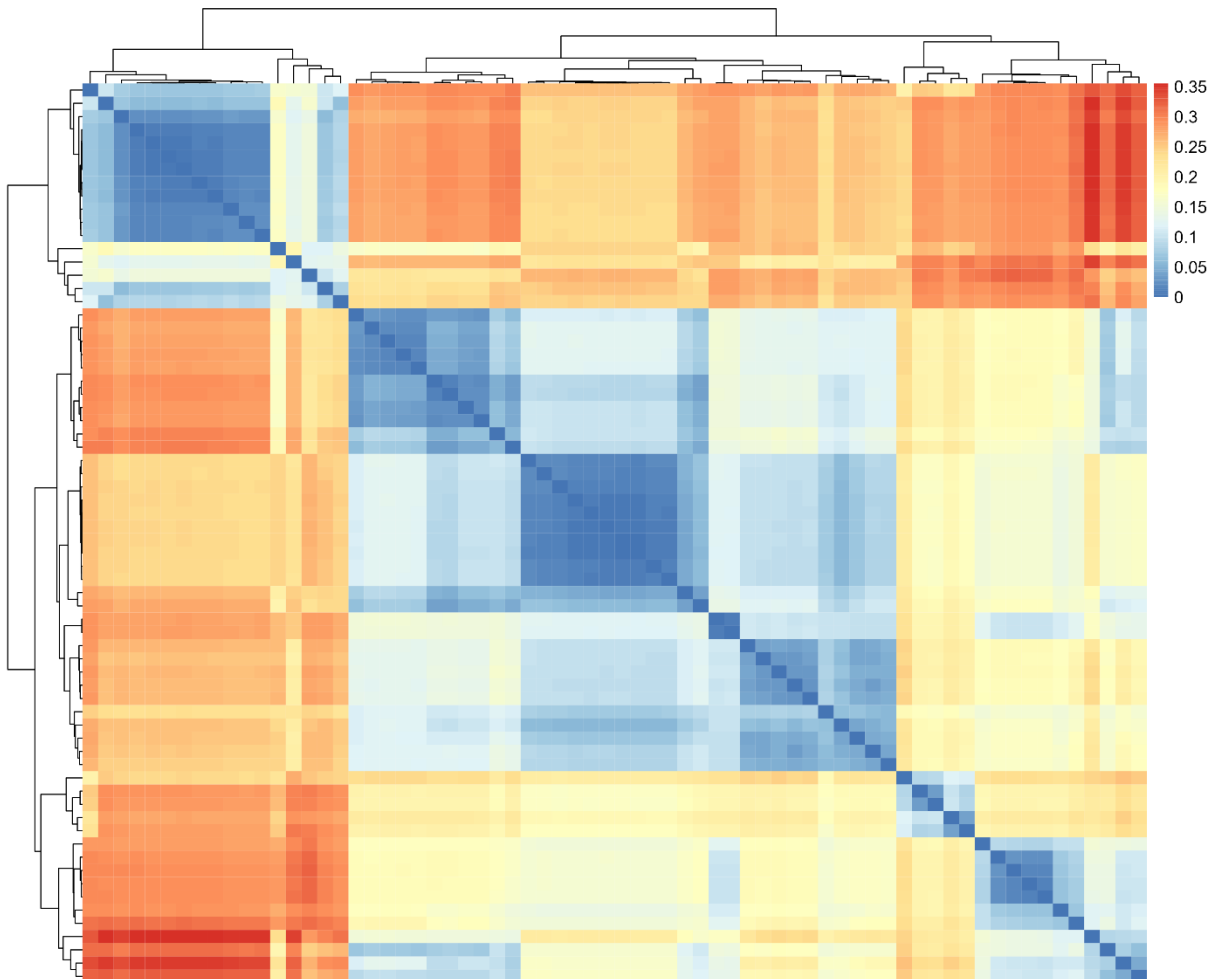


Figure 4. Heatmap visualizing Prevosti's genetic distance matrix between 67 genotyped individuals. Genetic distance increases from blue (0) to red (0.35). The integrated tree (based on the genetic distance matrix) indicates relatedness between individual samples.

The minimum spanning network (MSN) visualizes the differentiation of the MLGs into three groups (**Figure 5**). Individuals from Denmark and Germany are present in all three branches of the tree, exhibiting a large genetic distance between MLGs. Denmark samples contribute to the majority of MLGs. Finnish individuals are only present in two larger MLGs, with a small genetic distance, as indicated by the thick dark grey connections.

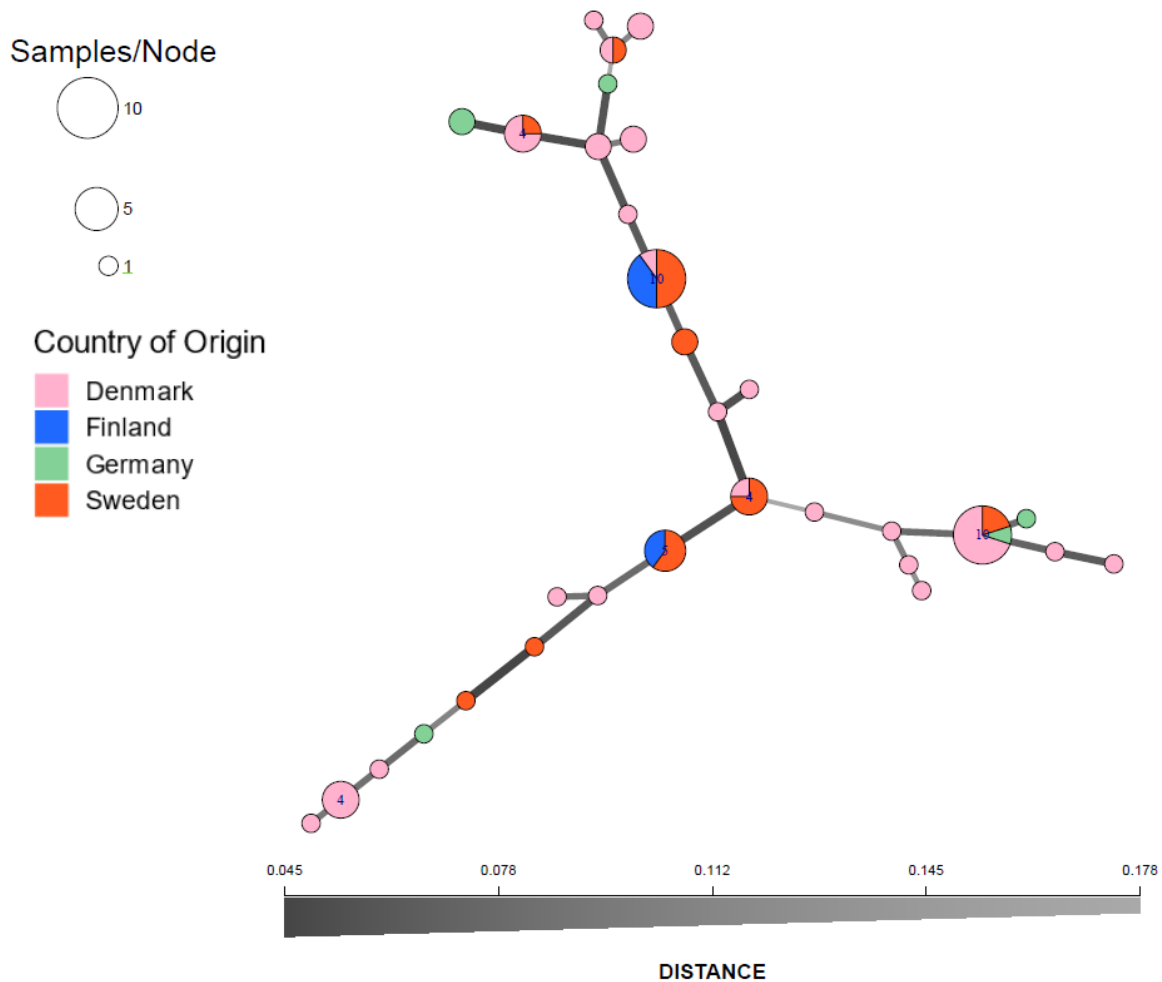


Figure 5. Minimum Spanning Network (MSN) of 67 genotyped individuals of *Ulva linza*. The nodes represent multilocus genotypes and the color represents the country of origin of each individual. The node size represents the number of individuals per MLG. Genetic distance is indicated by different shades of grey and darker grey indicates smaller genetic distance.

The genotypic richness (R) was lowest in Finland followed by Sweden (**Table 1**). On the other hand, Denmark and Germany had higher genotypic richness. R was highest in Germany with a value of 0.8, which means that almost all sampled individuals constitute a unique genotype.

Table 1. Genotypic richness (R) for the countries of origin.

Country	nInd	nMLG	R
Denmark	36	23	0.63
Finland	6	2	0.2
Germany	6	5	0.8
Sweden	19	9	0.44

R: genotypic richness $(nMLG - 1)/(nInd - 1)$.

The individual's mean observed heterozygosity (H_o ; **Figure 6**) ranges from 0.0068 to 0.5168. The average H_o for all individuals of all countries combined is 0.215. The average H_o is lowest in Finland with 0.098. Many individuals from Denmark exhibit high values of H_o . Individuals of German origin show two cohorts, with either high or low H_o . The proportion of missing data (due to poor polymorphism discovery) is rather low, below 0.04.

Linkage disequilibrium (LD; **Figure 7A**), was significant in all populations, however Finland showed the highest LD relative to the other countries, coinciding with the lowest R value (indicating highest clonality). Furthermore, the fixation index (F_{IS} ; **Figure 7B**) for the dataset containing all 67 individuals had a higher excess of heterozygotes in Finland and Sweden compared to the dataset containing only one individual per MLG and country of origin.



Figure 6. Genome-wide individual's mean observed heterozygosity for 67 genotyped individuals of *Ulva linza*. Individuals are divided into their country of origin and the panel to the right shows all individuals together. The point size indicates the proportion of missing genotypes based on poor polymorphism discovery due to sequencing errors. The dotted lines show average heterozygosity values and the dashed lines show the outlier threshold.

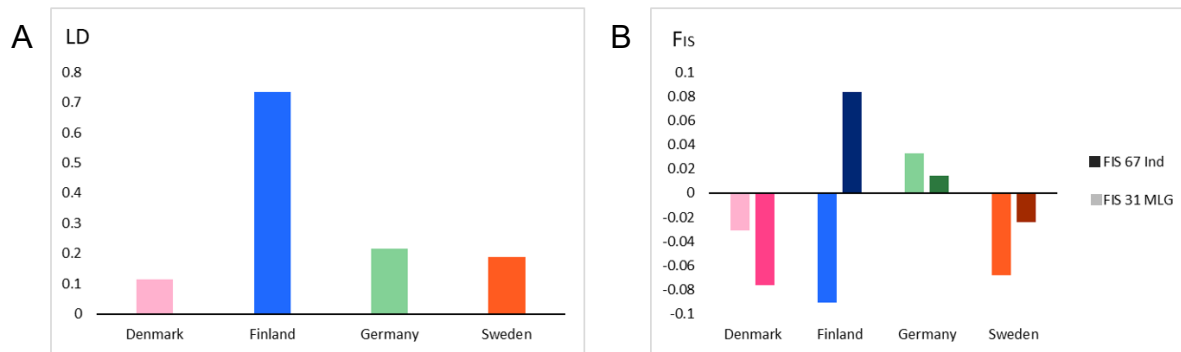


Figure 7. A) Linkage disequilibrium (LD) and **B)** inbreeding coefficient (F_{IS}). Brighter colors: calculated for dataset containing all 67 genotyped individuals; darker color: calculated for dataset containing only one individual per MLG and country of origin.

5.2 Sampling- and Genotyping Effort

To validate the minimum number of loci necessary to discriminate between MLGs, a genotype accumulation curve was created (**Figure 8**). The curve reaches a plateau after identifying ~400 loci. A total of 67 MLGs was identified, which correspond to the number of individuals in the data set. The allele accumulation curves (**Figure 9**) indicate that the highest genetic diversity is observed in Denmark, with alleles continuing to accumulate until approximately 35 sampled individuals. In Sweden, alleles accumulate up to 18 sampled individuals. Notably, in Finland, only a few alleles are added after only 2 genotyped individuals.

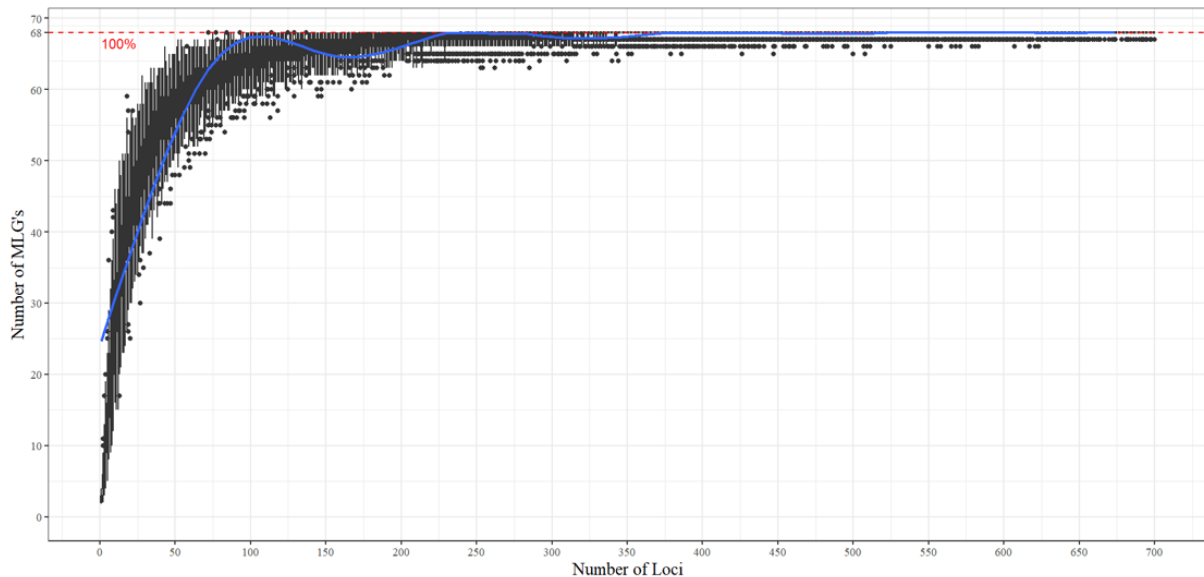


Figure 8. Genotype accumulation curve for 67 genotyped individuals of *Ulva linza* genotyped over 21,205 SNP loci. The curve visualizes the genotyping effort. The x-axis shows the number of randomly sampled loci and the y-axis shows the number of observed multilocus genotypes (MLGs). The red dashed line indicates 100 % of the genotyped individuals.

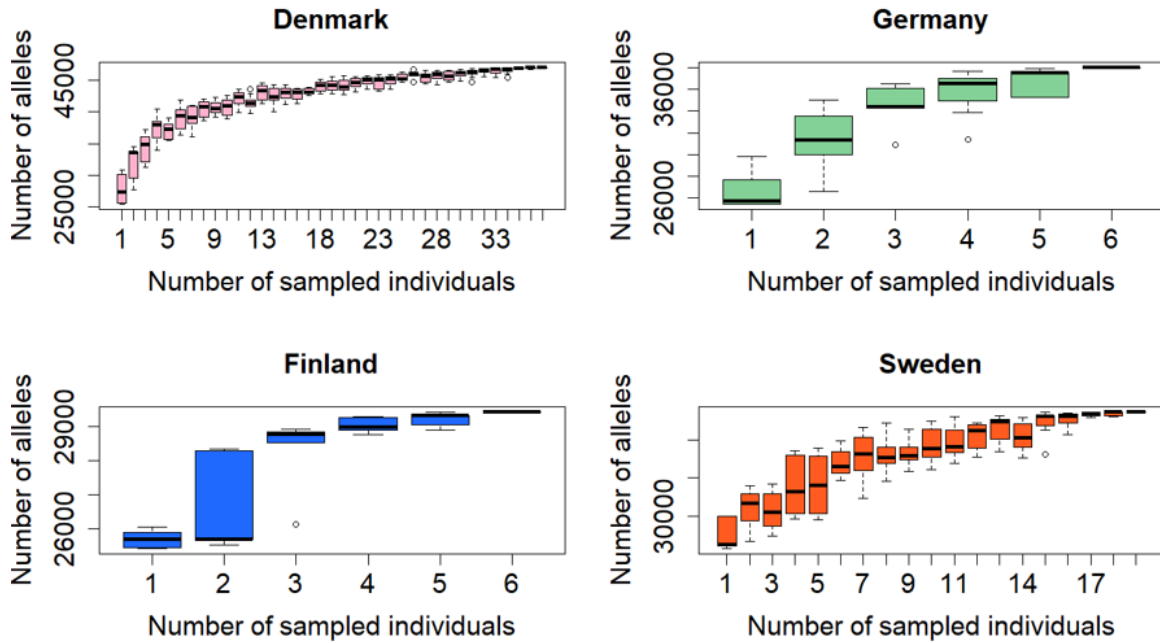


Figure 9. Allele accumulation curves for individuals on *Ulva linza* in Sweden, Denmark, Germany and Finland. The curves show the accumulation of alleles relative to the number of samples individuals within each country.

5.3 Distribution of Genetic Diversity

To investigate genetic differentiation among the MLGs, a PCA was carried out (**Figure 10**). The first PC axis represents 32.1 % of inertia in the data and the second PC axis explains 13.7 % inertia. Most variation is found within Denmark. Danish samples showed genetic differentiation along both axes. The German and Swedish MLGs also spread along both PC axes, whereas MLGs found in Finland, show little genetic differentiation and cluster together.

The distribution of individuals belonging to the same MLG and individuals with a unique genotype over the study area is shown in the map in **Figure 11**. The highest diversity of MLGs is located in Kattegat and Öresund, which is also where unique genotypes are predominantly found. Individuals in the Baltic Sea are clonally propagated, with one exception. Individuals belonging to the same MLG show a broad geographic distribution.

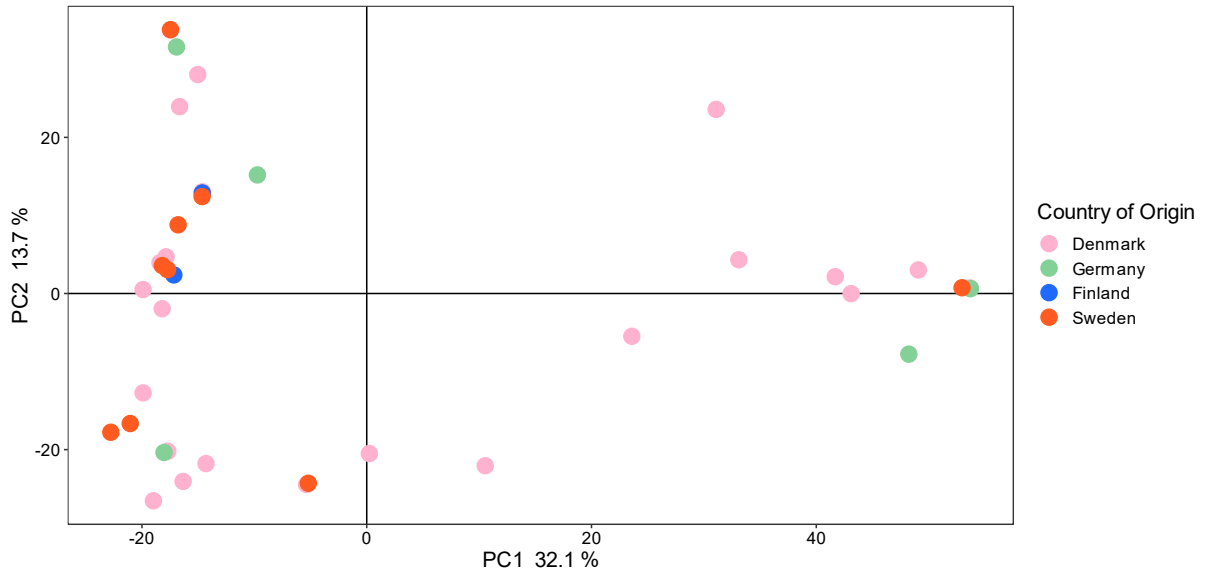


Figure 10. Principal component analysis of 31 multilocus genotypes with one individual per country of origin. The first PC axis explains 32.1 % and the second PC axis explains 13.7 % of the total variation found in the data. The colors represent the individual's country of origin.

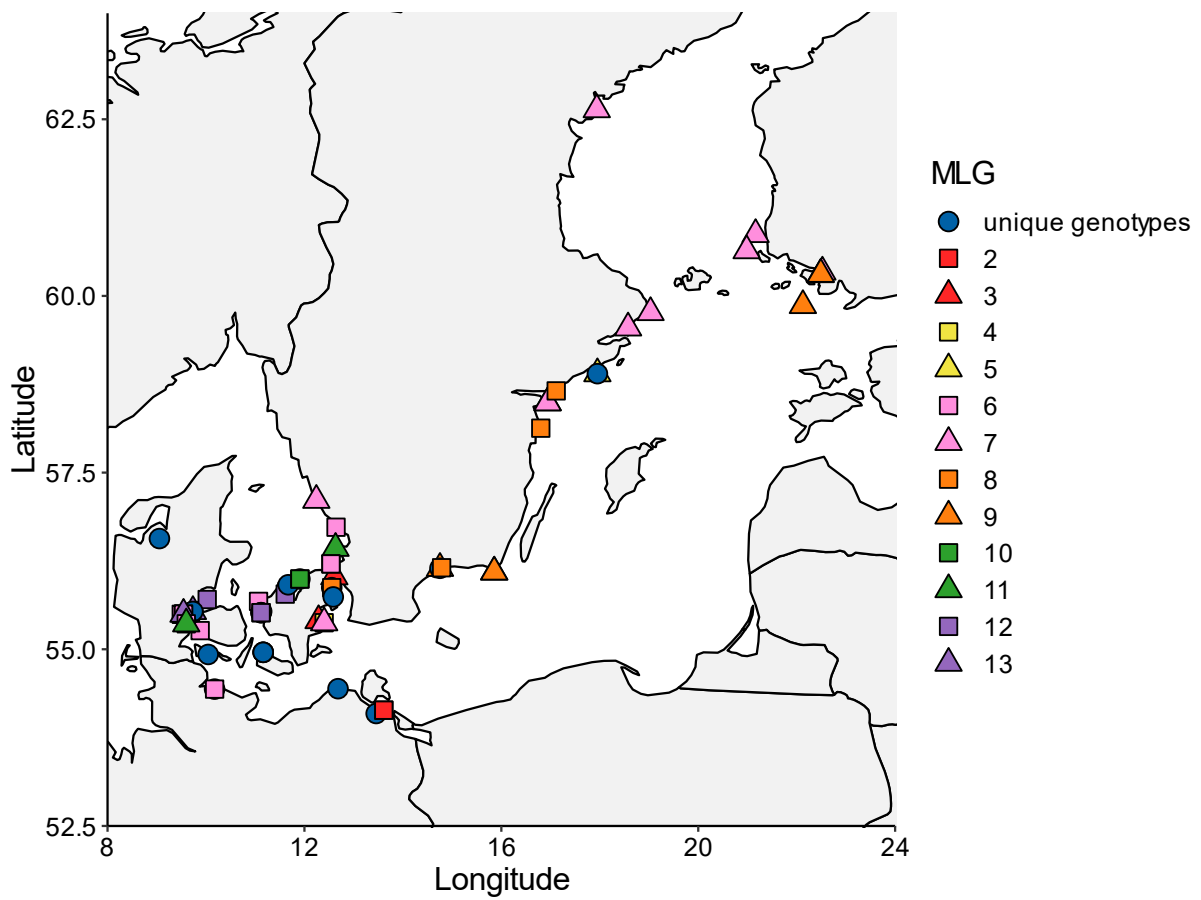


Figure 11: Distribution of the multilocus genotypes (MLG) over the study area. Individuals belonging to the same MLG are indicated by the same color and shape. Unique genotypes are represented by blue circles. Coordinate reference system: EPSG:4326.

6 Discussion

U. linza is a promising species for cultivation in aquaculture. With its short generation time and fast growth rate it bears good potential and can find applications in various fields (Brakel et al., 2021; Simon et al., 2022). However, little is known about the intraspecies genetic diversity distribution and clonality and the impact the reproduction mode may have on this. This study provides insights into the distribution of genetic diversity of *U. linza* in Skagerrak, Kattegat, Öresund and the Baltic Sea, and characterizes clonality. Clonality impacts the amount of genetic variation which in turn is an important foundation for healthy populations (Guillemin et al., 2008). Among 67 genotyped individuals, 31 MLGs were discovered. Finally, this study provides recommendation to establish a sustainable cultivation of *U. linza* by identifying gene pools that can serve as donor material for aquaculture.

6.1 Genetic diversity

In a pilot study by Nordfeldt Oswaldson (2023) based on 23 individuals of *U. linza*, three clonal lineages were discovered in Kattegat. These findings are corroborated and extended in this study. Although the sampling strategy was designed for taxonomy purposes with limited number of samples for population genetic estimates, the level of clonal detection was considerably high. As hypothesized, the genetic diversity showed a heterogeneous geographical distribution. Low genetic diversity was identified along the Swedish east coast and in Finland. These findings correspond with the high incidence of clonal reproduction in these areas. Individuals from Finland, in particular, show little genetic diversity, as indicated by the low genotypic richness (R , **Table 1**) and low differentiation between clonal lineages, visualized in the MSN (**Figure 5**) and PCA (**Figure 10**). Moreover, the low genetic diversity patterns in Finland are confirmed by low values of observed heterozygosity (H_o , **Figure 6**). Low values of heterozygosity (H_o) can result from repeated population bottlenecks during founding events (Allendorf, 1986; Nei et al., 1975). Genetic diversity is also influenced by the reproductive mode and clonal population can suffer similar consequences as inbred, sexual populations (Halkett et al., 2005).

The high values of genotypic richness for Denmark and Germany are in line with the higher sexual reproduction. The higher level of sexual reproduction and the occurrence of several MLGs contribute to the high genetic diversity in Kattegat. Moreover, the high values of observed heterozygosity predominantly found in Denmark are in line with the observation of recent high diversity.

Despite the low sample size in Germany, high genetic diversity was discovered. The allele accumulation curves for Germany, Denmark, and Sweden (**Figure 9**) show that as the sample size increases, more alleles are added, and thereby capturing more diversity. In Finland on the other hand, the allele accumulation curve indicates that most of the genetic variation was captured and an increased number of samples would have contributed little to uncover further diversity, as the curve starts to saturate at around three or four samples. In general, the allele accumulation curves corroborated the observations of higher genetic diversity in Kattegat and Öresund.

6.2 Clonality

Determining the genetic cut-off value to distinguish between unique genotypes and individuals belonging to the same MLG, is a crucial step for the identification of clones and different approaches have been used in previous works about clonality (Grünwald et al., 2003; Kamvar et al., 2014, Kamvar et al., 2015). The cut-off value used in this study was confirmed by two different aligning approaches, the largest genetic distance between a pair of technical replicates and the calculations of UPGMA clustering, which is also well known to biologists (Kamvar et al., 2015).

In this study, two major clones with 10 ramets each were identified. Moreover, several smaller clones are present in the study area and ramets belonging to the same MLG show a broad geographic distribution. The observation of clones was confirmed by different estimates, i.e., the LD, which showed significant values for all countries of origin. The high LD value for Finland aligns with the low value of genotypic richness (R ; 0.2), which is close to complete clonal dominance ($R = 0$), and the excess of heterozygotes (F_{IS}). This is in accordance with findings of Krueger-Hadfield et al. (2021), who report a negative correlation between R and LD. Moreover, the finding of significant LD despite sexual reproduction (as in Denmark or Germany) was also reported by Krueger-Hadfield et al. (2021) and hypothesized to result from a small neighborhood size with the occurrence of both selfing and inbreeding.

The dominance of clonality along the Swedish east coast and in Finland is in accordance with findings of other species, that also show high clonality in the Baltic Sea (Johannesson & André, 2006; Pereyra et al., 2023; Ries et al., 2023). The low salinity of the Baltic Sea poses extreme environmental conditions which may lead to the loss of sexual reproduction and favor clonality (Tobler et al., 2006). The influence of salinity on the reproductive mode has been shown for other algae (Davidovich et al., 2016; Serrão et al., 1996). Moreover, all organisms in the Baltic Sea, must have colonized the area postglacial (~8000 years ago) which makes it relatively recent on an evolutionary timescale (Björck, 1995; Gabrielsen et al., 2002). Baker's Law (Baker, 1955) suggests that clonal reproduction is favorable during range expansion, like the colonization of the Baltic Sea, as reproduction does not depend on encountering potential mating partners. This mechanism has been corroborated for partially clonal species by Pereyra et al. (2023). Nevertheless, clonality has not only been observed in the Baltic Sea, but also in Kattegat.

6.3 Biology of *U. linza*

The complex life history of the study organism of haplodiplonty together with partial clonality poses challenges for the analysis and interpretation of genetic diversity. Organisms with a haplodiplontic life cycle that are also facultative sexual have long been neglected in population genomic analyses (Krueger-Hadfield et al., 2021; Stoeckel et al., 2021). The ploidy determination in different studies has often been based on morphological characters, but also based on the number of alleles at a locus (M.-L. Guillemin et al., 2008; Krueger-Hadfield et al., 2016; Pardo et al., 2019). In this study however, it was challenging to establish a threshold of genome wide mean observed heterozygosity, to distinguish haploids from diploids, because of the continuous values of heterozygosity and sequencing errors. Based on the largest difference in heterozygosity of a pair of technical replicates, only one individual from Sweden can be identified as haploid. However, this individual belongs to the same

MLG as three other individuals, who do not fall under the threshold. Therefore, the method for identification of haploids should rely on experimental set up if there are no reliable genetic estimates or markers.

A possible explanation for the dominance of diploids is the high level of asexual reproduction observed in the study area. A shift in the ploidy ratio as a consequence of asexual reproduction has also been shown for the red seaweed *Gracilaria vermiculophylla* (Krueger-Hadfield et al., 2016). However, the available literature on mechanisms generating variation in haploid-diploid ratios for macroalgae is quite limited (Guillemin et al., 2014; Guillemin et al., 2013; Thornber & Gaines, 2004). A study by Alström-Rapaport et al. (2010) investigated the abundance of diploids and haploids of *U. intestinalis* in the Baltic Sea and found that the ratio changes with the seasons, but with a higher abundance of diploids throughout the year.

6.4 Recommendations for aquaculture practice

The mode of reproduction, sexual versus asexual, has consequences on the genetic composition of populations and thus on their genetic response to selection (Hamilton, 1980). In clones, selection on a trait of human interest will not only act on the gene controlling the trait, but on the whole genome. Together with selection, bottleneck effects imposed during the cultivation process, will reduce genetic diversity (Guillemin et al., 2008).

To maximize the yield potential of seaweed cultivation, the use of inbred lines or individuals capable of clonal reproduction may be a good strategy for aquaculture. However, research on clonality has shown the tendency of these lineages to accumulate deleterious mutations, which can make them vulnerable to environmental stressors and jeopardize their adaptive potential (Orive et al., 2017).

Historical events of collapses of genetically homogeneous food crops, such as the potato late blight epidemic in the 1840s (Provan et al., 1999) or the outbreak of Southern corn leaf blight in the 1970s (Tanksley & McCouch, 1997), demonstrate the critical importance of plant genomic resources (Govindaraj et al., 2015).

Hence, it is essential to find the balance between high biomass production and a diverse genetic base, which serves as the foundation for genetic improvement, enhances resilience against pest epidemics or diseases, and ensures a high fitness of crops (Laikre et al., 2020). This motivates the present study, which aims to increase the knowledge to understand the conditions and mechanisms that control and determine the clonal reproduction, as well as finding a balance between clonal donor individuals while including resilient genetic variants with broad environmental tolerances to maximise the cultivation success.

6.5 Conclusion

This study explored the genetic diversity and clonality of *U. linza* in the Nordics using genomics. High levels of clonality and a heterogeneous distribution of genetic diversity were observed over the entire study area. The highest genetic diversity was observed in the Kattegat region and two major clonal lineages, as well as several smaller ones have been uncovered. Multilocus lineages show a broad geographic distribution and sexual reproduction is almost completely absent in the Baltic Sea. The complex live

cycle of the study organism poses complications for the calculations and interpretations of population genomic parameters, as only a few studies have investigated the effects of partial clonality along with a haplodiplontic life cycle. This study contributes to the sustainable cultivation of *U. linza*.

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9 Supplementary

9.1 Popular Science Abstract

Sea Lettuce Secrets: Unlocking Genetic Diversity for Aquaculture Success

As the global human population is growing, so is the demand for food. In response, cultivating macroalgae holds great potential to meet the food demands, as it does not occupy farmland nor depend on good soil or rainfall. Among the marine flora, the green algae *Ulva linza*, commonly known as sea lettuce, is an ideal candidate for aquaculture due to its many applications as food, but also in the pharmaceutical- or the chemical industry.

Why is genetic diversity important?

To cultivate sea lettuce sustainably, it is important to identify suitable donor populations for aquaculture. To do so, I performed genetic analyses to quantify genetic diversity of possible populations. Genetic diversity is a broad term and describes the genetic variability within or among species or populations. It is an important aspect to ensure the long-term viability of cultivated sea lettuce. Populations with high genetic diversity are more resilient to environmental stressors, e.g., an outbreak of a disease in the aquaculture population.

Sea lettuce can reproduce clonally

The genetic diversity found within a species depends, among other factors, on its mode of reproduction. Sea lettuce possesses the ability to reproduce not only sexually, through the production of gametes and spores, but also clonally. Clonal reproduction refers to the ability of an organism to reproduce asexually from a single parent, and thereby producing an exact copy of its genome, like identical twins. Thus, high levels of clonality will lead to many individuals in a population with the same genetic makeup.

Sea lettuce has a complex life cycle

Additional to partially clonal reproduction, sea lettuce alternates the number of sets of chromosomes in a cell, also referred to as ploidy. We humans are diploid and have two sets of chromosomes, one from the mother and one from the father. Sea lettuce and many other organisms can develop into adults even with only one set of chromosomes. The ploidy state also influences the genetic composition of a population.

What do I want to do in this study?

The goal of this study is to analyze the genetic diversity of sea lettuce in the Kattegat, Öresund, and Baltic Sea region and to identify clones.

How to analyze clones?

I started in the laboratory, where DNA was extracted from plant tissue. To get measures of genetic diversity and to assess the level of clonality, the DNA has to be deciphered. Once the genetic code is available, I was able to detect clones, by investigating the similarities of the individual genomes. If two genomes are identical, the corresponding individuals are clones.

What did I find?

In this study I found two major clonal strains and several smaller clonal strains, indicating the presence of clonal as well as sexual reproduction. The clonal strains are not limited to one geographical area, but rather distributed over the entire study area. Also, Denmark, Germany, and the Swedish west coast show the highest level of genetic diversity found within sea lettuce in the study region, whereas all diversity found in Finland is likely caused by migration.

In this study, I provide insightful information about the genetic diversity of sea lettuce. This information can be used for the recruitment of individuals for aquaculture practice. In this way, future crops of sea lettuce will be healthy and resilient.

9.2 Popular Science Abstract (German)

Geheimnisse des Meersalats: Erschließung der Genetischen Vielfalt für eine erfolgreiche Aquakultur

Mit dem weltweiten Anstieg der menschlichen Bevölkerung wächst auch die Nachfrage nach Nahrungsmitteln. Eine Möglichkeit, um den Nahrungsmittelbedarf zu decken, ist die Kultivierung von Makroalgen, da es weder Ackerland beansprucht, noch auf gute Böden oder ausreichen Niederschlag angewiesen ist. Innerhalb der Meeresflora ist die Grünalge *Ulva linza*, gemeinhin als Meersalat bekannt, ein idealer Kandidat für die Aquakultur, da sie als Nahrungsmittel, aber auch in der Medizin oder chemischen Industrie Verwendung findet.

Warum ist genetische Diversität wichtig?

Um Meersalat nachhaltig anzubauen, ist es wichtig, geeignete Populationen für die Aquakultur zu identifizieren. Zu diesem Zweck habe ich genetische Analysen durchgeführt, um die genetische Diversität möglicher Populationen zu quantifizieren. Genetische Diversität ist ein weitreichender Begriff und beschreibt die genetische Variabilität innerhalb und zwischen Populationen oder Arten. Es ist eine wichtige Grundlage, um die langfristige Lebensfähigkeit von kultiviertem Meersalat zu gewährleisten. Populationen mit hoher genetischer Diversität sind widerstandsfähiger gegen Umweltstressoren, z.B. gegen den Ausbruch einer Krankheit.

Meersalat kann sich klonal fortpflanzen

Die genetische Diversität innerhalb einer Art hängt unter anderem von ihrem Fortpflanzungssystem ab. Meersalat kann sich nicht nur sexuell, durch die Produktion von Gameten und Sporen, fortpflanzen, sondern auch klonal. Klonale Reproduktion bezieht sich auf die Fähigkeit eines Organismus, sich ungeschlechtlich von einem einzigen Elternteil zu reproduzieren und dabei eine exakte Kopie seines Genoms zu produzieren, ähnlich wie bei eineiigen Zwillingen. Somit führt ein hoher Anteil von klonaler Reproduktion dazu, dass viele Individuen in einer Population den gleichen genetischen Aufbau haben.

Meersalat hat einen komplexen Lebenszyklus

Zusätzlich zur teilweise klonalen Reproduktion wechselt Meersalat die Anzahl der Chromosomensätze in einer Zelle, auch als Ploidie bezeichnet. Wir Menschen sind diploid und haben zwei Chromosomensätze, einen von der Mutter und einen vom Vater. Meersalat und viele andere Organismen können sich sogar mit nur einem

Chromosomensatz zu Erwachsenen entwickeln. Der Ploidiezustand beeinflusst ebenfalls die genetische Zusammensetzung einer Population.

Was möchte Ich in dieser Studie tun?

Das Ziel dieser Studie ist es, die genetische Diversität von Meersalat in der Region Skagerrak, Kattegat, Öresund und in der Ostsee zu analysieren und Klone zu identifizieren und zu charakterisieren.

Wie findet man Klone?

Die Arbeit fing im Labor an, wo DNA aus Algengeweben extrahiert wurde. Um die genetische Diversität zu messen und das Ausmaß an klonaler Fortpflanzung zu bewerten, muss der genetische Code entschlüsselt werden. Dadurch kann die Ähnlichkeit der einzelnen Genome untersucht werden. Wenn zwei Genome identisch sind, sind die entsprechenden Individuen Klone.

Was habe ich dabei entdeckt?

Zwei große Klone und mehrere kleine Klone konnten entdeckt werden. Außerdem kommt sowohl klonale, als auch sexuelle Reproduktion im vor. Individuen eines Klons zeigen eine weite geographische Verbreitung über die gesamte Untersuchungsregion. Das höchste Vorkommen an genetischer Diversität befindet sich in Dänemark, Deutschland und der schwedischen Westküste, während Finnland wenig Diversität aufweist.

Die Ergebnisse dieser Studie können für die Rekrutierung von Individuen für die Aquakultur verwendet werden. Auf diese Weise kann der zukünftige Anbau von Meersalat gesund und widerstandsfähig gemacht werden.

9.3 Supplementary Figures

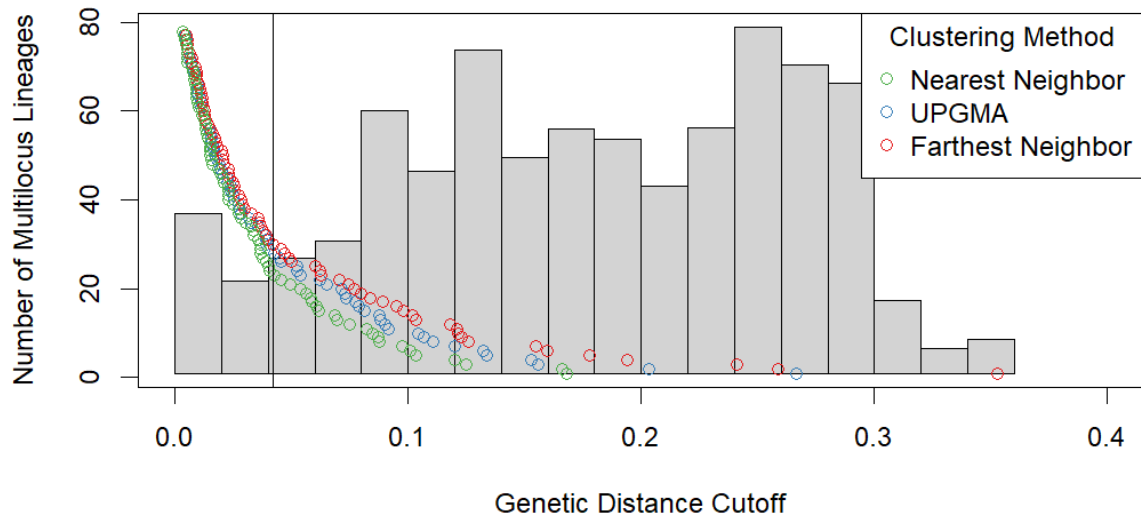


Figure S1. Genetic distance between 67 *Ulva linza* samples and 12 technical replicates based on Prevosti's genetic distance. The black vertical line shows the genetic distance threshold based on the maximum replicates distance (0.042). Individuals to the left of the threshold are potential clones. The "filter_stats" function embedded in the R package "poppr 2.9.5" was used.



Avtal för elektronisk publicering

§1 Upplåtelse för elektronisk publicering

Upphovsmannen/upphovsmännen/rättighetsinnehavaren/rättighetsinnehavarna, här kallad Författaren/Författarna, ger Göteborgs universitet rätt att publicera angivet arbete, här kallat Verket, i elektronisk form på Göteborgs universitets domän på Internet. Rätten att publicera innebär en rätt att göra Verket tillgängligt för allmänheten på Internet. Upplåtelsen medför inga inskränkningar i Författarens/Författarnas rätt att för egen del använda Verket.

§2 Giltighetstid och uppsägning

Upplåtelsen gäller till dess Författaren/Författarna skriftligen meddelar Göteborgs universitet att denna rätt skall upphöra. Vid ett upphörande har Göteborgs universitet inte längre rätt att tillgängliggöra Verket på Göteborgs universitets domän på Internet och skall med omedelbar verkan avlägsna Verket med följd att publiceringen upphör. Göteborgs universitet kan i sin tur med omedelbar verkan frånträda avtalet och avlägsna Verket med följd att publiceringen upphör. Något skäl för upphörandet behöver inte anges.

§3 Syfte med publiceringen

Den elektroniska publiceringen skall ske för universitetets forsknings-, utbildnings- och biblioteksverksamhet och ej för kommersiella ändamål.

§4 Verkets form

Författaren/författarna skall lämna över Verket i enlighet med de instruktioner som universitetet meddelar.

§5 Upphovsmannaskap

Författaren/Författarna ansvarar för att hon/han/de är upphovsman till Verket och att i förekommande fall inhämta erforderliga upphovsrättsliga medgivanden vad gäller i Verket eventuellt ingående material (bilder m.m.) som kan ha annan upphovsman, samt att Författaren/Författarna har rätt att disponera över Verket för publicering i enlighet med detta avtal.

§6 Ersättning

Inga ersättningar utgår mot bakgrund av detta avtal. Eventuellt uppkomna kostnader uppbärs av part som kostnaden hänförs till.

§7 Ikraftträdande

Detta avtal gäller omedelbart efter Författarens godkännande.

Uppsatsens titel/Thesis title

Genetic Diversity of the Green Macroalga *Ulva linza* in Kattegat and the Baltic Sea with Recommendations for Aquaculture

Studentens namn/Name of student

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Kurs/Course

BIO717

År kursen avslutades/Year course finished

2024

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