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Genome-Wide Adaptation to a Complex Environmental Gradient in a Keystone Phytoplankton Species

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ABSTRACT

Marine phytoplankton play essential roles in global primary production and biogeochemical cycles. Yet, the evolutionary genetic underpinnings of phytoplankton adaptation to complex marine and coastal environments, where many environmental variables fluctuate and interact, remain unclear. We combined population genomics with experimental transcriptomics to investigate the genomic basis underlying a natural evolutionary experiment that has played out over the past 8000 years in one of the world's largest brackish water bodies: the colonisation of the Baltic Sea by the ancestrally marine diatom *Skeletonema marinoi*. To this end, we combined target capture of the entire nuclear genome with pooled shotgun sequencing, and showed that the method performs well on both cultures and single cells. Genotype–environment association analyses identified > 1000 genes with signals of selection in response to major environmental gradients in the Baltic Sea, which apart from salinity, include marked differences in temperature and nutrient supply. Locally adapted genes were related to diverse metabolic processes, including signal transduction, cell cycle, DNA methylation and maintenance of homeostasis. The locally adapted genes showed significant overlap with salinity-responsive genes identified in a laboratory common garden experiment, suggesting the Baltic salinity gradient contributes to local adaptation of *S. marinoi*. Taken together, our data show that local adaptation of phytoplankton to complex coastal environments, which are characterised by a multitude of environmental gradients, is driven by widespread changes in diverse metabolic pathways and functions.

1 | Introduction

Given their essential roles in ecosystem functioning (Worden et al. 2015), understanding how marine phytoplankton adapt to changes in their environment is essential for making predictions about how they will be impacted by environmental change (Cavicchioli et al. 2019). Experimental work has shown that phytoplankton species can respond rapidly to environmental change, both through phenotypic plasticity and rapid evolution of metabolic traits (Padfield et al. 2016; Schlüter et al. 2014). However, the genetic underpinnings of adaptations in marine phytoplankton to new or changing environments are largely unknown (Filatov and Kirkpatrick 2024), including the evolutionary processes, molecular pathways and genes that drive adaptive change in these organisms. Experimental evolution combined with genome resequencing can provide detailed insights into the rate of adaptation in vitro, as well as the genes involved in the early stages of adaptation (Moerman et al. 2022; Schaum et al. 2018), but these experiments are often limited to individual strains of model species. Many of these have been grown in culture for decades, which can result in genetic change caused by artificial selection, loss of gene function and recombination (Bulankova et al. 2021; Helliwell et al. 2015; Rastogi et al. 2020). Phytoplankton species also exhibit high levels of intraspecific variation in their genetic make-up (Chaumier et al. 2024; Read et al. 2013) and physiology (Bishop et al. 2022; Pinseel et al. 2022; Schaum et al. 2012), which may not be captured by investigations of model strains. Finally, laboratory treatments cannot fully mimic the complexity of marine ecosystems where a multitude of biotic and abiotic parameters fluctuate and interact (Aranguren-Gassis et al. 2019; Zhong et al. 2021). Studies of natural populations can provide ecological context to laboratory studies of model strains and provide valuable new insights into adaptive change in microeukaryotes (Hattich et al. 2024; Nef et al. 2022).

To help fill this knowledge gap, we investigated how a marine diatom, *Skeletonema marinoi*, successfully colonised and adapted to the brackish Baltic Sea (Figure 1A–C). *S. marinoi* is a globally important phytoplankton species in terms of primary production, biogeochemical cycles and benthic-pelagic coupling, and thus presents an excellent opportunity to investigate the genetic basis of adaptation by marine phytoplankton to changes in their environment. The salinity gradient separating marine and freshwater environments is one of the principal ecological divides for microorganisms (Lozupone and Knight 2007), including diatoms (Nakov et al. 2019). Although many marine diatoms typically cannot survive low-salinity conditions, upon the inundation of the freshwater basin that is now the Baltic Sea by saline waters from the North Sea some 8000 years ago (Björck 1995) *S. marinoi* established a foothold in the area very early on (van Wirdum et al. 2019). Since then, it has become one of the most abundant phytoplankton species and prominent primary producers in the Baltic Sea (Godhe et al. 2016). Microsatellite DNA separated *S. marinoi* into high-salinity North Sea and low-salinity Baltic Sea populations, suggestive of reduced gene flow between the two regions (Sjöqvist et al. 2015). Although salinity is generally considered to be the major abiotic factor structuring diversity in the Baltic Sea (Johannesson et al. 2020), several other gradients, including temperature and nutrient availability, have likely imposed additional and possibly equally important selective pressures on *S. marinoi* in the Baltic Sea.

We designed and applied a novel approach for microbial population genomics—combining genome capture, single-cell genomics and pooled sequencing (pool-seq)—to understand how *S. marinoi* adapted to the Baltic Sea over the past 8000 years (Figure 1A). Our study sheds new light on the evolutionary genetic underpinnings that allow phytoplankton to adapt to complex coastal environments, providing novel and timely insights into the tempo and mode of local adaptation in microeukaryotes (Filatov and Kirkpatrick 2024).

2 | Materials and Methods

Between 2010 and 2018, we collected surface sediments from nine localities spanning the Baltic Sea salinity cline (Figure 1A, Table S1) and germinated resting cells of *S. marinoi* into monoclonal cultures. The taxonomic identity of each strain was confirmed by sequencing the LSU rRNA gene. Next, we pooled extracted DNA from 18 to 41 strains per locality into a single sample (Table S1). For some localities, cultures grew poorly, resulting in insufficient biomass for DNA extraction (Table S1). For these localities, we performed whole-genome amplification on single cells. After pooling the extracted DNA of all strains or single cells per locality, we performed target capture using a custom-designed probe kit developed to capture the entire nuclear genome of *S. marinoi*. Following DNA sequencing of pooled individuals on an Illumina HiSeq 4000, we (i) mapped quality-controlled and trimmed reads [obtained via Atria v3.1.0 (Chuan et al. 2021)] to the *S. marinoi* strain RO5AC reference genome v1.1.2 (available on Zenodo) with BWA-MEM v0.7.17 (Li 2013), (ii) removed ambiguously mapped reads and PCR duplicates with SAMtools v1.10 (Li et al. 2009) and Picard v2.26.10 (http://broadinstitute.github.io/picard/), respectively, (iii) performed indel realignment in GATK v3.5 (Van der Auwera and O'Connor 2020), (iv) removed indel regions with PoPoolation2 (Kofler, Pandey, et al. 2011), (v) called single nucleotide polymorphisms (SNPs) with PoolSNP at a minimum coverage of 20×, a minimum allele count (MAC) of 4, a minimum allele frequency (MAF) of 0.1% and a maximum coverage that equaled twice the average sequencing depth, individually defined per contig per pool (Kapun et al. 2020) and (vi) removed multiallelic SNPs and calculated allele frequencies with R (code available on Zenodo). We then further filtered the dataset to two sets of SNPs: (i) a minimum coverage of 20× and MAF of 5% (liberal SNP set) and (ii) a minimum coverage of 40× and MAF of 5% (conservative SNP set). We performed all downstream analyses on both sets of SNPs.

We used F_{ST} values calculated in Poolfstat (Gautier et al. 2022) to construct isolation by distance plots, and characterised genome-wide patterns of genetic variation with PoPoolation (Kofler, Orozco-terWengel, et al. 2011). We evaluated dispersal trajectories and potential barriers to gene flow for *S. marinoi* in the Baltic Sea using a seascape connectivity model based on a Lagrangian particle-tracking model, TRACMASS (Vries and Döös 2001). The model was parameterised with two drift depths

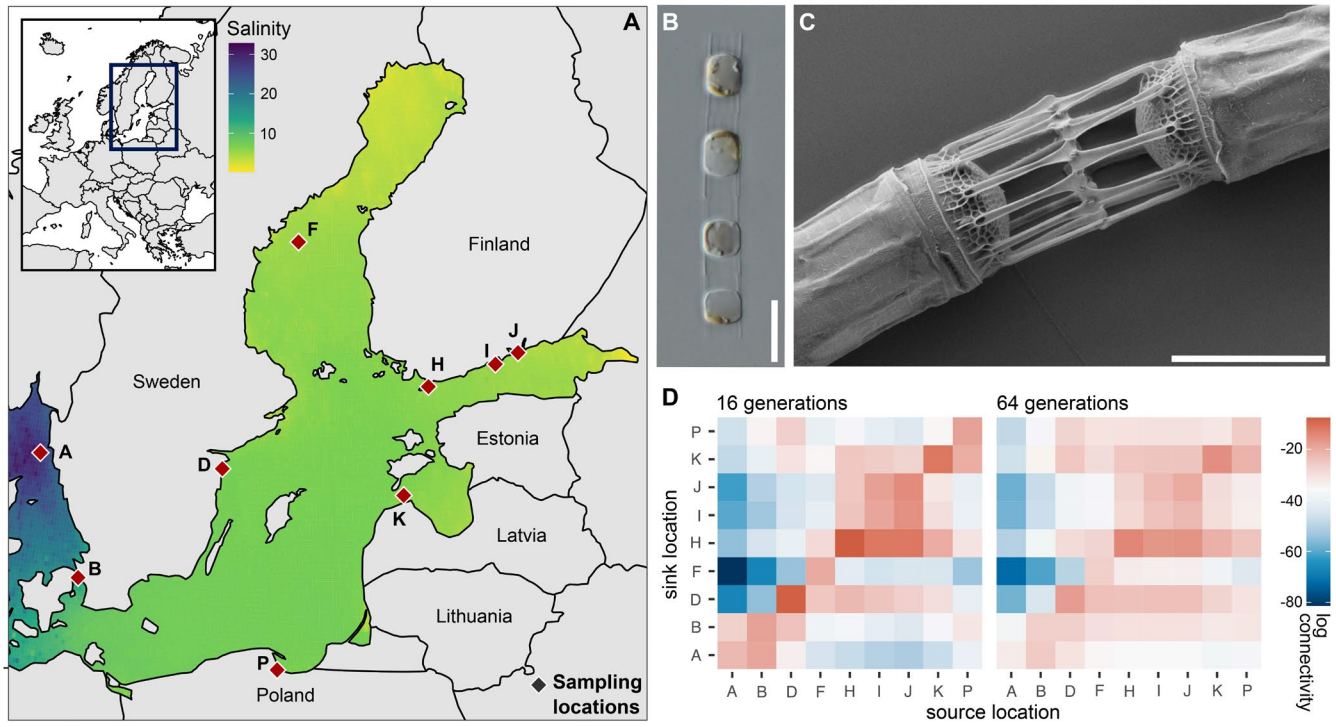


FIGURE 1 | Sampling locations and study system. (A) The North Sea–Baltic Sea salinity gradient, with sampling locations for *S. marinoi*. Salinity measurements for the period 2010–2018 used for the interpolation were downloaded from ICES (ICES Ocean Hydrography, 2020. ICES. Copenhagen) and Sharkweb (https://sharkweb.smhi.se/hamta-data/). The inset map in the top-left corner shows the broader geographic region. Our Zenodo repository contains replicates of the Baltic Sea map for several other environmental gradients, including temperature, pH and nutrient concentrations. (B) Light micrograph of a *S. marinoi* culture (scale bar = 10 μm). (C) Scanning electron micrograph of *S. marinoi* strain RO5AC (scale bar = 5 μm). The micrograph shows the linking spines that connect individual cells, resulting in chain formation. The SEM image was obtained by the Centre for Cellular Imaging at the University of Gothenburg and the National Microscopy Infrastructure, NMI (Sweden, VR-RFI 2016-00968). (D) Heatmaps showing multigenerational stepping-stone connectivity (16 and 64 generations) between the sampling locations for a particle size of *S. marinoi*, calculated from the seascape connectivity model. Data were averaged across all months, drift depths and drift durations. Multigenerational connectivity represents the probability to go from locality X to Y using stepping-stone dispersal over *n* generations. Dark blue values represent lowest connectivity, whereas dark red values represent highest connectivity. Dispersal from locality X to Y is different than going from Y to X, because of possible asymmetric water transport. Connectivity values for the 64-generation heatmap are generally lower than those for the 16-generation heatmap, despite allowing for more generations: In consecutive iterations of the model, particles are lost when dispersing out of the domain (and no new particles are generated), and therefore, multigenerational connectivity needs to be interpreted in a relative sense. This is also true within localities (e.g., from locality X to locality X), as the probability of local retention or self-recruitment differs among sites in the seascape due to spatial differences in oceanographic circulation patterns.

(surface at 0–2 m and a deeper layer at 10–12 m), two drift durations (10 and 20 days) and with the release of virtual particles once every month, modelling the transport of suspended *S. marinoi*. Results were obtained for both one- and multigeneration connectivity across 16, 32 and 64 generations and thus assumed stepping-stone dispersal, which can uncover long-term connectivity between sites (White et al. 2010). The resulting connectivity matrices were summarised by averaging the data across the drift depths, durations and months, and subsequently visualised in R (Figure 1D, Zenodo).

We interpolated environmental data from the study area's surface layer (0–10 m depth, 2010–2018), covering data from both coastal and non-coastal monitoring stations, obtained from ICES (https://www.ices.dk/data/data-portals/Pages/ocean.aspx) and Sharkweb (https://sharkweb.smhi.se/hamta-data/) across the North and Baltic seas, extracted mean seasonal and annual variables for each locality, and corrected the dataset for collinearity. To detect outlier SNPs in the Baltic Sea, we ran genotype–environment association (GEA) analyses in LFMM using the lfmm_ridge model of the R package lfmm v1.1 (Jumentier 2021) and BayPass v2.3 (Gautier 2015), using the first two axes of a PCA on the environmental variables of the Baltic Sea. To detect outlier SNPs between the North Sea and Baltic Sea, we ran the BayPass C2 model (Olazcuaga et al. 2020) which contrasts allele frequencies between population ecotypes with a binary trait (here: 'North Sea' or 'Baltic Sea'), and evaluated allele frequency differences (as F_{ST}) between the North Sea and Baltic Sea. Outlier SNPs were annotated with SnpEff v5.1 (Cingolani et al. 2012). Gene Ontology (GO) enrichment on relevant outlier genes was performed in TopGO v2.46.0 (Alexa and Rahnenführer 2009). We reanalyzed the previously obtained transcriptome data of *S. marinoi* in low salinity using the *S. marinoi* reference genome v1.1.2, given the original study used an earlier version (v1.1) (Pinseel et al. 2022). This reanalysis detected virtually the same set of differentially expressed genes, but found a larger number of 'core response genes' deemed essential for low-salinity acclimation in *S. marinoi* (33 vs. 27) (Pinseel et al. 2022). We then tested whether the overlap

in differentially expressed and outlier genes was significant. 4A more detailed overview of the methodology can be found in the Data S1.

3 | Results and Discussion

3.1 | Leveraging Target Capture for Population Genomics

Population genomics on microeukaryotes is challenged by many factors, including large or unknown genome sizes, methodological challenges with culturing and sequencing large numbers of individuals, contamination by mutualistic bacteria, and the limited resolution of traditional methods, such as microsatellites (Rengefors et al. 2021). As a result, relatively few genome-wide population studies have been carried out on protists (Nef et al. 2022; Postel et al. 2020; Rengefors et al. 2021), which stand in stark contrast to macrobiota. Inspired by previous work on plants and animals (Rudman et al. 2019; Slimp et al. 2021), we obtained genome-wide nuclear SNPs from hundreds of individuals in a protist species. Our approach combined target capture of the complete nuclear genome, followed by pool-seq to minimise costs. Target capture avoids issues with bacterial contamination and over-sequencing of organellar DNA, redirecting the sequencing effort to the target genome. Pool-seq also significantly reduces the cost of laboratory consumables, as library preparation only needs to be performed on a handful of samples instead of hundreds of individual genomes. In addition, the small genome size of *S. marinoi* (estimated 54.6 Mb) allowed us to design baits for the entire nuclear genome, in contrast to RAD-seq or microsatellite studies that typically sample a small and untargeted (RAD-seq) or functionally unimportant (microsatellites) fraction of the genome.

We established *S. marinoi* strains from resting stages in surface sediments collected from the North Sea and Baltic Sea in the years 2010–2018. The genetic composition of these diatom seed banks is locally stable over decades (Godhe and Härnström 2010; Härnström et al. 2011), so sample collection over a 9-year period should not significantly impact inferences on population structuring or selection. In total, 245 cultured strains were included in pools from localities B, F, I, J, K and P, as well as 121 single cells for localities A, D and H (Figure 1), where germination success of resting stages was lower (Table S1). It generally took multiple attempts to resurrect *S. marinoi* from the core materials. We were able to resurrect a substantial number of cells on the first attempt for some localities (e.g., locality F), but other localities required upwards of 1000 isolations to establish sufficient numbers of viable strains. Variation in resting stage viability between sites, possibly caused by biological or physicochemical differences between localities or differential impact of sample transport, might explain the observed variation in germination success. Although not enough for the pools, we retrieved a handful of viable cultures from the single-cell localities that could be used for experimental work (Pinseel et al. 2022).

We achieved read-mapping rates of 96.6%–99.0% and recovered 2,197,240 filtered biallelic SNPs across the nine pools with a minimum coverage of 20×, a MAC of 4 and a MAF of 0.1% (Figures S1 and S2). To ensure reliable inferences of allele frequencies, we further filtered this dataset to (i) a liberal set of SNPs at minimum coverage of 20× and MAF of 5% (1,059,738 SNPs) and (ii) a conservative set of SNPs at a minimum coverage of 40× and MAF of 5% (355,715 SNPs). These filtering strategies were based on a literature survey which showed that (i) 20× (or lower) is a commonly used minimum coverage threshold in pool-seq studies (Alshwairikh et al. 2021; Kapun et al. 2020) and (ii) 40× coverage across pools of 25–50 individuals recovers allele frequencies with high accuracy (Czech et al. 2022). Although more outlier genes were detected at 20× coverage, the two SNP sets did not reveal meaningful differences in diversity estimates, population structuring, or signals of local adaptation. Unless otherwise indicated, our manuscript therefore reports the results of the conservative filtering strategy (40× coverage).

Altogether, the high SNP coverage retrieved in our study, even with our highly stringent filtering strategy, is well-suited for detecting signatures of selection. Pools assembled from single cells and cultures had similar sequence coverage, though it was on average slightly lower for the single cells (Figure S1). Importantly, the number of SNPs retrieved at sufficient read coverage to be retained for the analyses did not differ substantially between pools (Figure S2B,C) underscoring the utility of our single-cell method for uncultivable microbes. Our approach thus performs equally well as a recently designed SAG-RAD protocol that combines single-amplified genomes with RAD-seq (Gollnisch et al. 2023). However, our protocol has the additional advantage of (i) eliminating bacterial contaminants, which might be difficult to discern from the host nuclear genome and (ii) retrieving a higher number of SNPs than typically possible with RAD-seq, which might make it difficult to reliably detect loci under selection in the latter (Lowry et al. 2017). One drawback of our method is the requirement of a reference genome or transcriptome for bait design. For species with large genomes, such as many dinoflagellates, it will be too costly to capture the entire genome, making target capture of specific genes or genomic regions a more useful approach in these organisms (Slimp et al. 2021). A second drawback of pool-seq is that it does not recover individual genotypes, which restricts the population and speciation genomic analyses that can be done and might make it more difficult to reliably detect rare variants and patterns of gene flow. That said, novel approaches that account for the specific sources of noise associated with pool-seq continue to open new questions that can be answered with pool-seq (Carvalho et al. 2023).

3.2 | The North Sea and Baltic Sea Are Home to Distinct Populations of *S. marinoi*

Using genome-wide SNP data, we found major population structuring of the combined North Sea and Danish Straits (localities A and B: hereon referred to as 'North Sea') versus the Baltic Sea (all other localities) (Figure 2A,C). Specifically, our principal component analysis (PCA) suggested the presence of two major populations in the dataset based on a broken stick criterion (Patterson et al. 2006), and as such, the population structure of *S. marinoi* mirrors that of other Baltic organisms, including mussels, fish and seaweeds (Johannesson et al. 2020). Yet, it is also evident from both the F_{ST} values and structuring in the PCA plot that substantial subpopulation structuring exists within the North and Baltic Seas (Figure 2A,C), suggesting these populations might experience internal barriers to gene flow as well. Microsatellite studies

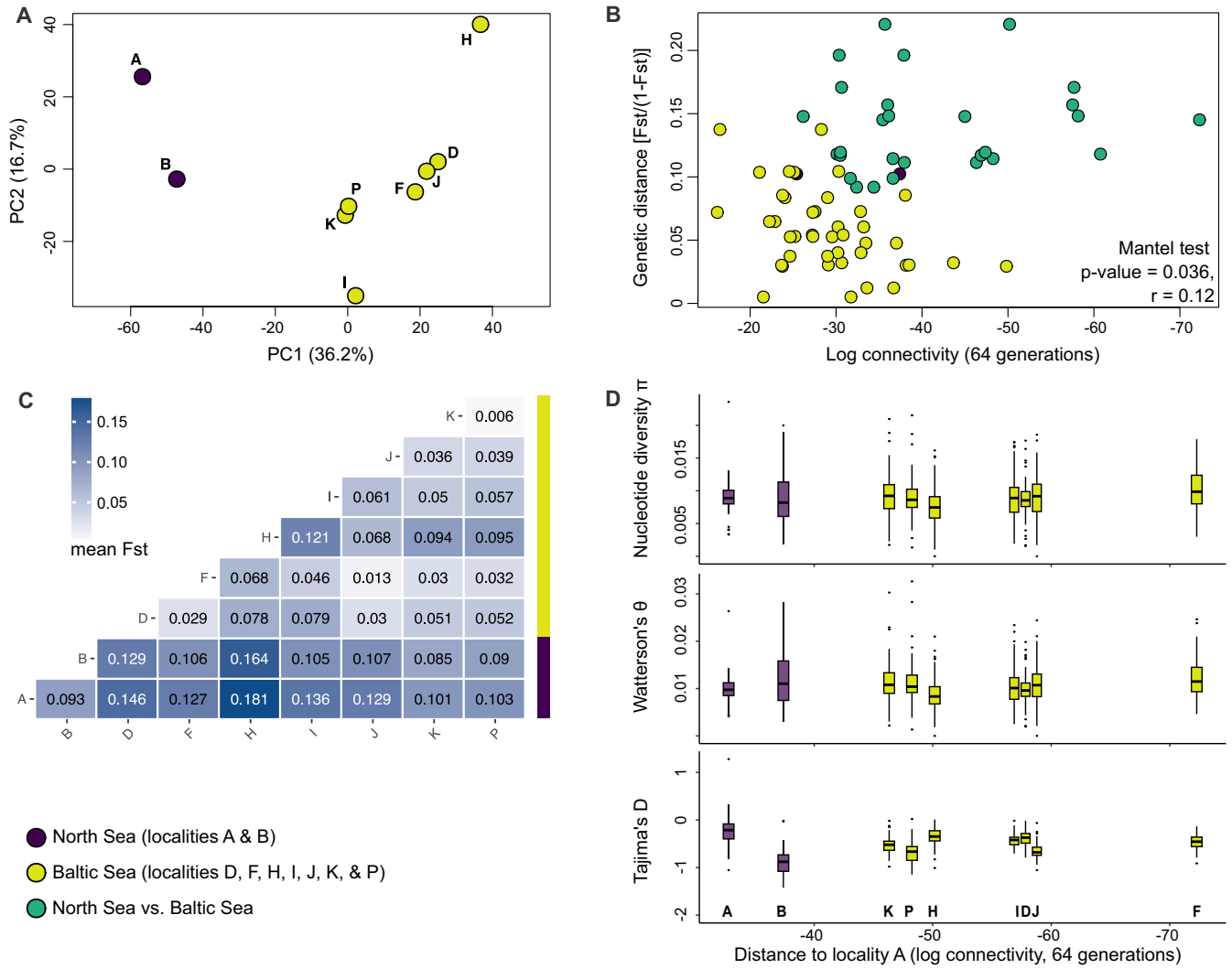


FIGURE 2 | Population structure of *S. marinoi*. (A) PCA of the allele frequencies, showing clear distinction between samples from the North Sea and the Baltic Sea. (B) Isolation-by-distance plot. Distance is measured as the multigenerational stepping-stone connectivity, across 64 generations (see Figure 1D). Each pair of localities is plotted twice due to asymmetric water transport between localities. (C) Pairwise genome-wide F_{ST} between all localities, showing the lowest levels of population differentiation between localities from the Baltic Sea. (D) Measures of genetic variation: Nucleotide diversity π, population mutation rate θ_W (Watterson's theta) and Tajima's *D*. Values were averaged across each contig for each locality, and visualised as boxplots. Outlier SNPs were removed prior to creating the plots in panels (A–C). These plots show results from SNPs filtered at a minimum coverage of 40×. The colours from the legend in the bottom-left corner refer to corresponding colours in panels (A–D). The green colour ('North Sea vs. Baltic Sea') compares the genetic distance between sites in the North Sea and the Baltic Sea.

on Baltic *S. marinoi* similarly showed pronounced genetic differentiation along a southwest to northeast trajectory during a single bloom period (Godhe et al. 2016), and revealed significant genetic differentiation over only tens of kilometres (Sefbom et al. 2018). It is important to note that although our sample localities were all in coastal areas, previous research using microsatellites found similar levels of genetic differentiation in non-coastal areas of the North and Baltic Seas as well (Godhe et al. 2016).

3.3 | Similar Levels of Genetic Diversity in the North and Baltic Seas

We expressed genetic diversity for each sample locality as the nucleotide diversity π and the population-scaled mutation rate θ_W. Both are measures of the degree of nucleotide polymorphism within a population. We found that π and θ_W did not differ between the North Sea and Baltic Sea populations (paired *t*-test; *p*-value = 0.53 and 0.74, respectively), indicating similar levels of genetic diversity in the two regions (Figure 2D). Next, we calculated Tajima's *D*, which captures deviations from neutral-equilibrium processes including selection and population expansion or contraction. Although Tajima's *D* was significantly lower in the North Sea (*p*-value = 0.002), this pattern was driven by locality B (Figure 2D). To assess whether our Tajima's *D* estimates were biased upwards due to our MAF of 5%, we also calculated Tajima *D* using an MAF of 0.1%, but did not observe a significant difference between these two datasets. Thus, despite population differentiation and its relatively confined setting, the Baltic Sea population does not

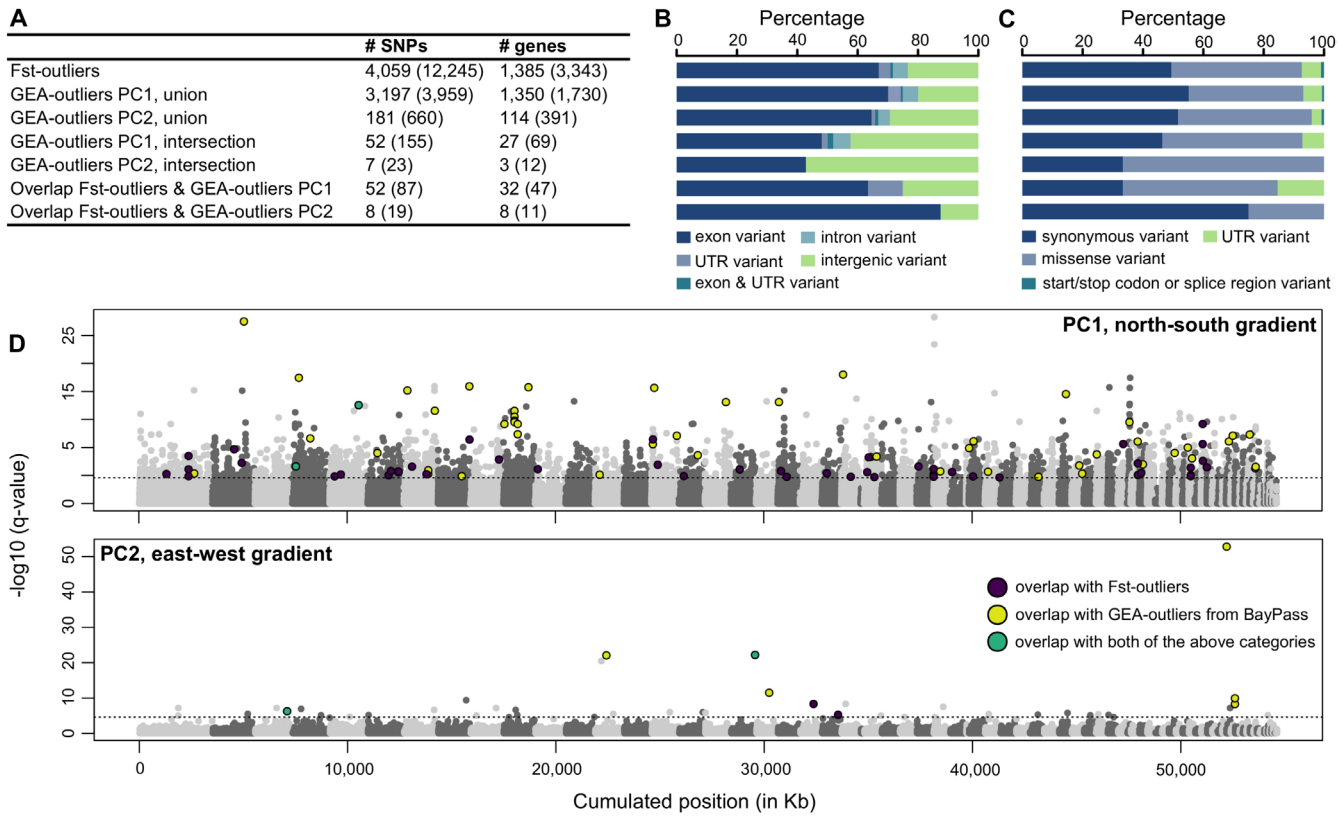
exhibit reduced genetic diversity compared to its source population in the North Sea. This suggests that if *S. marinoi* experienced a population bottleneck when colonising the Baltic Sea some 8000 years ago, the bottleneck was either too small, or too long ago, to be detected today. Although microeukaryotes have short generation times and are assumed to harbour high levels of intraspecific variation (Godhe and Rynearson 2017), the genetic diversity of *S. marinoi* in our study area (i.e., nucleotide diversity π averages 0.009 in both the North and Baltic seas) is comparable to populations of small mammals, insects, and plants (Leffler et al. 2012). Our dataset thus provides additional evidence for Lewontin's Paradox in phytoplankton (Filatov 2019; Filatov and Kirkpatrick 2024). Theoretically, larger populations are expected to harbour more genetic diversity, yet the range of population sizes far exceeds the range of genetic diversity in natural populations (Lewontin 1974). Altogether, the high genetic diversity of Baltic *S. marinoi* combined with evidence for reduced gene flow from the North Sea to the Baltic Sea from our biophysics model suggests that Baltic *S. marinoi* is not a sink population, i.e., one that is maladapted to the Baltic Sea and sustained only through constant migration from a diverse source population in the North Sea.

3.4 | Seascape Connectivity Reveals (Lack of) Barriers to Gene Flow

To assess the potential barriers to gene flow suggested by the F_{ST} values, we used a biophysical model that accounts for oceanographic currents to estimate the degree of seascape connectivity and predicted dispersal of *S. marinoi* between sample localities (Figure 1D). This included the calculation of one- and multigeneration connectivity across 16, 32 and 64 generations and assumed stepping-stone dispersal, which can uncover long-term connectivity between sites (White et al. 2010). Our model highlighted the geographical isolation of the Baltic Sea, as few modelled trajectories of *S. marinoi* can reach the Baltic Sea from the North Sea through surface currents, even when allowing for multigenerational dispersal (Figure 1D). In contrast, there is high connectivity between localities within each sea (Figure 1D). We found a significant isolation-by-distance pattern: Mantel test p-value = 0.036 for the 64-generation connectivity model (Figure 2B) and p-value = 0.007 for the shortest distance over the sea (Figure S3). However, this pattern was driven entirely by the contrast between the North Sea and the Baltic Sea, as there is no significant isolation by distance within the Baltic Sea: Mantel test p-value = 0.11 for the 64-generation connectivity model and p-value = 0.48 for the shortest distance over the sea (Figure S3). Together, these analyses indicate that the narrow Danish Straits impose a strong dispersal barrier for *S. marinoi*, as it does for other micro- and macrobiota in the area, including mobile and sessile organisms, as well as drifters (Johannesson et al. 2020).

The high levels of oceanographic connectivity within the Baltic Sea suggest a panmictic population of *S. marinoi* should be present. Indeed, we observed relatively low genetic differentiation among several distant localities (e.g., D, F, J) (Figure 2A,C). However, we also observed relatively high F_{ST} values between localities in the Baltic Sea, as discussed previously (Figure 2C). Two Baltic localities (H and I, located in the Archipelago Sea) in particular showed high F_{ST} differentiation from the other Baltic sites, and each other (Figure 2C), despite high oceanographic connectivity and close geographic proximity (Figure 1D). This confirms previous microsatellite work that also found high genetic differentiation over small spatial scales (i.e., 6–152 km) in the Archipelago Sea (Sefbom et al. 2018). These observations suggest that even though dispersal between localities is frequent, it is not necessarily accompanied by gene flow, though it is unclear why. Possibly, differences in the local environment drive population differentiation in the Archipelago Sea. However, patterns of genetic differentiation/similarities between sites do not correlate with sampling year, nor with local environmental conditions (Table S1, Figure S5). In fact, localities H and I, which are genetically highly distinct, have similar environmental conditions in the parameters measured, whereas localities with distinctly different environmental conditions (e.g., K and P) are genetically highly similar. However, many environmental variables remain unmeasured. For instance, biotic interactions, such as predator–prey dynamics, synergistic/antagonistic interactions between diatoms and bacteria, or the impact of viral activity on diatom blooms, are important for diatom community dynamics by influencing cell densities and health (Frada et al. 2014; Koedooder et al. 2019). We cannot rule out that these or other latent variables vary significantly across the Baltic Sea, including the topographically complex Archipelago Sea (Sefbom et al. 2018). For example, copepods are a typical grazer for diatom blooms, yet their biomass varies across the Baltic Sea (Selander et al. 2019; Vuorinen et al. 1998). In addition, our interpolation method might have overlooked small, but significant, variations in abiotic conditions in the study area. Importantly, previous experimental work suggested that historic effects, namely local adaptation in combination with priority effects, can drive genetic differentiation in *S. marinoi* (Sefbom et al. 2015; Sildever et al. 2016). Such a scenario entails rapid population growth after initial colonisation, which saturates the open niche, followed by rapid local adaptation (Sundqvist et al. 2018). In turn, later arrivals, especially ones maladapted to the local conditions, face a competitive disadvantage and will contribute relatively little to gene flow. Given that *S. marinoi* forms local seed banks that can survive decades to millennia (Härnström et al. 2011; Bolius et al. 2025), local adaptation may be further promoted by increasing standing genetic variation and seed banks might sustain locally adapted variants through time, buffering against later immigrants and maintaining population differentiation (Sundqvist et al. 2018). Laboratory experiments focused on strains from localities H and I could resolve whether local adaptation and priority effects are at play here.

Finally, it is important to note that (i) *S. marinoi* shows high census population sizes in our study area, with spring phytoplankton blooms reaching densities up to 10,000 cells/mL (Godhe et al. 2013; Godhe and Härnström 2010), (ii) diatoms have a life cycle that alternates between asexual and sexual reproduction, though sexual reproduction is common (Audoor et al. 2024; Bilcke et al. 2021; De Decker et al. 2018; Filatov and Kirkpatrick 2024; Kim et al. 2020; Poulíčková et al. 2007), including in *S. marinoi* (Ferrante et al. 2019; Godhe et al. 2014) and (iii) *S. marinoi* in our study area shows high levels of genetic diversity, as most strains isolated from the same bloom tend to have different microsatellite profiles, suggestive of frequent sexual reproduction (Godhe and Härnström 2010). As a result,



	# SNPs	# genes
Fst-outliers	4,059 (12,245)	1,385 (3,343)
GEA-outliers PC1, union	3,197 (3,959)	1,350 (1,730)
GEA-outliers PC2, union	181 (660)	114 (391)
GEA-outliers PC1, intersection	52 (155)	27 (69)
GEA-outliers PC2, intersection	7 (23)	3 (12)
Overlap Fst-outliers & GEA-outliers PC1	52 (87)	32 (47)
Overlap Fst-outliers & GEA-outliers PC2	8 (19)	8 (11)

FIGURE 3 | Outlier SNPs and genes associated with the Baltic Sea environmental gradients. (A) Table showing the number (#) of outlier SNPs and genes in each tested category for the 40× minimum coverage dataset (20× minimum coverage between brackets). For the GEA analyses, *union* refers to the full set of SNPs or genes found by one or both approaches (i.e., LFMM/BayPass), and *intersection* refers to outlier SNPs and genes that were part of the overlap of both approaches. The bottom-two rows refer to outlier SNPs or genes that overlapped between the F_{ST} outliers and the union lists of the GEA outliers. (B) Types of outlier SNPs for the categories in (A). The labels of the vertical axis correspond with the labels in (A). (C) Types of outlier SNPs for the categories in (A), only showing SNPs located in exons. The labels of the vertical axis correspond with the labels in (A). (D) Manhattan plots showing outlier SNPs associated with the environment of the Baltic Sea as estimated by LFMM, shown separately for both PC axes. The dotted line represents the 1% FDR significance threshold. Different contigs are indicated with alternating shades of grey. The coloured dots represent outlier SNPs that overlap with F_{ST} outliers (dark blue), BayPass' GEA test (yellow) or both (green). Plots (B–D) show results obtained at a minimum coverage of 40×.

the effective population size is expected to be high, reducing the impact of genetic drift and suggesting that natural selection should be highly effective during diatom blooms (Filatov and Kirkpatrick 2024). If so, we should find distinct signals of local adaptation in the genomes of Baltic Sea *S. marinoi*.

3.5 | Signatures of Local Adaptation to the Baltic Sea

We detected outlier SNPs correlated with seasonal environmental variables in the study area using GEA, focusing on surface waters (0–10 m) and the time period matching our strain collection (2010–2018). These environmental variables were obtained from publicly available monitoring datasets (see Section 2). The full dataset with environmental variables, including visualisations of data interpolations across the Baltic Sea are available in our Zenodo repository. The environmental differences between the North and Baltic seas are correlated with *S. marinoi* population structure (Figure S4), which challenges the ability of GEA approaches to control for neutral patterns of population structuring (Forester et al. 2016; Lotterhos 2023). Therefore, we restricted all GEA analyses to the Baltic Sea. To avoid issues with collinearity between environmental variables, we used the first two principal components of a PCA on the Baltic environmental variables, which explained 72% of the variation (Figure S5), as input for a latent factor mixed model, LFMM (Jumentier 2021) and a Bayesian approach, BayPass (Gautier 2015). PC1 and PC2 correlated with the north–south and east–west environmental gradients of the Baltic Sea, respectively (Figure S5). Although the environmental dataset was not exhaustive, this focus on a north–south and east–west gradient in our GEA analysis indirectly accounts for unmeasured variables. Sea-ice variation/composition, for instance, follows a north–south gradient in the Baltic Sea (Granskog et al. 2006). Similarly, predator abundance is correlated with salinity in the Baltic Sea (Vuorinen et al. 1998).

Most outlier SNPs were associated with the north–south gradient, suggesting that selection was stronger along the north–south compared to the east–west environmental gradients in the Baltic Sea, and that differences in salinity, summer temperature and nutrient availability impose the greatest selective pressures in the area (Figure 3A). In turn, the east–west gradient was correlated with differences in salinity, temperature

(winter, spring, autumn), pH, alkalinity, light availability, oxygen, nitrate and silicate. Outlier SNPs were distributed across the genome (Figure 3D, Figure S6C). Most GEA outliers were located in exons, and about half of these were missense SNPs, i.e., nonsynonymous SNPs resulting in amino acid changes that may cause structural or functional changes in the protein (Figure 3B,C). These outliers are the most likely to be either under selection or linked to SNPs under selection. Despite the challenges of distinguishing the two, here and elsewhere in our study we focus on these missense SNPs as targets of selection. We also explored SNPs in untranslated regions (UTRs), as these regions can affect the translation, degradation and localisation of mRNAs (Mignone et al. 2002). A large proportion of the outlier SNPs (> 50%) were located in genes with either no or very limited functional annotation (i.e., in the whole genome, 53% of the proteins have no hits in Swissprot), challenging our ability to link signals of local adaptation with biological function. This is a common issue in non-model organisms with poorly characterised genomes and gene functions. Nevertheless, careful examination of GEA outlier SNPs and GO enrichments on their corresponding genes revealed numerous biological processes associated with local adaptation.

The overlap between outlier SNPs detected by LFMM and BayPass is relatively small but significantly larger than expected by chance (hypergeometric test, p-value < 0.001, Figure 3A). These SNPs carry the strongest evidence for association with the Baltic environmental gradients. Outlier SNPs detected by applying a 40× minimum coverage were part of genes involved in diverse cellular functions including signal transduction (histidine kinase), proteolysis, the cell cycle and transmembrane activities, including an ABC transporter. These processes are known to be involved in diatom stress and acclimation responses (Downey et al. 2023; Pinseel et al. 2022). We also detected a chitin synthase under selection along the north–south gradient. Chitin is thought to be important for low-salinity tolerance by affecting cell wall remodelling and/or buoyancy adjustments, as was suggested for the euryhaline diatom *Cyclotella cryptica* during acute hyposalinity stress (Downey et al. 2023). In addition, chitin might play a role in cell linkage and chain formation in *S. marinoi* (Amato et al. 2018). Given the importance of chain length to grazer susceptibility (Bergkvist et al. 2012), selection on chitin genes may suggest that variation in predation (Vuorinen et al. 1998) imposes variable selection pressures across the Baltic Sea (Selander et al. 2019). When screening for outliers at a minimum coverage of 20×, we naturally found more candidate SNPs. Most notably, these additional outliers included transcription factors, cation channels and a heme chaperone.

Only focusing on the intersection between LFMM and BayPass (see above) can introduce a bias towards detecting only strong selective sweeps because all methods must agree. In a system that is dominated by recent selection or selection on standing genetic variation—which typically leaves a weaker signal—only analysing the overlap between multiple GEA approaches will not effectively detect all loci under selection (Forester et al. 2018). Given that adaptation in our system is clearly polygenic, it is unlikely that many individual loci carry the signal of a strong selective sweep. Furthermore, it is important to note that when combining multiple GEA methods, results based on the intersection will be biased towards the weakest method used, which again limits insights and undermines the robustness of the results (Forester et al. 2018). Therefore, we also explored the full set of GEA outliers (> 3000 SNPs) regardless of overlap between LFMM and BayPass, focusing primarily on SNPs detected with a minimum coverage of 40×, and looking at individual genes with outlier SNPs and GO enrichment results (Figures 3A and 4A, Figures S7–S9). In general, genes with GEA outlier SNPs, and thus possible signals of local adaptation, had functions that were clearly associated with the Baltic environmental gradients. For instance, along the north–south gradient we found selection on genes involved in cation and ammonium transmembrane transport, fatty acid metabolism, heat shock transcription factors and oxidative stress (i.e., superoxide dismutase), indicative of changes in how the diatom responds to nutrient availability and osmotic (salinity) stress (Figure 4A, Figures S7 and S8). We also detected enrichment for genes involved in 'posttranscriptional regulation of gene expression' along the north–south gradient (Figure 4A), suggesting adaptive change in regulation of gene expression. Notably, along the north–south gradient we found four outlier genes with missense SNPs that are likely involved in polyamine metabolism. Apart from a role in silica precipitation and cell wall biosynthesis in diatoms (Kröger et al. 2000), polyamines can increase antioxidant enzyme activity, trigger the stress signal transduction chain and serve an osmolyte function, and as such have been found to play key roles in abiotic responses in vascular plants and diatoms, including salinity and heat stress/acclimation (Chen et al. 2018; Liu et al. 2016; Pinseel et al. 2022; Scoccianti et al. 1995). Outlier genes along the east–west gradient were, among others, involved in urea and amino acid transport, transcription and translation, DNA mismatch repair and polyamine metabolism (Figure 4A, Figures S7 and S8). In addition, we found two outlier genes involved in glutathione metabolism, which defends against external stressors, including reactive oxygen species (Berglund and Ohlsson 1995). Similar to the observation that epigenetic modifications play a role in local adaptation in vascular plants (Dubin et al. 2015; Platt et al. 2015), we detected a total of four outlier genes along the north–south and east–west gradients that were involved in histone modification (i.e., methylation and acetylation), consistent with selection on the epigenetic machinery in Baltic *S. marinoi*.

Diatoms undergo progressive cell size reduction by mitosis until the cell size reaches a species-specific size threshold which, often together with an environmental trigger, initiates sexual reproduction to restore maximal cell size (Round et al. 1990). In *S. marinoi*, shifts to lower salinities can trigger sexual reproduction (Godhe et al. 2014). Similarly, unfavourable conditions induce the formation of resting stages that can survive in bottom sediments for at least 7000 years (Bolius et al. 2025). Although many of the genes involved in these processes are unknown, they likely involve cell cycle and cell division genes, several of which were under selection along both the north–south and east–west gradients. In addition, we found several meiotic genes under selection (e.g., genes involved in flagella development). Altogether, our data suggest that local adaptation to the Baltic Sea involves selection on some combination of growth rates, resting cell formation and sexual reproduction. This is consistent with laboratory experiments which found that Baltic strains have evolved higher growth rates relative to North Sea strains to cope with the more stressful Baltic Sea environment, but these higher growth rates have made Baltic strains less fit in their ancestral

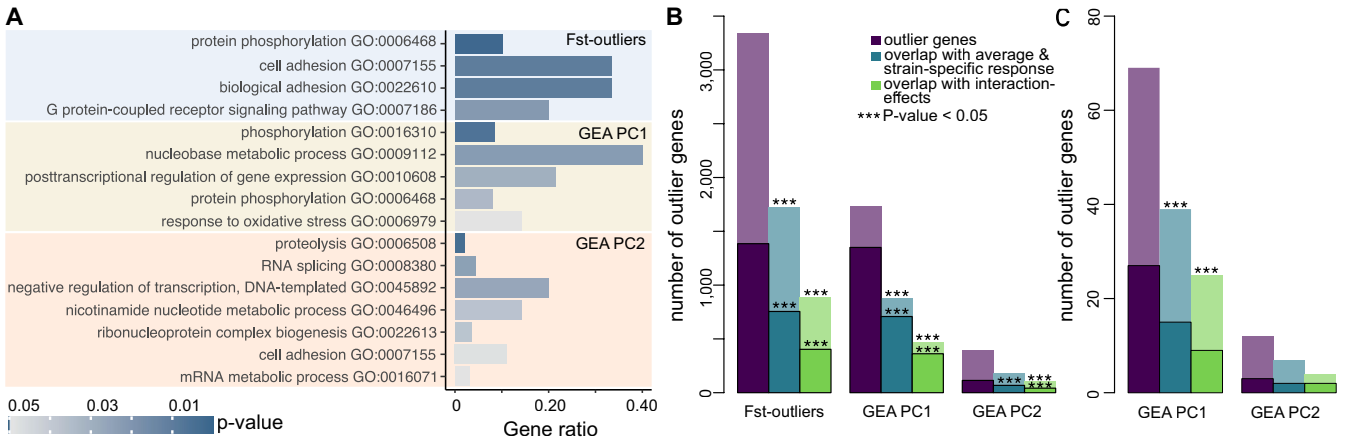


FIGURE 4 | (A) GO enrichment results (biological process) for the outlier genes. The GO enrichment results shown in this figure are based on the outliers detected using a minimum coverage of 40× and only include outlier genes (i.e., genes with outlier SNPs) that had at least one outlier missense SNP. Three categories are shown: (i) F_{ST} outliers (outliers between the North Sea and the Baltic Sea), (ii) GEA outliers for PC1 (north–south gradient in the Baltic Sea) and (iii) GEA outliers for PC2 (east–west gradient in the Baltic Sea). For the GEA outliers, GO enrichment is based on the union of both GEA approaches (LFMM/BayPass) For the GEA PC2 set, GO terms were summarised with REVIGO (Supek et al. 2011). Bars are coloured by topGO's *p*-value. The height of the bars indicates the proportion of genes with a given GO term that are enriched relative to the total number of genes with this GO term in the genome of *S. marinoi*. (B and C) Barplots showing the overlap between the outlier genes and differentially expressed genes in *S. marinoi* in response to low salinity. Outlier genes are subdivided into three categories: F_{ST} outliers, GEA outliers for PC1 and GEA outliers for PC2. Panel (B) indicates the F_{ST} outliers and the full set of GEA outliers detected by the union of both approaches (LFFM/BayPass). Panel (C) indicates the GEA outliers detected by both LFMM and BayPass. The dark shades indicate results from outliers detected at a minimum coverage of 40×, whereas the light shades correspond with outliers detected at a minimum coverage of 20×. Gene expression data were obtained from eight strains, originating from localities A, B, D, F, I, J, K and P, which were exposed to salinities mimicking the Baltic Sea salinity cline (24, 16 and 8). We tested for differentially expressed (DE) genes for each salinity contrast (8–16, 16–24 and 8–24) within each strain and by combining data from all strains, resulting in a total of 7676 differentially expressed genes (= *average & strain-specific response* in the plot). For all combinations of strains, we also tested for interaction effects for each salinity contrast, thus testing for significant strain-specific responses: This resulted in 3958 differentially expressed genes (= *interaction effects* in the plot). A significant overlap between the outlier and differentially expressed genes is indicated with asterisks.

North Sea waters (Sefbom et al. 2023). This is an example of countergradient selection, where genetic effects counteract environmental effects. In summary, the genome-wide signals of natural selection in Baltic Sea *S. marinoi*—including selection on cell cycle genes and previous findings of phenotypic differentiation in growth rates observed (Sefbom et al. 2023; Sjöqvist et al. 2015)—further support the hypothesis that Baltic *S. marinoi* is adapted to local environments across the Baltic Sea.

3.6 | What Enabled *S. marinoi* to Colonise the Baltic Sea?

We found a large number of SNPs with significantly different allele frequencies in the North versus Baltic seas. Outlier SNPs between the North Sea and the Baltic Sea were detected using a dual approach: (i) application of the C2 statistic as implemented in BayPass, which contrasts allele frequencies between population ecotypes with a binary trait (in this case: 'North Sea' or 'Baltic Sea') while correcting for population structure (Olazcuaga et al. 2020) and (ii) screening of the dataset for the top 10% differentiated SNPs identified by F_{ST} that are also significant in Fisher's Exact Test. We then accepted only those outlier SNPs between the North Sea and Baltic Sea detected by both approaches, resulting in >4000 outlier SNPs in >1300 genes (Figure 3A) (hereon referred to as 'F_{ST} outliers'). Similar to the GEA tests, SNPs were distributed across the genome and were mostly located in exons, with about half representing missense SNPs (Figure 3B,C, Figure S10). Also here, we focus on SNPs associated with changes in protein sequence (missense) or located in UTRs.

The small number of SNPs that were both F_{ST} and GEA outliers (Figure 3A) are the strongest candidates for a role in the range expansion of *S. marinoi* into the Baltic Sea, as they show large allele frequency differences between the regions and are also associated with the Baltic environmental gradients (Figure S10). The majority of these outliers did not have a functional annotation, but those with annotations and minimum 40× coverage included: a heat stress transcription factor, a cotranscriptional regulator and an ascorbate peroxidase involved in the oxidative stress response. At 20× minimum coverage, we additionally detected a heme chaperone, and genes involved in signal transduction and ion transport.

We also explored the larger set of F_{ST} outliers, regardless of overlap with GEA, to gain a general idea of genes and pathways that might have enabled colonisation of the Baltic Sea (Figures 3A, 4A, Figures S7–S9). Most strikingly, these F_{ST} outliers were enriched for signal transduction, cell communication, cell adhesion and homeostasis (Figure 4A, Figures S7–S9). This includes various protein kinases: (i) calcium/calmodulin-dependent protein kinases which are differentially expressed in *S. marinoi* in response to hyposalinity (Pinseel et al. 2022) and which might play a role in osmotic sensing in diatoms (Helliwell et al. 2021), (ii) serine/threonine protein kinases, including *TOR*, which have been linked to stress responses and resting cell formation

in diatoms (Chen et al. 2014; Pelusi et al. 2023), (iii) cGMP-dependent protein kinases, which play a role in the salt stress response of plants (Shen et al. 2019) and are differentially expressed in diatoms in response to copper exposure and deficiency (Suzuki et al. 2022), (iv) histidine kinases, which are involved in signal transduction across the cell membrane, nutrient sensing, light perception, and oxidative and osmotic stress response in plants, fungi and algae (Kabbara et al. 2019) and lastly, (v) cAMP-dependent protein kinases, which rely on the ancient signalling molecule cAMP that serves as a stress indicator across the tree of life (Berman et al. 2005). Several of the histidine kinases were also GEA outliers. Importantly, protein kinases were also found to be highly responsive to hyposalinity stress in *S. marinoi* (Judy et al. 2024). Clearly, widespread changes in signal transduction pathways that are involved in various metabolic processes, including the stress response and osmotic regulation, could have played a central role in the colonisation of the Baltic Sea by *S. marinoi*.

The Baltic Sea is naturally vulnerable to nutrient enrichment due to stratification and long retention times (Andersen et al. 2017). As a result, during its initial colonisation, *S. marinoi* may have been confronted with both lower salinities and higher nutrient concentrations. Furthermore, during the last century, the Baltic Sea has undergone large-scale eutrophication due to increased anthropogenic inputs (Andersen et al. 2017), and it is conceivable that these changes add additional selection pressures on Baltic *S. marinoi* today. We found several genes involved in nitrogen, phosphorus, or molybdate transport and/or metabolism (Figures S7–S9). Similar to the GEA approach on the Baltic Sea, we detected several North Sea–Baltic Sea outliers that play a role in stress and acclimation responses in diatoms, including *S. marinoi* (Downey et al. 2023; Pinseel et al. 2022). This included heat stress transcription factors and heat shock proteins, genes involved in oxidative stress management (e.g., peroxiredoxin, glutathione metabolism, violaxanthin-de-epoxidase), transmembrane transport of ions and amino acids, and genes involved in polyamine and chitin metabolism. We also detected selection on genes involved in amino acid metabolism, including the osmolytes proline and taurine. Osmolytes are used by diatoms to mitigate hyperosmotic stress (Krell et al. 2007; Nakov et al. 2020), although it is still uncertain whether proline serves a universal role as an osmolyte in diatoms, including in *S. marinoi* (Kamakura et al. 2024; Pinseel et al. 2022). Notably, several genes involved in histone modifications (e.g., methyltransferases) are under selection, which, similar to the GEA analyses, provides a link with the epigenetic machinery. We also detected various cell cycle genes in the F_{ST} outliers, which again underscores a clear agreement with laboratory experiments that showed distinct differences in growth rates between North Sea and Baltic Sea *S. marinoi* strains. Altogether, our data indicate that adaptation of *S. marinoi* to the Baltic Sea balanced an array of selection pressures, involving differences in osmotic pressure, nutrient availability and the general impact of a suboptimal, potentially stressful, environment. Although the F_{ST} and GEA outliers overlap little in the exact outlier SNPs and genes, the distinct overlap with the biological processes identified in both sets suggests a common biological strategy underlies both the initial colonisation of the Baltic Sea and the subsequent local adaptation to environmental differences within the Baltic Sea.

3.7 | What is the Role of Salinity in Adaptation to the Baltic Sea?

Salinity represents one of the most important environmental gradients structuring biodiversity in our study area (Johannesson et al. 2020). However, given that salinity is correlated with other environmental variables, it is challenging to disentangle the specific role of salinity in driving adaptation and population differentiation. To help overcome this issue, we combined our SNP dataset with a common garden experiment that we performed previously. In this experiment, we exposed eight *S. marinoi* strains originating from the same sampling localities as this study (all, but site H) to salinity treatments that mimicked the Baltic Sea salinity cline (24, 16 and 8 ppt) (Pinseel et al. 2022). For most outlier categories, there was significant overlap between the outlier genes identified in this study and ones that were differentially expressed in response to salinity (hypergeometric test, p-value < 0.05) (Figure 4B). However, we did not find a relationship between the strength of gene expression differences and the magnitude of between-locality allele frequency differences of SNPs within these shared genes (Figure S11). Thus, genes with outlier SNPs that showed the largest allele frequency differences between localities did not consistently show the strongest differences in gene expression between *S. marinoi* strains originating from the same localities.

Among the set of 33 differentially expressed genes considered crucial to low-salinity acclimation in *S. marinoi* (Pinseel et al. 2022), 11 were recovered as outlier genes at a minimum coverage of 40×, of which 7 had missense and/or UTR SNPs. This indicates that at least a subset of crucial genes that enable acclimation of *S. marinoi* to low salinities in laboratory conditions are also under selection in our study area over multi-millennial timescales. This included genes involved in the metabolism of storage molecules (i.e., fatty acids and the polysaccharide chrysolaminarin), polyamine metabolism and transporters for amino acids, polyamines and cations. The full set of differentially expressed genes identified in our common garden experiment that also contained outlier SNPs were involved in several other processes that are important for hyposalinity stress mitigation and acclimation (Downey et al. 2023; Judy et al. 2024) in diatoms (Figures S12 and S13), many of which were already discussed above. Briefly, these included stress response genes such as heat shock proteins, genes involved in oxidative stress management (e.g., thioredoxin, peroxiredoxin), as well as ion transporters and osmolytes (e.g., taurine) and signal transduction protein kinases. Genes involved in chlorophyll biosynthesis were also under selection. *S. marinoi* is known to upregulate chlorophyll biosynthesis during hyposalinity acclimation (Pinseel et al. 2022), which might drive elevated photosynthesis in suboptimal conditions (Shetty et al. 2019). Our data suggest that the photosynthetic machinery itself is under selection in Baltic Sea *S. marinoi*. Although several of these pathways could be important for responses to other stressors, our data suggest that salinity is likely an important, though not sole, driver of local adaptation to the Baltic Sea. Finally, a phylogenomic study of Thalassiosirales, the diatom lineage to which *S. marinoi* belongs, identified 532 hemiplasious genes, i.e., ones with persistent ancestral polymorphisms associated with marine–freshwater transitions (Roberts et al. 2023). A total of 52 and 109, at minimum coverages of 40× and 20× respectively, of

our outlier genes were also present in this set of hemiplasious genes, suggesting that some of the genes underlying ancient (i.e., millions of years) marine–freshwater transitions may have been similarly important for salinity adaptation over the microevolutionary (i.e., thousands of years) timescales of this study.

4 | Conclusion

The adaptive potential of phytoplankton species is routinely studied through laboratory experiments. We investigated the genetic basis of adaptation in a globally significant marine planktonic diatom to its natural environment using a combination of high-resolution population genomics and experimental transcriptomics. Our data showed that the complex environment of the Baltic Sea, which is characterised by multiple, interacting environmental gradients, drives genome-wide changes in diverse pathways in *S. marinoi*. Given the existence of seasonal variation in most abiotic parameters, it is likely that selection is mostly fluctuating in nature, resulting in multiple coexisting genotypes adapted to different environmental conditions (Godhe et al. 2016). Although transcriptome data indicated that the marine–brackish salinity gradient is a major driver of adaptive change, it is clear from the patterns of selection across the genome that other factors, including nutrient availability, are also important. Thus, our data indicate that the adaptive potential of marine planktonic diatoms hinges at least partially on the ability to rapidly adapt through complex polygenic changes. In addition, by integrating genome resequencing with transcriptome data, we found evidence that gene expression, either through plastic or evolved changes (Pinseel et al. 2022), and sequence evolution work hand-in-hand to enable persistence of phytoplankton in complex, changing environments. Our study suggests that understanding the adaptive potential of phytoplankton populations must account for the polygenic nature of adaptation to complex environments. Ultimately, insights into the genetic drivers behind microeukaryote adaptation in natural environments will contribute to understanding and predicting their responses to environmental change and vulnerability to extinction (Cortés et al. 2020). The latter is especially important for marginal populations in areas that are forecasted to experience massive environmental change in the coming decades (Pinseel et al. 2021; Trubovitz et al. 2020).

Author Contributions

A.J.A. and A.G. conceived the project. A.J.A., E.C.R., T.N., A.G. and M.W.H. designed the study. O.K., A.K., C.S., M.T. and A.G. performed fieldwork and aided with experimental design. A.G., M.T. and M.I.M.P. provided the annotated genome of *S. marinoi*. E.C.R. performed laboratory work, including culturing and molecular work, with support from T.N. T.N. wrote the machine learning script for estimating the number of cells per chain. P.R.J. designed and ran the biophysics model for seascape connectivity. E.P. conceived the data analysis workflow, with support from M.W.H. and W.R.R. E.P. performed the data analysis, wrote the manuscript and prepared the figures. All authors read and commented on the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Pool-seq data are available from the Sequence Read Archive (NCBI) under project number PRJNA950465. The *S. marinoi* reference genome (v1.1.2) used for read mapping and reanalysis of the *S. marinoi* salinity transcriptome, as well as all datasets and scripts needed to reproduce the analyses and figures, are available from Zenodo (https://zenodo.org/records/11200281).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.