



Review article

Population evolution of seagrasses returning to the ocean

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ABSTRACT

Seagrasses are higher flowering plants that live entirely in marine environments, with the greatest habitat variation occurring from land to sea. Genetic structure or population differentiation history is a hot topic in evolutionary biology, which is of great significance for understanding speciation. Genetic information is obtained from geographically distributed subpopulations, different subspecies, or strains of the same species using next-generation sequencing techniques. Genetic variation is identified by comparison with reference genomes. Genetic diversity is explored using population structure, principal component analysis (PCA), and phylogenetic relationships. Patterns of population genetic differentiation are elucidated by combining the isolation by distance (IBD) model, linkage disequilibrium levels, and genetic statistical analysis. Demographic history is simulated using effective population size, divergence time, and site frequency spectrum (SFS). Through various population genetic analyses, the genetic structure and historical population dynamics of seagrass can be clarified, and their evolutionary processes can be further explored at the molecular level to understand how evolutionary processes contributed to the formation of early ecological species and provide data support for seagrass conservation.

1. Introduction

Characterizing the population history of a species and identifying local adaptations are critical in functional ecology, evolutionary biology, biological conservation, and agriculture. There has been a proliferation of genomic studies elucidating population evolution over the last decade [1], including understanding migration and adaptation [2], the origin of domesticated species [3], and the genetic basis of local adaptation [4].

Speciation is an ongoing process, and understanding it is one of the main goals of evolutionary biology. The effect of evolutionary processes on population genetic diversity to drive differentiation and ultimately species formation, has been a hot topic [5–8]. The “neutral theory” of molecular evolution holds that most polymorphisms within species are in mutation-drift equilibrium, and that differentiation is not the result of adaptation [9,10]. Based on population structure, the identification of genome-wide variation loci to indicate the impact of natural selection on specific loci can contribute to a better understanding of polymorphism and adaptation [11, 12].

Population genetics studies initially focused on model organisms and less on natural populations with complex life histories. However, many key questions in ecology and evolutionary biology cannot be addressed using model organisms alone. Recent advances

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in computational biology, genomics, and phylogenetics have made it possible to study the genetic evolution of non-model systems [13]. Using next-generation sequencing techniques, genetic heterogeneity has been identified in several biological species, enriching the study of speciation and divergence, such as higher plants [1,8,14], insects [15], fishes [16,17], mammals [18], shellfish [19], and birds [20,21].

To date, most plant species do not conform to the population statistical assumptions of standard equilibrium neutral models and deviations from neutral expectations are relatively common [11]. Analyzing highly divergent genomic regions and exploring how selection drives speciation have been a hot spot in speciation and population evolution studies [8,16]. Selection can lead to changes in traits and related genes that affect the relative fitness of individuals and the differences between populations, which in turn drive speciation [22]. Recent population genomics studies have shown that selective traits have existed during the evolution of many species [23,24] and that genomic landscapes are largely influenced by natural selection [5]. The development of high-throughput sequencing technologies has facilitated the generation of genome-wide data for a wide range of species, which can help distinguish between neutral processes and selective features. Population genomics provides methods to better integrate selection into population history.

Seagrasses, composed of 60 monocotyledonous angiosperms, are the only flowering plants that live entirely in a marine environment [25–27] and play an important ecological role in coastal ecosystems [28,29]. The “return to the ocean” is one of the biggest changes in the habitat of vascular plants, and ecologists and evolutionary biologists are increasingly interested in the ecological and evolutionary significance of seagrasses. On an evolutionary scale, genetic diversity provides the basis for adaptive evolution, enabling populations to respond to their environment [30]. Maintaining genetic diversity to support adaptation is particularly important in light of increased environmental pressures such as rapid climate change and habitat loss or fragmentation [31]. With the development of next-generation sequencing technology, the global evolution of seagrass populations has been studied extensively. For example, genetic analysis based on eight microsatellite markers assessed the genetic and physical connectivity of *Zostera Noltei* population, enhancing our understanding of the population dynamics of important coastal base species [32].

Studies on the population genetic diversity and systematic geographic relationships of 32 *Thalassia Testudinum* samples from the Caribbean and Gulf of Mexico have shed light on its evolutionary and ecological history [33]. A 5–12 year resampling of *Zostera marina* from Bodega Bay, San Francisco, and coastal Bays in Virginia to determine the genetic diversity and structural variation within and among meadows [34] showed that neither diversity nor differentiation has changed over time. A 10-year survey of genetic structure and diversity could provide an accurate description of populations and increase the utility of published genetic data for the restoration and design of protected area networks.

Oceans cover 71% of the world’s surface area, and the conservation of marine biodiversity has become one of the United Nations Sustainable Development Goals [35]. Genetic diversity provides the basis for adaptive evolution and underpins ecosystem resilience and function [36,37]. In recent decades, global seagrasses have declined dramatically and their degradation rate has accelerated owing to climate change and anthropogenic factors [38–40]. Studies have shown that increasing genetic diversity can promote the resilience and productivity of seagrasses [37]. Population genetic results from *Zostera marina* in the Channel Islands and adjacent coastal areas of California suggest that seagrass restoration and transplantation must identify species populations and consider the degree of genetic and genotypic variation in candidate donor populations [41]. Studies on the genetic patterns of hydrodynamically related populations of *Thalassia Hemprichii* widely distributed in the Western Indian Ocean, have also been used to improve seagrass management in the

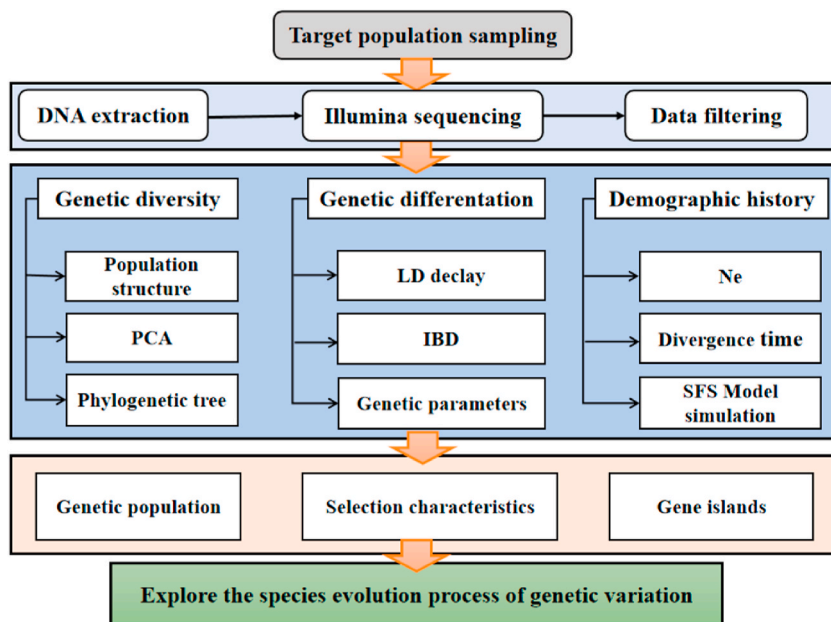


Fig. 1. Typical research ideas for studying the evolution of higher plant populations.

region to maintain healthy seagrass populations [42]. The clonal diversity, fine spatial genetic structure, and flowering yield of *Enhalus acoroides*, which is widely distributed on the eastern coast of Hainan Island, China, were evaluated for population conservation and restoration [43]. The genetic diversity of *Z. marina*, widely distributed in the Shandong Peninsula, China, was found to be different at each site owing to different population distributions and sampling ranges, which raised new thoughts on local population conservation [44,45]. Information on the genetic structure and variation of seagrasses can provide important data for coastal management [42]. It also plays a significant role in the successful transplantation and reconstruction of seagrasses [37].

Therefore, more details on the speciation history and comparisons between lineages with different degrees of divergence and geographical distributions are required to infer the population adaptation of seagrasses [8]. Exploring the relationships between different evolutionary clades of seagrasses will help clarify their origin [46] and reveal the evolutionary mechanisms underlying the transition of seagrass from land to sea [47,48].

2. Materials and methods

Seagrasses form different subspecies or subpopulations under different survival conditions due to natural selection, genetic drift, and other factors. To parse these processes and infer the evolutionary mechanisms by which populations adapt to radiation [49], detailed information on the speciation history and comparisons between lineages with different degrees of divergence and geographical distribution is required [8]. Understanding the correlations and genetic structure within and among populations is an important starting point for inferring selection or demographic history. The population evolution of seagrasses relies on research methods of terrestrial higher plants (Fig. 1).

- (1) CASE 1: Wang et al. used whole-genome resequencing data from 517 *Arabidopsis* relatives of *Bochera stricta* to determine the genetic population and demographic history; combined with population genetic characteristics, three genomic regions with high π exhibited long-term balancing selection. Moreover, F_{ST} islands that had undergone directional selection were also identified [8].
- (2) CASE 2: Ke et al. analyzed five species of *Primulina* from Karst limestone habitats and Danxia habitats, with a total of 132 individuals. The results showed that gene flow and linkage selection influenced the genome-wide variation landscape, and identified several gene islands affected by the divergent sorting of ancient polymorphisms involved in the speciation of endemic plants in special soil habitats and their adaptation to specific habitats [1].

2.1. Population sampling, genome sequencing, and single nucleotide polymorphism (SNP) calling

The sampling sites are set according to the distribution locations of the research objects. To maintain genetic diversity, at least 10 samples are randomly selected from each site with a certain distance [50], and the total sample size should not be not less than 30. The extraction of DNA from samples has been described in previous studies on the physiological ecology of seagrasses [38].

Libraries are prepared for each sample and sequenced on the Illumina platform. Trimmomatic V.0.36 is used to remove read connector sequences and trim bases [51], and FastUniq is used to remove duplicate reads [52]. Clean reads for each genotype are compared to the reference genome using BWA V.0.7.15 [53]. The sequences are recalibrated using HaplotypeCaller [54]. GATK V.3.8 was used to filter raw SNP [55]. Genomic variant call format (GVCF) files are generated using HaplotypeCaller for each individual and are combined and genotyped using CombineGVCFs and GenotypeGVCFs in GATK.

2.2. Population genetic diversity

To clarify the genetic diversity and selection of natural seagrass populations, genotypes and subpopulations should first be determined, followed by the genetic relationships among populations. Genetic diversity and genome-wide variation are affected by effective population size and population structure. The three methods used to verify and jointly elucidate the genetic structure and phylogenetic relationships are population structure analysis, principal component analysis (PCA), and phylogenetic tree construction.

Population structure analysis can be performed using different software packages [1,7] such as Admixture [56], FastSTRUCTURE [57], sNMF [58] and NGSadmix [59]. During the operation, genotypes can be called using GATK with K values and repeated 30 times for different samples. The ΔK method is used to determine optimal K value [60]. Ten-fold cross-validation and cross-entropy criteria are used to evaluate the Admixture and sNMF with different K values.

PCA is mainly performed using EIGENSOFT V.6.0 [61] in PLINK [62]. For each SNPs cluster, the heterozygosity of each individual is estimated as the ratio of heterozygous to homozygous SNP.

Phylogenetic trees for nuclear data can be constructed using three different methods: a maximum likelihood (ML) tree using an IQ-tree [63], a neighbor-joining (NJ) tree using bootstrap values of MEGA V.7 [64], and a window-based gene tree using Astral-III [65].

2.3. Linkage disequilibrium (LD)

LD decay is the evolutionary process from LD to linkage equilibrium between loci, and the mean square correlation (r^2) per SNP pair is used to represent the LD level of the population, which assists in the analysis of the evolution and selection processes [66]. The LD decay distance can be used to describe the decay rate [66], and the LD decay expression varies greatly among different subgroups. To

explore the degree of LD in different subgroups, SNPs with minor allele frequency (MAF) > 0.05 are first extracted and r^2 is estimated using PLINK V.1.90 [67]. The decay of LD with physical distance is estimated using nonlinear regression in equation (1) of Hill and Weir [68]. The LD in nonoverlapping windows is calculated using Vcftools. The window-LD value is averaged for each biallelic locus in each window.

2.4. Isolation by distance (IBD)

VEGAN in the R package is used to reorder permutations, and the Mantel test is conducted for the correlation between the genetic and geographic matrices of the permutations [69]. The intensity of IBD in each population should be quantified. Considering the problem of uneven sampling, the genotype group pairs are used to calculate the average genetic distance between each group. On this basis, a weighted linear regression is used to estimate the slope and intercept of the genetic distance to the geographical distance. The rate of increase in pairwise diversity at a geographical distance can be estimated from the slope of the mean of the pairwise diversity in each group.

2.5. Demographic history

The dynamics of marine connectivity is complex, and seagrasses undergo significant habitat changes after returning to the ocean. Detailed information on their evolutionary histories is important for understanding the process of genome differentiation after speciation. The change in the effective population size over time will be studied. The divergence time and sequence evolution should be estimated to determine the population dynamics, construct a phylogenetic tree, evaluate the site frequency spectrum (SFS) to model the demographic history, and select the optimal population statistical model to simulate the population dynamic history of seagrasses.

2.5.1. Effective population size (N_e)

Pairwise sequentially Markovian coalescent (PSMC) is one of the most popular and widely used methods for N_e calculation [70]. SMC++ V.1.15.2 is used to track the change in individual effective population size over time based on non-phase data [71].

2.5.2. Estimation of divergence time

The Markov chain Monte Carlo (MCMC) module in BEAST V.1.8.0 [72] is used to infer phylogenetic relationships and merge dates. The divergence time is estimated using HKY + G + I and BEAUti. Due to the lack of accurate data records to calibrate the mutation rate, the generation time and mutation rate (μ) is set based on fossil or related species of target species [73]. The best-fit model for sequence evolution is jModelTest 2.1.4 [74], and all samples are tested in Tracer V.1.6 to generate the final population trajectories [75]. All the runs are combined in LogCombiner 1.4.8 [76] and the resulting tree is visualized using FigTree 1.2 [77].

2.5.3. Site frequency spectrum (SFS)

The probability method in ANGSD V.0.919 is used to evaluate SFS [78]. Considering the missing data, downsampling is performed to reduce errors [19]. Folded SFS is used to reduce bias in determining the ancestral allele status [79]. Population statistical models are constructed using the coalescence-based simulation method in Fastsimcoal V.2.6.0.3 to infer the demographic history of seagrasses [79].

2.6. Population-genetic parameters

Identification of differential selection regions or infiltration barrier regions and natural selection characteristics can be achieved using genetic parameters such as Tajima's D , Fay and Wu's H , F_{ST} [80], d_{XY} [81], da [81]), and nucleotide diversity (π) [82]. To test the overall recombination rate ($\rho = 4Nec$) for the entire dataset and each population [7], the software package LDhelmet V.1.10, running with default parameters can be used, and a weighted average should be performed [83]. The impact of effective population size on estimated ρ is explained in terms of ρ/π [7].

2.7. Outlier screening and gene ontology (GO) analysis

We can standardize π in every window of each population and define high π windows with $Z-\pi \geq 2$ as π islands. According to formula $Z-F_{ST} = (F_{ST} \times F_{ST}') / \text{std-}F_{ST}$, F_{ST} of each window in each paired group is normalized to the Z-score [84]. High F_{ST} windows with $Z-F_{ST} \geq 2$ are defined as F_{ST} islands. To examine the functional classes of the overexpressed genes in the islands, GO analysis is performed using TBtools [85] and multiple tests are corrected using Benjamini-Hochberg (B-H) [86]. After B-H correction, GO items with a false discovery rate (FDR) < 0.05 are considered to be significantly enriched.

3. Results and discussion

As sequencing technologies develop and costs decrease, marine scientists have begun to use genomic technologies to address long-standing and emerging questions in evolutionary biology, thereby improving their understanding of marine biodiversity and evolution. To better explore the evolutionary mechanisms of genetic variation and understand the causality among biology, genetics, developmental science, and ecology, current research approaches use a combination of multiple information aggregated statistics [6,13],

which allows an integrated analysis of demographic history and selection to learn more about the population structure and evolutionary process [6]. It provides a comparative study of lineages with different degrees of divergence and geographical distributions [6]. For example, high-quality genomic information from zebrafish has been increasingly used to study the evolution of the human genome and for disease diagnosis [87]. RNA-seq has been used to investigate the evolutionary mechanism of *Tigriopus californicus* [88] and to reveal the population history dynamics of *Nematostella vectensis* [89]. Plastid genome studies have provided new insights into the early origins of green algae [90].

To measure genetic differentiation among subpopulations, genome-wide single-copy nuclear loci can be used to study LD, which can elucidate the extent of linkage, recombination, and mutation, and determine whether migration-drift equilibrium exists among populations [66]. Different statistics are used to find the selected loci and identify selection features. Selection can generate highly differentiated regions and determine the selection process by searching for gene islands and examining the functional classes of overexpressed genes on the island, which can infer the evolutionary mechanism that affects the genomic landscape.

Genetic heterogeneity among multiple species has been identified using next-generation sequencing technologies [7]. Balanced selection can result in increased π and decreased F_{ST} in the offspring. Positive and purifying selection favor single dominant alleles and reduce genetic diversity [91]. With changes in geographical location, selection is more likely to be based on locally adapted alleles in offspring; that is, regions with strong linkage selection have low recombination rates and a high density of functional loci with peak diversity and divergence, resulting in increased F_{ST} but decreased or unchanged d_{XY} [7]. Further predictions of linkage selection include positive and negative correlations between recombination rate and π/F_{ST} , respectively, and negative correlations between π/F_{ST} and genetic density in the selection target region. Because of the stress induced by background selection and periodic selection gaps, genetic diversity in low-frequency recombination regions decreases, F_{ST} increases, and d_{XY} remains the same or decreases. The combination of F_{ST} and d_{XY} can distinguish linkage selection from other evolutionary processes affecting genomic divergence [6]. Differential selection for gene flow and divergent sorting using ancient polymorphisms can produce genomic regions with elevated F_{ST} and d_{XY} . Other processes also produce highly divergent regions. For example, genetic drift can lead to peaks in genetic diversity and divergence [8]. It is difficult to distinguish population genetic patterns [49], as they are not mutually exclusive and can produce similar “footprint” across the genome [6].

The diversity and distribution of genetic variation within and among populations can reflect the interaction of complex processes and thus infer the genetic mechanisms of local adaptation and the adaptive potential of species. In other words, plant adaptation can be maximized only by identifying variation sites at the gene level, dividing genetic variation, and exploring the impact of natural selection on population evolution [24].

4. Conclusion and outlook

Whole-genome sequencing is performed in different geographical regions or subpopulations of target species to obtain hundreds of genotypes that represent a considerable geographical range. With large SNP data, population genetic questions can be solved, such as identifying selection characteristics and differences in the genome or introgression disorders and determining whether there is a mutation-drift balance [6,13]. Synthetic analysis of effective population size, divergence time, and SFS evaluation allows model simulations of demographic history to clarify the molecular nature of speciation, drive the evolutionary mechanism of genetic variation due to habitat changes, and thus reveal the theoretical genetic basis of population dynamics.

Currently, restoration of seagrasses generally focuses on burying plants underground to maximize coverage and density, with less attention paid to the preservation and restoration of genetic variation. Genetic structures can reveal the potential mechanisms of population dynamics and sustainability, and information on genetic diversity and differentiation is crucial for conservation of seagrasses conservation. Based on this bioevolutionary information, suitable donor plants and the most vulnerable extinction areas can be identified as conservation targets [92].

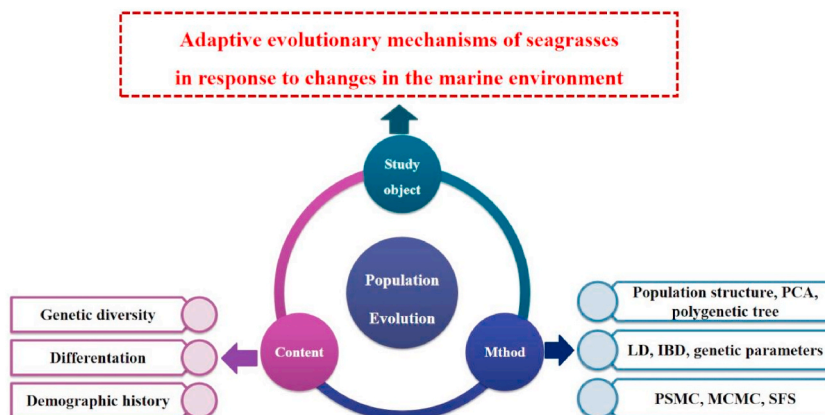


Fig. 2. Ideas for studying the evolution of seagrass populations.

In conclusion, information on the population evolution of seagrasses is essential for conservation of population diversity and policymaking. Seagrasses, as marine higher plants, can be used to explore the population structure and discuss the evolution of genetic variation (Fig. 2) on the basis of previous approaches to terrestrial higher plants (Fig. 1), to identify and assess the different processes that drive evolutionary differences and the formation of genetic variation patterns, which provide a genetic basis and data support for the conservation of seagrasses, and to further elucidate the adaptation of seagrasses from land to sea.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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