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# Population genomics of Posidonia sinuosa

**Theme:** Benthic Habitats and Communities

WAMSI Westport Marine Science Program

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## ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a $13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.





**FUNDING SOURCES**
The $13.5 million WAMSI Westport Marine Science Program was funded by the Western Australian Government, Department of Transport. WAMSI partners provided significant in-kind funding to the program to increase the value to >$22 million.


**DATA**
Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).




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**FRONT COVER IMAGE**
**Theme:** Benthic habitats and communities
**Front cover image:** Seagrass (Posidonia australis) in Cockburn Sound. Photo courtesy of Rachel Austin (The University of Western Australia).

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*The WAMSI Westport Marine Science Program is a $13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.*

# 1 Population Genomics in *Posidonia sinuosa*

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**Project 2.2 Pressure-response relationships, building resilience and future proofing seagrass meadows**

Diver collecting shoot samples in a healthy *Posidonia sinuosa* meadow near Garden Island, Cockburn Sound. Photo credit: Nicole Said, Edith Cowan University.

# 2 Executive Summary

This study focused on understanding genetic diversity and connectivity in the *Posidonia sinuosa* seagrass populations along the west coast of Western Australia, as part of Project 2.2 'Building Resilience and Future Proofing Seagrass Meadows'. Seagrass populations were sampled across the latitudinal gradient from Geraldton (northern range edge) to Geographe Bay (southernmost populations on the west coast) between May and December 2022. Sampling was clustered in three geographic areas (northern, central, southern) and included long term seagrass monitoring sites, with greater intensity of sampling conducted within the Cockburn Sound area at a range of depths (1.2 - 11.8 m). A population genomics approach was undertaken to assess levels of diversity within meadows (including clonal diversity), patterns of genetic structure among meadows (admixture and gene flow), and discovery of putative adaptive variants associated with local environmental conditions (local adaptation). These data will provide important baseline information for understanding patterns of genetic diversity in natural *P. sinuosa* populations along the west coast, as well as enable informed decisions for incorporating resilience into future seagrass restoration activities.

Adult shoot samples were collected from 30 meadows, sequenced and Single Nucleotide Polymorphisms (SNPs markers) generated. All meadows were growing in oceanic conditions (~36 salinity units) across a range in ocean temperatures at the time of collection (15 – 24 °C). After sequencing and bioinformatics filtering steps, the dataset consisted of 287 multilocus genotypes for 7,777 SNP loci from 28 meadows.

**The key outcomes from this research include:**

- Extremely high clonal diversity was detected, with almost every shoot sampled having a unique multilocus genotype ($R$ = 283/287 = 0.99). A single multilocus genotype was shared between Burns Rocks and North Beach (~16 km apart). Similar levels of genetic diversity were identified within all sampled meadows (observed heterozygosity = 13.3 % ± 0.019; allelic richness = 1.217 ± 0.02). Levels of inbreeding were high in all meadows ($F_{IS}$ = 0.336 ± 0.109), suggesting pollen dispersal is limited leading to an excess of non-random mating amongst close relatives.

- There was weak, but significant, genetic structure across the sampled latitudinal range ($F_{ST}$ = 0.080). Significant genetic structure occurred at all hierarchical levels and was associated with two mesoscale IMCRA bioregions (Central West Coast, Leeuwin-Naturaliste), geographical sampling clusters (northern, central, southern), and six sampling areas (Geraldton, Jurien Bay Marine Park, Marmion Marine Park, Cockburn Sound, Shoalwater Islands Marine Park, Ngari Capes Marine Park). Most of the variation was attributed to variation among individuals within populations (~40 %) or within individuals (~54 %).

- Genetic structure analyses suggest there were four genetic populations ($K$ = 4). However, high levels of admixture were indicative of spatial and temporal gene flow across the species' latitudinal range along the WA coastline.

- There was weak overall genetic structure among 11 meadows within Cockburn Sound ($F_{ST}$ = 0.057), with most populations forming a single genetic cluster. Connectivity was high among populations at the northern end of Cockburn Sound. Population towards the southern end of Cockburn Sound were more genetically discrete and not well-connected, a pattern also identified for *Posidonia australis* meadows.

- Asymmetric, or contemporary directional migration was detected along the WA coastline. A predominantly northward flow was consistent with the prevailing sea surface Capes Current driven by summer south-westerly winds. The most northerly (Geraldton) and southern (Ngari Capes Marine Park) populations were not as well connected. Populations in Perth metropolitan waters and Jurien Bay Marine Park were well-connected. Marmion Marine Park were well-connected with Owen Anchorage, while Becher Point appears to act as a sink for southward migration.

- Putatively adaptive loci (up to 4.7 % of SNPs) were strongly associated with sea surface temperature variables. The most common genotype-by-environment associations were with maximum sea surface temperature in summer ($SST_{max}$) and mean sea surface temperature in the warmest month ($SST_{summer}$). Significant allelic turnover for temperature related variables occurred between Jurien Bay Marine Park and Geraldton (n = 3), Perth metropolitan waters and Jurien Bay Marine Park (n = 5), and Ngari Capes Marine Park and Perth metropolitan waters (n = 5). These genotypic changes which occurred over less than 1.4 °C change may be indicative of local adaptation to warmer ocean conditions compared to populations further south.

- Genes were identified for 15 putatively adaptive loci, suggesting they may be associated with temperature adaptation.

- Our results highlight the importance of using genomic tools in determining genetic diversity, connectivity, and assessment of putative adaptation in seagrass meadows. Results support the potential for implementing resilience building options in Cockburn Sound to future-proof meadows to warming oceans and extreme heatwave events. Empirical studies, through the use of common garden experiments, will be needed to determine whether assisted gene migration might be a viable option for evolutionary change to escape extinction. A risk assessment and appropriate approvals would be needed before this could be actioned.

- Conservation of the fragmented northern warmer-adapted meadows is critical for long term persistence of *P. sinuosa* along the west coast of Australia. Plant material (transplants or seeds) could be sourced from more northerly, naturally warmer water adapted meadows to increase resilience in southern populations.

# 3 Introduction

Climate change and anthropogenic stressors present challenges to global biodiversity with loss of species, declines in populations and many plant species exhibiting signs of stress (Jordà et al., 2012; Christmas et al., 2015; Anderegg et al., 2019; Gibson and Newman, 2019). These impacts continue to manifest with rapid climate change, including increases in the frequency, intensity, and duration of environmental extremes such as heatwaves (Allen et al., 2010; IPCC, 2018). Such species and population declines can also lead to reductions in genomic diversity that could influence the resilience of species including their ability to adapt to change. Understanding the genomic diversity of plant species over their range can help inform local conservation actions to address these global pressures.

The genomic diversity of a species is a result of its evolutionary past, demographic history and natural selection (Savolainen and Pyhäjärvi, 2007; Daniell et al., 2016; Dai et al., 2019). Understanding the distribution of genomic diversity within a species and the presence of adaptive variants can provide useful insights into the past and how a species might adapt to future environmental conditions (Frankham, 2010). Adaptive variants are maintained in populations via a balance between selection, drift and gene flow and variation within populations can also arise through new mutations or from standing genomic variation. Detection of adaptive loci is often possible across geographic and environmental gradients and may allow for the prediction of populations vulnerable or resilient to future conditions (Bay et al., 2018; Hoffmann et al., 2021; Rellstab et al., 2021). Many adaptive genomic studies, to date, have focussed on terrestrial species (Fahrenkrog et al., 2017; Jordan et al., 2017; Gugger et al., 2021; Hopley and Byrne, 2022; Whale et al., 2024a), though the number of studies for aquatic and marine environments is increasing (Barceló et al., 2022; Marshall et al., 2022). Genotype-environment association (GEA) analyses can provide estimates of vulnerability to current environmental conditions and in association with projected environmental conditions provide managers with real-world applications to enhance conservation and restoration outcomes (Hoffmann et al., 2015).

Marine ecosystems along the West Australian coastline were significantly impacted by the 2010/2011 marine heat wave (e.g. Wernberg et al., 2013, Strydom et al., 2020). Future projections indicate continuing ocean warming leading to tropicalisation of some temperate ecosystems. The habitat-forming temperate seagrass meadows dominated by *Amphibolis* and *Posidonia* spp., are projected to experience range contractions of approximately 200 – 400 km southwards by 2100 (Hyndes et al., 2016). This could mean that *P. sinousa* meadows within the Perth metropolitan waters may be close to a new northern range limit, highlighting the conservation value of these meadows. These projections do not, however, take local adaptive variation into account. Species resilience can be enhanced to counteract the effects of rapid climate change through management strategies such as assisted (gene) migration (Aitken and Whitlock, 2013) or climate-adjusted provenancing (Prober et al., 2015). Climate-adjusted provenancing is the selection of genotypes from a donor population that will likely increase fitness of the recipient under a future climate scenario. The introduction of plants already adapted to future conditions, e.g. a particular temperature range, may enhance both genomic and phenotypic diversity in potentially vulnerable populations. An understanding of genomic and adaptive diversity in natural populations is necessary for either of these strategies to be implemented.

Seagrasses are flowering plants commonly found in shallow, protected coastal waters. They are sensitive to anthropogenic pressures (Cambridge and McComb, 1984; Cambridge et al., 1986; Coles et al., 2007; Gaylard, 2014) and climate change including heatwaves (Strydom et al., 2020). Many seagrass species have wide distributions over latitudinal gradients, so populations grow under different sets of environmental conditions. Latitudinal variation gives rise to differences in the temperature range, but environmental conditions can also vary due to depth gradients or the level of coastal development and associated eutrophication. These environmental differences have the

potential to give rise to local adaptation that, like in other plant species, could leave some populations vulnerable and some more resilient to rapid environmental change (Cao et al., 2020; Sang et al., 2022), although few studies have assessed this in seagrasses to date (e.g. Procaccini et al., 2017; Phair et al., 2019). Seagrasses can reproduce both clonally and sexually (Paulo et al., 2019), and through sexual reproduction, recombination can lead to new, potentially adaptive variants that may increase genetic diversity and enhance fitness. Clonal diversity is highly variable in seagrasses, with most meadows having low to moderate levels of clonality (e.g. McMahon et al., 2018; Hernawan et al., 2023; Sinclair et al., 2023), although there are some exceptions where a single clone dominates an entire meadow (e.g. Evans et al., 2014; Bricker et al., 2018; Edgeloe et al., 2022). Dispersal of seagrass propagules (fruit, seeds, seedlings) can occur from distances of 10's to 100's of km (reviewed in Kendrick et al., 2012), with buoyant *Posidonia australis* fruits dispersing 100 - 150 km (Ruiz-Montoya et al., 2015). The scale of dispersal among populations determines their connectivity and how potentially adaptive variants may be shared and maintained. Understanding this connectivity, in conjunction with environmental pressures, allow for the determination of how populations (at the fine-scale) and species (broad-scale) may respond to future climate change (Gaines et al., 2007). Consequently, it is important to understand the existing genomic diversity, the presence and spatial patterns of adaptive genomic diversity and the connectivity between populations to inform the effective conservation and management (including restoration) strategies.

In this study, we focused on *Posidonia sinuosa*, a widely distributed keystone species in temperate Western Australian waters with significant environmental and economic value to the wider ecological community. We conducted a population genetic study along a latitudinal gradient (560 km) to (1) Estimate genetic diversity, structure and connectivity for 28 populations and (2) search for local adaptation along this latitudinal gradient and if this putatively adaptive genomic variation is associated with environmental traits through genotype-environment outlier tests. We hypothesise that *P. sinuosa* will exhibit signatures of local adaptation to temperature, depth, and light. The outcomes from this study can be used to enhance *P. sinuosa* meadow resilience and inform management and conservation actions such as through assisted gene migration.

# 4 Materials and Methods

## 4.1 Species, site selection, and sample collection

*Posidonia sinuosa* Cambridge & Kuo 1979 is a perennial, temperate seagrass endemic to western and southern coastlines of Australia, ranging from the Abrolhos Islands in central Western Australia (WA) to Kingston, South Australia (Cambridge and Kuo, 1979, 1982). Ocean temperatures typically range from winter lows of 17 °C to summer maximum of 23 °C (NOAA Coral Reef Watch, 2022). The species is typified by non-fibrous leaf sheaths and narrow leaves that distinguish it from other Australian *Posidonia* species. *P. sinuosa* flowers towards the end of winter and produces fruit in late spring. Mature fruit, each containing a single seed, are released and rapidly float to the water surface (i.e. are positively buoyant), where they are dispersed by surface currents. Little information is available on sexual reproduction in *P. sinuosa,* although Smith and Walker (2002) found meadows in Warnbro Sound had fewer flowers on shorter stems (within the leaf canopy) than its sister species, *P. australis*. *Posidonia sinuosa* has a naturally disjunct distribution, preferring sheltered coastal embayments where it forms large, dense meadows to a depth of ~20 metres. *P. sinuosa* is listed as vulnerable on the IUCN Red List of Threatened Species (IUCN, 2010) with population declines, particularly in Cockburn Sound, linked to human activity (Short et al., 2011). The majority of seagrass loss in Cockburn Sound occurred between 1960 and 1970 (Cambridge and McComb, 1984; Cambridge et al., 1986). There has been limited natural recovery in following decades, despite improvement in water quality (Kendrick et al., 2002).

*Posidonia sinuosa* was sampled throughout its latitudinal range along the west coast from Geraldton, at the most northern species range edge to Ngari Capes Marine Park in Geographe Bay (Figure 1, Table 1). This range extends across two IMCRA mesoscale bioregions (Central West Coast, Leeuwin-Naturaliste), as defined under Integrated Marine and Coastal Regionalisation of Australia (IMCRA v4.0; Commonwealth of Australia, 2006; Last et al., 2010). This inshore bioregionalisation was derived from biological (distribution of demersal fishes, marine plants and invertebrates) and physical data (sea floor geomorphology and sediments, and oceanographic). Our sampling resulted in three broad geographic clusters (northern, central, southern), and six sampling areas (GERO = Geraldton; JBMP = Jurien Bay Marine Park; MMP = Marmion Marine Park; CS = Cockburn Sound; SIMP = Shoalwater Islands Marine Park; NCMP = Ngari Capes Marine Park), reflecting the naturally disjunct distribution of meadows. The marine environment along the west coast is influenced by a dominant warm, offshore southward flowing Leeuwin Current (Godfrey and Ridgeway, 1985; James and Bone, 2011), with a cooler, seasonal wind-driven northwards counter-current that flows along the inner shelf (Pearce and Pattiaratchi, 1999) facilitating sea surface dispersal. Sample sites were selected in areas where dense meadows form and to align with historical monitoring sites (see Webster et al., 2024). A Moran's *I* test in R (R Core Team, 2019) was used to assist with site selection to capture the range of temperatures experienced by *P. sinuosa* meadows. Environmental variables derived from NOAA (2022) including mean annual sea surface temperature (SST), mean maximum SST and SST annual range were used in the analysis. This method determines the spatial autocorrelation of the environmental gradients between sites using the '*Moran.I'* function in the APE package (Paradis and Schliep, 2019). Sites were selected, where possible, to cover the depth range of the species in each location (1.2 - 18.0 m; Australian Height Datum AHD). A greater number of sites were selected within Perth metropolitan waters, including Cockburn Sound, as this is an area with a large investment in seagrass restoration and a strategic initiative to explore and implement resilience building opportunities.

Cockburn Sound is an industrialised coastal embayment approximately 16 km long and ~7 km wide where significant declines in seagrasses. Several *Posidonia* species have been impacted (Cambridge and McComb, 1984; Cambridge et al., 1986; Kendrick et al., 2002), and further industrial development

is anticipated. The embayment provides a sheltered environment for ten seagrass species (Cambridge and McComb, 1984) and is an important spawning ground for fish (Breheny et al., 2012). Jurien Bay Marine Park (JBMP) is an area approximately 200 km north of Perth, ~90 km long running from Green Head in the north to Wedge Island in the south, encompassing 824 $km^2$ (Edgar et al., 2007; Fairclough et al., 2011). Coastlines within JBMP are protected from prevailing swells by a series of islands, structured reefs, and limestone pavements (Edgar et al., 2007) that provide suitable habitat for nine seagrass and three wrasse species (Fairclough et al., 2011), and breeding grounds for sea lions (Gales et al., 1992). Ngari Capes Marine Park (NCMP) is located in southwestern WA and wraps around Cape Naturaliste and includes Geographe Bay in the north and Flinders Bay in the south, the marine park extends 1200 $km^2$ (Hastings and Ryan, 2017). NCMP supports abundant fish biodiversity (e.g. Scanlon et al., 2024), migrating marine mammals (e.g. Double et al., 2014), and large continuous seagrass meadows (McMahon et al., 2007).

A total of 588 mature shoots were collected from 30 independent meadows via SCUBA. Sampling within each meadow occurred from a central point and individuals collected at 5 m intervals along radiating angles at the 0°, 90°, 180°, and 270°. A 5 m interval was selected to reduce the chance of re-sampling shoots from the same clone. All collected shoots were kept cold (~4 °C) for a maximum of 24-hours before processing. Non-photosynthetic shoot meristem tissue was removed from each individual and placed into a 2 mL microcentrifuge tube and frozen at -80 °C prior to DNA extraction. Samples were collected between May and December 2022. All samples were checked by Dr Marion Cambridge before processing, as there can be challenges with species identification, especially between *P. sinuosa* and *P. angustifolia* (Cambridge and Kuo, 1979). This resulted in some collected samples being removed and resampling from some meadows to increase the sample number.

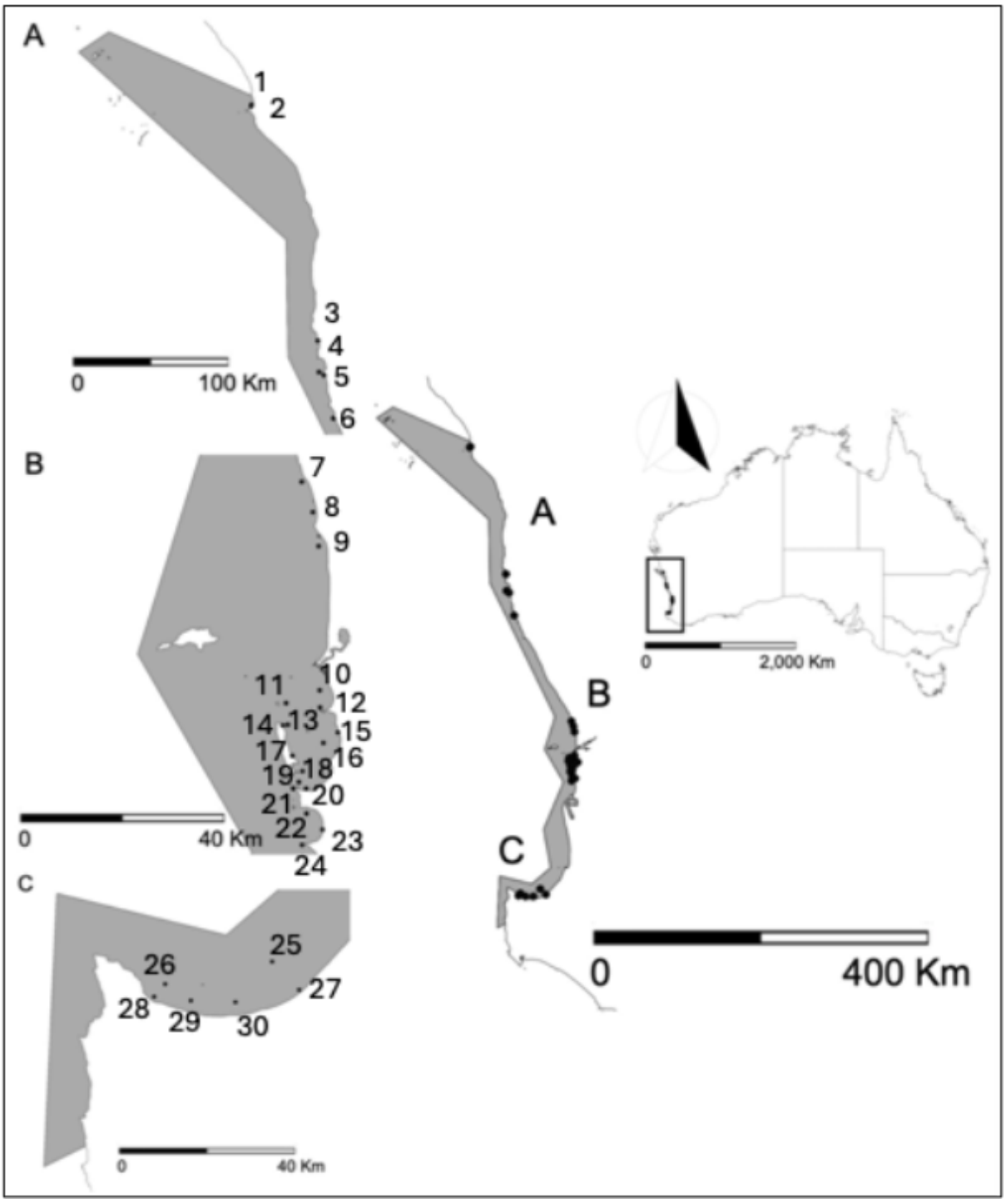


**Figure 1:** Location of sampled *P. sinuosa* populations along the west coast of Western Australia. Grey shaded area represents the approximate *P. sinuosa* distribution along the west coast (Atlas of Living Australia, 2022). Black circles represent individual meadows sampled across three geographic clusters: A. northern (Geraldton and Jurien Bay Marine Park); B. central (Marmion Marine Park, Cockburn Sound, and Shoalwater Islands Marine Park); and C. southern (Ngari Capes Marine Park). Site numbers match code in Table 1.

**Table 1.** Sampling locations of *P. sinuosa* in Western Australia. IMCRA mesoscale bioregions: CWC = Central West Coast; LNE = Leeuwin-Naturaliste; Geographic sampling clusters (northern, central, southern); Sampling areas: GERO = Geraldton; JBMP = Jurien Bay Marine Park; MMP = Marmion Marine Park; CS = Cockburn Sound; SIMP = Shoalwater Islands Marine Park; NCMP = Ngari Capes Marine Park. *, denotes long-term seagrass monitoring site; D seagrass meadow is adjacent to a restoration site.

| No. | Sample location | Abbr. | IMCRA bioregions (n = 2) | Geographic clusters (n = 3) | Sampling areas (n = 6) | Latitude (S) | Longitude (E) | Depth (m) |
|---|---|---|---|---|---|---|---|---|
| 1 | Beresford, Geraldton | BSFD | CWC | northern | GERO | -28.74845 | 114.6103 | 8.8 |
| 2 | Point Moore, Geraldton | POMO | CWC | northern | GERO | -28.75465 | 114.6076 | 10.8 |
| 3 | *Fishermans Island | FISH | CWC | northern | JBMP | -30.12717 | 114.9959 | 4.7 |
| 4 | *Boulanger Island | BOUL | CWC | northern | JBMP | -30.31245 | 115.0021 | 3.6 |
| 5 | *Jurien Bay | JUIS | CWC | northern | JBMP | -30.33013 | 115.0314 | 3.9 |
| 6 | *Kangaroo Point | KAPO | CWC | northern | JBMP | -30.58201 | 115.0856 | 4 |
| 7 | Burns Rock | BURO | CWC | central | MMP | -31.7274 | 115.7057 | 10.7 |
| 8 | Mullaloo | MULL | CWC | central | MMP | -31.78143 | 115.7253 | 10.3 |
| 9 | North Beach | NOBE | CWC | central | MMP | -31.84165 | 115.7354 | 8.9 |
| 10 | ^Owen Anchorage | S4S2 | LNE | central | CS | -32.09812 | 115.7372 | 5.8 |
| 11 | Carnac Island | CAIS | LNE | central | CS | -32.12077 | 115.6775 | 4 |
| 12 | *Woodman Point | WOOD | LNE | central | CS | -32.12859 | 115.73774 | 3.8 |
| 13 | *Garden Island Deep | GNDP | LNE | central | CS | -32.15896 | 115.68086 | 10.8 |
| 14 | *Garden Island Shallow | GRDN | LNE | central | CS | -32.16023 | 115.6721 | 3.1 |
| 15 | *Jervoise Bay | JERV | LNE | central | CS | -32.1723 | 115.77004 | 4.5 |
| 16 | *Kwinana | KWIN | LNE | central | CS | -32.19153 | 115.74389 | 5.2 |
| 17 | *Garden Island Settlement | GDNS | LNE | central | CS | -32.21371 | 115.68872 | 1.2 |
| 18 | *Southern Flats | SOFL | LNE | central | CS | -32.24155 | 115.70708 | 2.1 |
| 19 | *Causeway | CSWY | LNE | central | CS | -32.26048 | 115.70012 | 5.1 |
| 20 | *Mangles Bay | MANG | LNE | central | CS | -32.27244 | 115.71357 | 3.4 |
| 21 | Point Peron | POPE | LNE | central | SIMP | -32.27235 | 115.6905 | 3 |
| 22 | *Warnbro Sound | WNBR | LNE | central | SIMP | -32.3162 | 115.71393 | 6 |
| 23 | *Warnbro Sound Deep | WBDP | LNE | central | SIMP | -32.34505 | 115.74273 | 11.8 |
| 24 | *Becher Point | BEPO | LNE | central | SIMP | -32.37232 | 115.70608 | 3.4 |
| 25 | *Outer Shelf near Forrest Beach, Geographe Bay | OFOB | LNE | southern | NCMP | -33.54437 | 115.36998 | 16.1 |
| 26 | *Outer Shelf near Dunsborough, Geographe Bay | ODUN | LNE | southern | NCMP | -33.59011 | 115.15085 | 18 |
| 27 | *Vasse-Wonnerup, Geographe Bay | VAWO | LNE | southern | NCMP | -33.60188 | 115.42545 | 4.9 |
| 28 | *Dunsborough, Geographe Bay | DUBO | LNE | southern | NCMP | -33.61654 | 115.12865 | 3.6 |
| 29 | *Mid-Shelf near Marybrook, Geographe Bay | MMRY | LNE | southern | NCMP | -33.62426 | 115.20375 | 8.3 |
| 30 | *Mid-Shelf near Broadwater, Geographe Bay | MBRD | LNE | southern | NCMP | -33.62757 | 115.29517 | 9.7 |

## 4.2 DNA extractions, library preparation, and bioinformatics

Genomic DNA was extracted from frozen shoot meristems using a Qiagen DNeasy Plant Pro Kit (Qiagen, Germany) following the extraction method outlined by Edgeloe et al. (2022) using a Qiagen Tissuelyser II instead of a pestle and mortar, for 478 of the samples across the 30 populations (13-20 individuals per population, not all samples were extracted in case extracts did not meet quality control but were retained). Genomic DNA samples were stored at -80 °C. Genomic DNA quantity was measured using a Qubit 3.0 Fluorometer (Invitrogen, Australia) and fragment size quantified using an Agilent 4150 Tapestation (Integrated Sciences, Chatswood, NSW, Australia).

All genomic DNA extracts were submitted to the Australian Genome Research Facility (AGRF, Melbourne, Australia) for double digest restriction-site associated sequencing (ddRAD-seq, Peterson et al., 2012). Samples were digested using a Pst*I*/Mse*I* restriction enzyme combination with DNA fragments cleaved by both enzymes, and ligated with sequencing adapters. Sequencing was performed on a NovaSeq 6000 sequencing machine. Sequences of 150 base pairs were filtered with a minimum quality threshold phred score of greater than 35, read depth across all sequences were an average of 19X, and sequencing data were run through the AGRF Ipyrad and NSGEP pipelines using default parameters. Quality of the sequencing data was assessed based on the sequencing of duplicate samples. Locus filtering was conducted in R using the DARTR package (Gruber et al., 2018). Loci were removed if they were not bi-allelic, had more than 35 % per locus of missing data and where loci were under high linkage disequilibrium greater than 0.5 The minor allele frequency (MAF) was set to 0.02, one random locus per sequence read was retained. Individuals with greater than 30 % missing data were also removed.

## 4.3 Genomic diversity and clonal richness within populations

Estimates of clonality of multi-locus genotypes (MLGs) within populations were conducted in Genodive v3.06 (Meirmans and Van Tienderen, 2004) using the 'assign clones' function. We generated a distance matrix using the infinite alleles model with a genetic distance threshold of 314, then estimated the observed clonal diversity based on Nei's corrected diversity index (Nei, 1987) which incorporated the 'randomise alleles over individuals within populations' method. Final clonality estimates from Genodive were calculated with 999 bootstrap permutations. Estimates of clonality were independently verified in POPPR (Kamvar et al., 2014; Kamvar et al., 2015) in R. A distance matrix was generated and a bitwise distance threshold of 0.054 was used to detect clones as this method is suitable for a SNP dataset with missing data (Kamvar et al., 2014). Clonal richness (*R*) was estimated from the number of unique genotypes (MLG) relative to the number of sampled individuals per population (N); (MLG-1)/(N-1) (Dorken and Eckert, 2001). Shannon-Weiner Diversity Index (H) using the '*locus_table*' function and Simpsons Diversity Index (D) were both estimated in POPPR, recommended as standardised measures of heterogeneity to capture clonal diversity and evenness (Arnaud-Haond et al., 2007).

Standard population genetic statistics were estimated for each population based on unique multilocus genotypes (that is, clonal replicates were removed from the data set) including Shannon-Weiner index (H) as a measure of evenness and Simpson's diversity index (lambda) which provided a measure of clonal heterogeneity were estimated using POPPR. Observed ($H_O$) and expected heterozygosity ($H_e$) were estimated using the '*gl.report.heterozygosity*' function in DARTR, and a standardised allelic richness (AR) using the '*allelic.richness*' function calculated in the HIERFSTAT package (de Meeûs and Goudet, 2007; Goudet, 2005). Loci for all samples were tested to determine whether they conformed to Hardy-Weinberg equilibrium (HWE) based on classical $X^2$ using the '*hw.test*' function in the PEGAS package (Paradis, 2010). An inbreeding coefficient ($F_{IS}$) was also estimated using the Weir and Cockerham method (Weir and Cockerham, 1984) in HIERFSTAT.

## 4.4 Genomic structure among populations

Population structure among meadows was investigated using multiple approaches. Firstly, a discriminate analysis of principal components (DAPC) analysis (Jombart et al., 2010) was used to visualise the spatial relationship among meadows and individual samples. The '*find.clusters'* function was used to estimate the number of clusters within the dataset using the ADEGENET package (Jombart, 2008; Jombart and Ahmed, 2011). Sub-populations were estimated using the lowest Bayesian Information Criterion (BIC) score. The '*dapc'* function was used to visualise population

structure without *a priori* knowledge of the number of clusters by transforming the data through Principal Component Analysis (PCA) followed by determination of clusters using discriminant analysis. Here we retained 120 principal components representing 69.8 % of the variation, with two discriminant functions retained. The DAPC was visualised as a 2D scatterplot. Separate analyses were performed for the complete set of sampled meadows, as well as a reduced data set focussing on the Cockburn Sound area where the majority of sampling sites were located. Population structure was tested using the sparse non-negative matrix factorisation (SNMF) method in the LEA package (Frichot and François, 2015). The analysis was run for each *K*-value between 1 and 28 populations. Each *K* value was run ten times, with ten iterations, and combined. Cross-entropy scores for each run were compared to determine the optimal *K*-value. The best *K*-value according to the cross-entropy criterion was selected when the cross-entropy value did not decrease for a greater *K*. A consensus plot for each *K-value* was generated in CLUMPP (Jakobsson and Rosenberg, 2007) with graphical visualisation in DISTRUCT (Rosenberg, 2004).

Overall and pairwise population differentiation was estimated using the Weir and Cockerham method ($F_{ST}$, Weir and Cockerham, 1984) in HIERFSTAT. Finally, a Hierarchical Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) was used to test genetic partitioning among individuals using the *poppr.amova* function in POPPR. The data set was partitioned based on different geographical hypotheses: 1. IMCRA mesoscale bioregions (Central West Coast (CWC) versus Leeuwin-Naturaliste (LNE), 2. Geographic sampling clusters (northern, central, and southern) and 3. $K$ = 4 - 6 populations as identified through PCA clustering (DAPC) and structure (SNMF) analyses. Significance was based on 999 permutations.

## 4.5 Contemporary migration among populations

Estimates of directional relative migration among populations was calculated using divMigrate through the *R* package *diveRsity* using the $G_{ST}$ genetic distance measure which is analogous to $F_{ST}$ (Sundqvist et al., 2016). Each estimate was conducted at a significance threshold of $\alpha$ = 0.05, with 1000 bootstraps and gene flow patterns were visualized using the *qgraph* package (Epskamp et al., 2012). Relative migration values range from zero (equal migration between populations) to one (representing increasing directional migration). Boundary detection of isolated populations was estimated using Monmonier's maximum difference algorithm (Monmonier, 1973) using the '*monmonier'* function in ADEGENET. This method identifies a barrier where genetic differences between pairs of populations are highest.

Isolation by distance (IBD) was estimated to determine the relationship between genetic similarity among populations over geographic distance (km). Genetic similarity was assessed through pairwise $F_{ST}$, calculated following Weir and Cockerham (1984), in *HIERFSTAT* at a significance threshold of $\alpha$ = 0.05, with 1000 bootstraps. Geographic distance among populations was calculated through the *Marmap* package (Pante and Simon-Bouhet, 2013) as the shortest overwater distance among populations through >1 m water depth. Due to the map resolution, coordinates for some populations were adjusted (maximum 1 km from original coordinates) to ensure they registered as being located in water >1 m in depth. It is often assumed that the greater the geographic distance between two populations, the greater the genetic difference, however, this assumption does not always hold true in complex marine coastal environments where local conditions influence (pollen and seed) dispersal patterns. Correlation between the genetic and geographic distance matrices was estimated using a Mantel test with Spearman rank correlation through the *mantel* function with 1000 permutations in the *vegan* package (Oksanen et al., 2020).

## 4.6 Genotype by Environment association

We determined the spatial dependence among variables across the sampled spatial distribution before testing for associations between environmental variables and genomic loci. A Pearson's correlation coefficient (*r*) was calculated for ten environmental variables: mean annual sea surface temperature ($SST_{MA}$), mean sea surface temperature of the warmest month ($SST_{max}$), mean summer sea surface temperature ($SST_{summer}$), mean sea surface temperature of the warmest quarter ($SST_{WARMQ}$), mean sea surface temperature of the coldest month ($SST_{min}$), mean winter sea surface temperature ($SST_{winter}$), mean sea surface temperature of the coldest quarter ($SST_{COLDQ}$), mean annual range of sea surface temperature (TAR), diffuse attenuation coefficient at 490 nm (turbidity of the water column, kd490), and depth. Temperature data were downloaded from the National Oceanic and Atmospheric Administration (NOAA; https://coastwatch.pfeg.noaa.gov/erddap/griddap/NOAA_DHW_monthly.html) at 5 km pixel resolution (NOAA Coral Reef Watch, 2022; Skirving et al., 2020). All temperature variables were the NOAA baseline data (January 1, 1985 to December 31, 1990, and 1993). $SST_{max}$ are temperature values recorded in February, while $SST_{min}$ are data from July. $SST_{summer}$ was classified as the mean sea surface temperature of the traditional Austral summer months (December, January, and February), while $SST_{WARMQ}$ are the three warmest months of the year (January, February, and March). Similarly, $SST_{winter}$ and $SST_{COLDQ}$ are data recorded from the traditional Austral winter months (June, July, and August) and the three coolest months of the year (July, August, and September). Water turbidity (kd490) was also downloaded from NOAA at 4 km pixel resolution (https://coastwatch.noaa.gov/erddap/griddap/noaacwNPPVIIRSSQkd490Monthly.html) and data were averaged for the total data period of December 1, 2012 to December 1, 2023. Depth data for each site was extracted from the Two Rocks to Cape Naturaliste Light Detection and Ranging (LiDAR) as reported in Webster et al. (2024). Bathymetry data was collected from airborne laser bathymetry, a technique used to map seabed topography (Doneus et al., 2013), adjusted to the mean sea surface, and reduced to the Australian Height Datum (AHD), the national vertical datum for mainland Australia. Geo-referenced surface image of bathymetry at a 10 m resolution were read into ArcGIS v10.7 and estimates of depth were quantified for each population (Webster et al., 2024).

We employed LFMM2 (Latent Factor Mixed Model 2; Caye et al., 2019), a univariate GEA method, to detect associations to environment among all retained populations of *P. sinuosa*. LFMM2 requires a full dataset with no missing data, therefore all missing data among individual genotypes were imputed using the *impute* function using the median method to complete the genotype matrix. Population structure was accounted for by using latent factors to estimate the discrete number of ancestral clusters (*K*) which contribute to genetic variation with a PCA. This *K* estimate was used within the '*lfmm_ridge'* function within the LFMM2 v1.0 package to fit the LFMM2 regression model to the number of *K* factors. Individual associations to each environmental variable were estimated using the '*lfmm_test'* function with *p*-values calibrated using the genomic inflation factor (gif) control method. A Bonferroni correction was applied to the calibrated *p*-values at a significance threshold of $\alpha = 0.001$ to limit the false discovery rate since higher thresholds have been found to generate more variable data and increase the number of false positives (Ahrens et al., 2021). These thresholds were applied to each environmental variable.

A multi-variate method was also conducted to detect relationships between SNP loci and environmental variables. The complete imputed data set (see above) was used as redundancy analysis requires no missing data (RDA; Forester et al., 2018). The *rda* function was used to perform the RDA in the VEGAN v2.6-4 package (Oksanen et al., 2020). The *anova.cc* function was used to estimate the overall significance of the analysis. If the overall model was significant, the significance of each environmental variable was then tested using 999 permutations also using the *anova.cc* function. All loci significantly associated with an environmental variable were then plotted in two-dimensional form in R.

We performed a generalised dissimilarity model (GDM), an approach used to determine the significance of the environmental variable on the genomic loci, providing a visualisation of the spatial interactions across the landscape (Ferrier et al., 2007). The GDM was used to identify the allelic turnover of putatively adaptive loci (change in allele frequency across environmental space) from the GEA analyses. A pairwise genetic matrix of associated loci per environmental variable was generated using the *genet.dist* function in HIERFSTAT. These matrices were used within the GDM model using the GDM package (Fitzpatrick et al., 2020) using the *gdm* function to estimate the deviance among climate space and environmental associations (change in allele frequency turnover across environmental space). DNA sequences for loci showing the greatest deviance across environmental space were compared to the *Arabidopsis thaliana* genome in The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/index.jsp ; Berardini et al., 2015) using the BLAST and Mapviewer tools to determine whether the loci may be in genic space. Ideally a *Posidonia* genome would be used for the alignment but although there are some genomes sequenced they are not annotated to the level required for these assessments (Bayer et al., 2022 preprint).

# 5 Results

## 5.1 SNP dataset and bioinformatics

Genotype-by-Sequencing of *P. sinuosa* samples returned 55,478 loci throughout the genome and resulted in 7,777 polymorphic SNP loci for 287 individuals after bioinformatic filtering. The number of individuals sequenced was reduced from 478 to 287 for a total of 28 sampled meadows. DNA and/or sequencing quality was poor, particularly for almost all samples collected within two Geographe Bay sites (MMRY and OFOB), that resulted in their exclusion from all analyses.

## 5.2 Genomic diversity and clonal richness within populations

All shoot samples genotyped had unique multilocus SNP profiles, except for three shoots (Table 2; Clonal diversity, $R$ = 0.99). Estimates of clonality from both Genodive and POPPR resulted in the detection of multiple copies of MLGs in Burns Rocks (BURO; 2 samples) and Vasse Wonnerup (VAWO; 2 samples), and a single MLG shared between Burns Rocks and North Beach (~16 km apart). Population genetic diversity parameters were remarkably similar across all sampled meadows (Table 2), with low overall observed heterozygosity ($H_O$ = 13.3 %). Observed heterozygosity was significantly lower than expected in all populations, leading to a highly positive ($F_{IS}$ = 0.336). Significant deviations from HWE were present in 5103/7777 loci (65.6 %) at α = 0.01 threshold.

**Table 2.** Genomic diversity parameters within sampled *P. sinuosa* populations and overall means. N = number of individuals sequenced per population; G = number of unique multilocus genotypes (MLGs); *R* = clonal richness; H = Shannon-Weiner Index; lambda = Simpsons Diversity Index; E.5 = Evenness; AR = allelic richness; $H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; $F_{IS}$ = inbreeding coefficient.

| No. | Population | N | G | *R* | H | lambda | evenness | AR | *H*o | *H*e | *F*IS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | BSFD | 5 | 5 | 1.00 | 1.61 | 0.80 | 1.00 | 1.20 | 0.126 | 0.160 | 0.304 |
| 2 | POMO | 6 | 6 | 1.00 | 1.79 | 0.83 | 1.00 | 1.21 | 0.138 | 0.180 | 0.289 |
| 3 | FISH | 9 | 9 | 1.00 | 2.20 | 0.89 | 1.00 | 1.22 | 0.138 | 0.193 | 0.307 |
| 4 | BOUL | 14 | 14 | 1.00 | 2.64 | 0.93 | 1.00 | 1.22 | 0.114 | 0.198 | 0.412 |
| 5 | JUIS | 15 | 15 | 1.00 | 2.71 | 0.93 | 1.00 | 1.23 | 0.125 | 0.212 | 0.404 |
| 6 | KAPO | 10 | 10 | 1.00 | 2.30 | 0.90 | 1.00 | 1.21 | 0.124 | 0.189 | 0.358 |
| 7 | BURO | 15 | 13 | 0.93 | 2.62 | 0.92 | 0.97 | 1.21 | 0.105 | 0.188 | 0.427 |
| 8 | MULL | 13 | 13 | 1.00 | 2.56 | 0.92 | 1.00 | 1.24 | 0.135 | 0.219 | 0.384 |
| 9 | NOBE | 11 | 11 | 1.00 | 2.40 | 0.91 | 1.00 | 1.24 | 0.161 | 0.220 | 0.286 |
| 10 | S4S2W | 12 | 12 | 1.00 | 2.48 | 0.92 | 1.00 | 1.24 | 0.143 | 0.220 | 0.353 |
| 11 | CAIS | 15 | 15 | 1.00 | 2.71 | 0.93 | 1.00 | 1.22 | 0.128 | 0.203 | 0.357 |
| 12 | WOOD | 9 | 9 | 1.00 | 2.20 | 0.89 | 1.00 | 1.21 | 0.112 | 0.185 | 0.401 |
| 13 | GNDP | 7 | 7 | 1.00 | 1.95 | 0.86 | 1.00 | 1.22 | 0.136 | 0.190 | 0.318 |
| 14 | GRDN | 12 | 12 | 1.00 | 2.48 | 0.92 | 1.00 | 1.23 | 0.131 | 0.210 | 0.367 |
| 15 | JERV | 14 | 14 | 1.00 | 2.64 | 0.93 | 1.00 | 1.21 | 0.118 | 0.199 | 0.398 |
| 16 | KWIN | 8 | 8 | 1.00 | 2.08 | 0.88 | 1.00 | 1.22 | 0.132 | 0.197 | 0.347 |
| 17 | GDNS | 8 | 8 | 1.00 | 2.08 | 0.88 | 1.00 | 1.21 | 0.149 | 0.183 | 0.236 |
| 18 | SOFL | 5 | 5 | 1.00 | 1.61 | 0.80 | 1.00 | 1.20 | 0.130 | 0.168 | 0.299 |
| 19 | CSWY | 13 | 13 | 1.00 | 2.56 | 0.92 | 1.00 | 1.22 | 0.127 | 0.202 | 0.362 |
| 20 | MANG | 6 | 6 | 1.00 | 1.79 | 0.83 | 1.00 | 1.20 | 0.126 | 0.171 | 0.325 |
| 21 | POPE | 15 | 15 | 1.00 | 2.71 | 0.93 | 1.00 | 1.20 | 0.156 | 0.189 | 0.227 |
| 22 | WNBR | 13 | 13 | 1.00 | 2.56 | 0.92 | 1.00 | 1.22 | 0.132 | 0.206 | 0.350 |
| 23 | WBDP | 13 | 13 | 1.00 | 2.56 | 0.92 | 1.00 | 1.22 | 0.125 | 0.197 | 0.366 |
| 24 | BEPO | 11 | 11 | 1.00 | 2.40 | 0.91 | 1.00 | 1.24 | 0.162 | 0.218 | 0.275 |
| 25 | ODUN | 9 | 9 | 1.00 | 2.20 | 0.89 | 1.00 | 1.23 | 0.122 | 0.211 | 0.414 |
| 26 | VAWO | 7 | 5 | 0.83 | 1.75 | 0.82 | 0.94 | 1.20 | 0.128 | 0.175 | 0.317 |
| 27 | DUBO | 6 | 6 | 1.00 | 1.79 | 0.83 | 1.00 | 1.22 | 0.141 | 0.185 | 0.302 |
| 28 | MBRD | 6 | 6 | 1.00 | 1.79 | 0.83 | 1.00 | 1.23 | 0.164 | 0.194 | 0.230 |
| **Overall** | | **287** | **283** | **0.99** | **5.64** | **1.00** | **0.99** | **1.22** | **0.133** | **0.195** | **0.336** |

## 5.3 Genomic structure among populations

The DAPC analysis for the entire data set with 145 PCA eigenvalues retained accounted for 68.1 % of the variation (Figure 2A). Individuals clustered by sampling location, indicating a closer relationship among samples within a site, although there were large areas of overlap, particularly among populations within the Jurien Bay Marine Park and the central geographic cluster (Marmion Marine Park, Cockburn Sound, Shoalwater Islands Marine Park). Interestingly, the two Geraldton populations (BSFD and POMO) clustered discretely more closely with southern populations (Ngari Capes Marine Park) than the Jurien Bay Marine Park, despite considerable geographic distance between them (~560 km). The BIC scores derived for the DAPC analysis indicate the optimal number of population clusters was 3 or 4 (Figure S1). Spatial genetic structure within the northern geographic cluster showed discrete non-overlapping clusters by population (Figure 2B). Geraldton populations clustered separately from Jurien Bay Marine Park populations. Populations from the central cluster were largely overlapping, with the exception of Garden Island settlement (GDNS), Point Peron (POPE), and Owen Anchorage (S4S2W), which formed discrete non-overlapping clusters (Figure 2C). Three of the four populations in Ngari Capes Marine Park clustered together, while the mid-shelf site near Broadwater (MBRD) was most genetically differentiated from all other southern populations (Figure 2D).

A DAPC analysis for Cockburn Sound populations formed a single cluster (overlapping 95% confidence intervals), with the exception of two populations, the most northernly site at Owen Anchorage (S4S2W) and the central Cockburn Sound site at Garden Island settlement (GDNS; Figure 3A). The single cluster included closely-related genotypes from Carnac Island in the north to Mangles Bay in the south. The Jervoise Bay (JERV) population also clustered separately, after the removal of Garden Island settlement (Figure 3B).

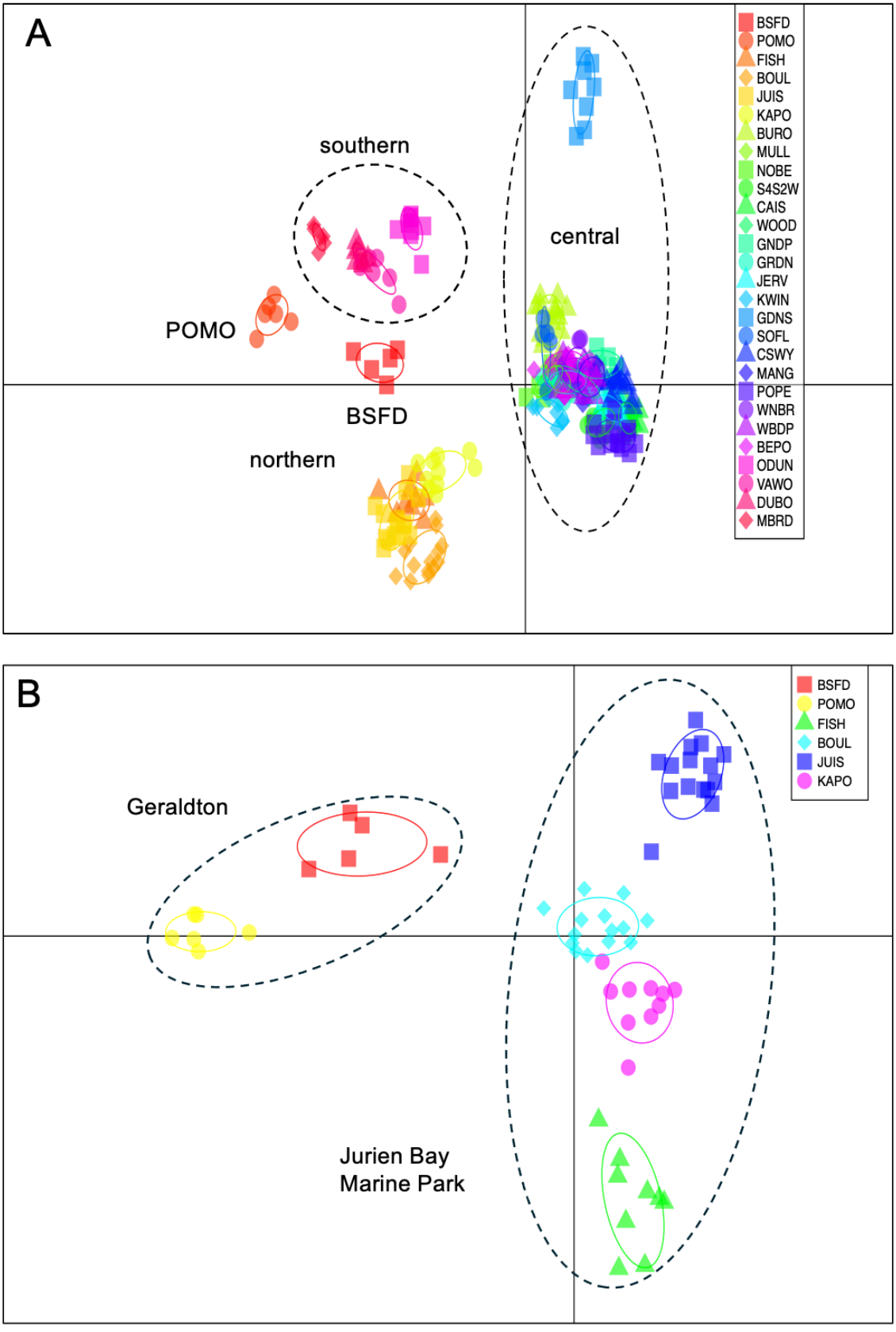


**Figure 2.** Discriminant analysis of principal component plots for all sampled *P. sinuosa* populations. A. All sampled populations (n = 28); B. northern populations only (n = 6); (continued over).

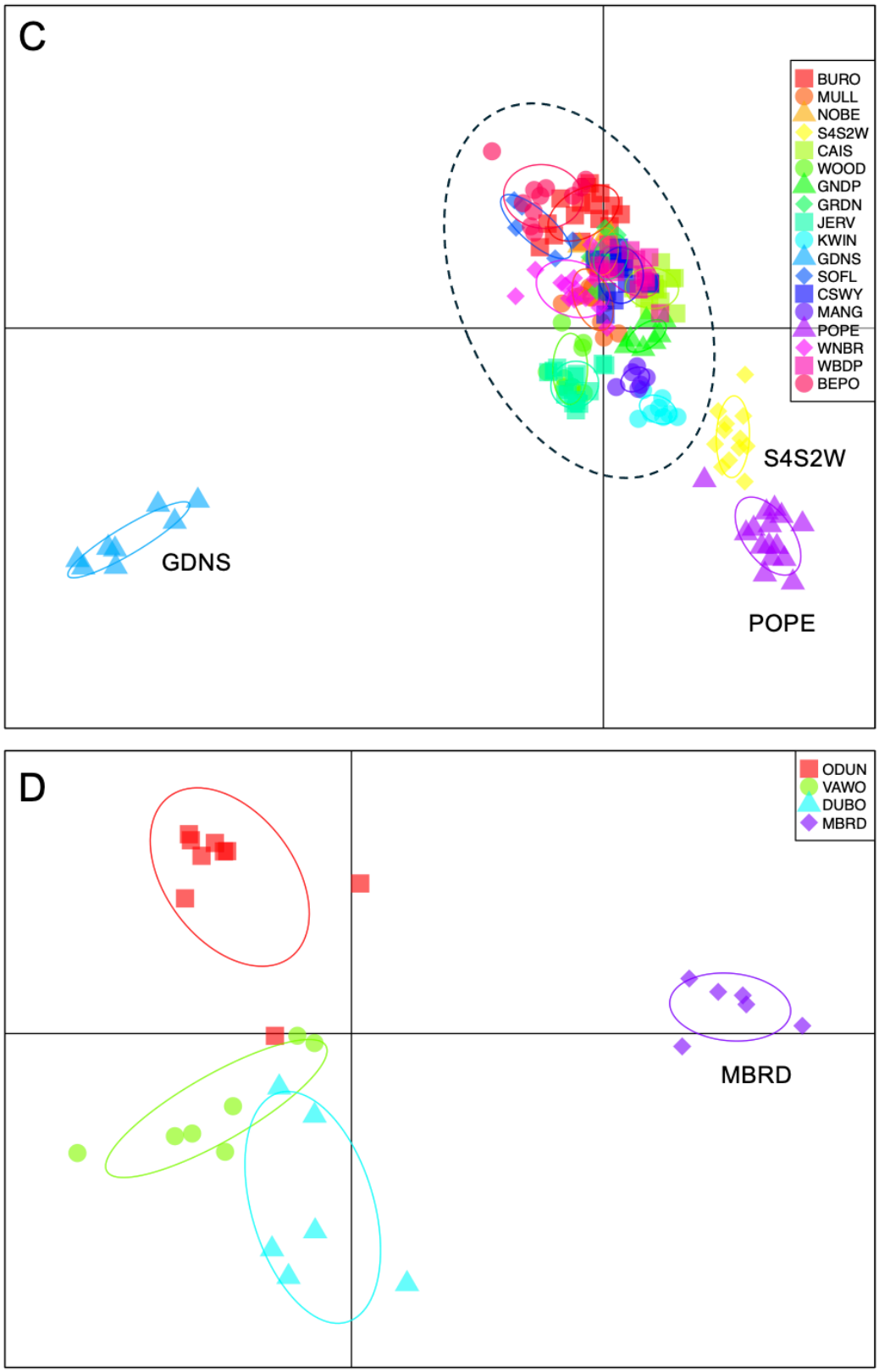


**Figure 2. continued;** C. central populations only (n = 18); D. southern populations only (n = 4). DAPC plots explained 68.1 %, 69.6 %, 66.9 %, and 69.9 % of the variation with 145, 35, 100, and 16 PCs retained, respectively. Coloured ellipses are 95% confidence intervals and dashed lines are drawn to aid interpretation.

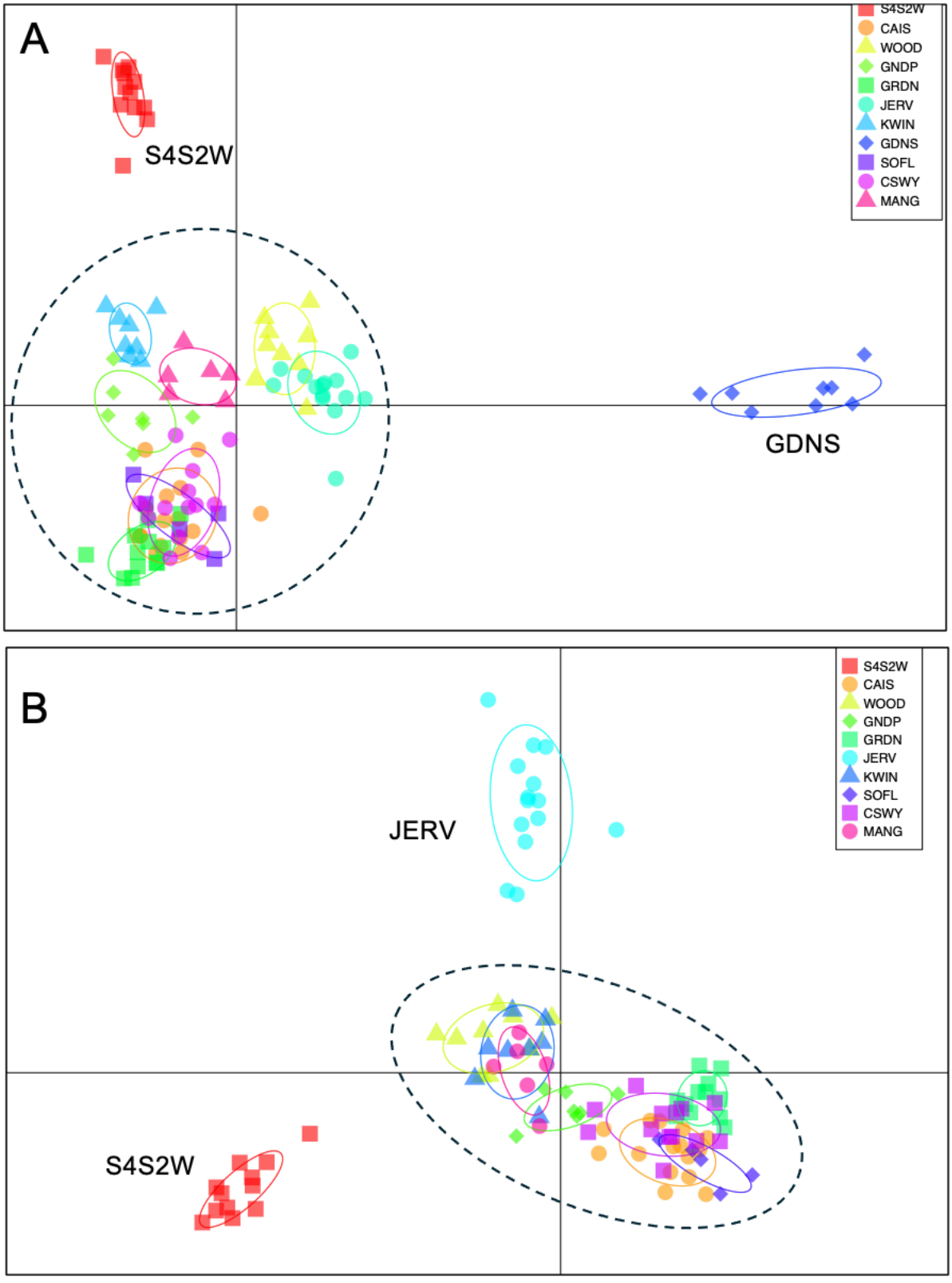


**Figure 3.** Discriminant analysis of principal component (DAPC) plot for *Posidonia sinuosa* populations sampled within Cockburn Sound. A. All Cockburn Sound populations; B. without the Garden Island settlement (GDNS). DAPC explained 66.0% of the variation with 55 PCs retained in the first two discriminant eigenvalues. Coloured ellipses are 95% confidence intervals and dashed lines are drawn to aid interpretation.

The cross-entropy scores for the SNMF analysis infer the optimal number of genetic clusters was *K* = 4 (Figure 4). The large drop in cross-entropy at *K* = 2 can be explained by the '*K* = 2 conundrum' and should be largely ignored (Janes et al., 2017). '*K*' population outputs from SNMF were plotted for *K* = 2 - 6. Overall, there was high admixture across most of the sampled populations, with the exception of three central populations at Burns Rock (BURO), North Beach (NOBE) and Point Peron (POPE; Figure 5). The pattern was consisent with a low level of genetic differentation across the latitudinal gradient.

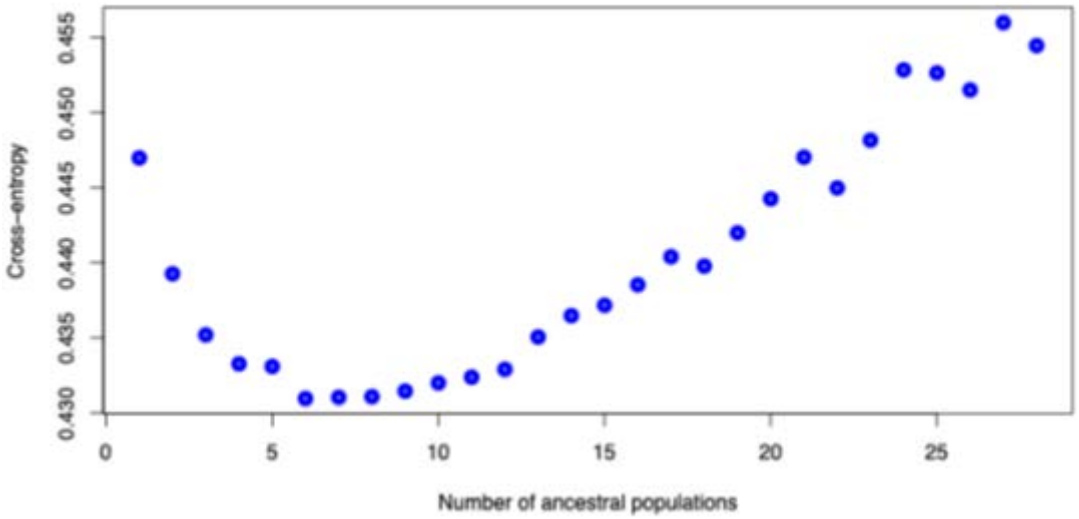


**Figure 4.** Values for the cross entropy plot for *K* = 1 to 28. An optimal value of *K* = 4 populations was inferred.

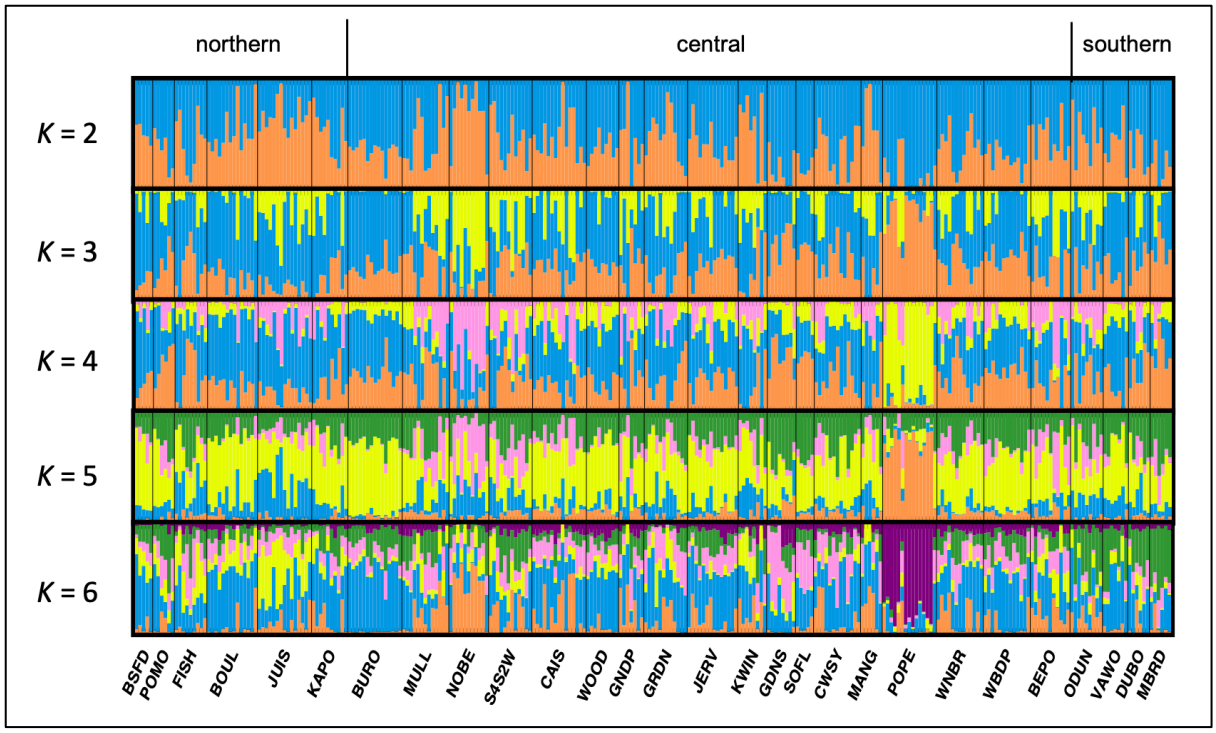


**Figure 5.** Structure plot of individual assignment of genetic clusters (*K* = 2 - 6) from SNMF analysis visualised with CLUMPP and DISTRUCT. Populations are labelled below the plot, indicated by vertical black lines, and orientated from north to south (left to right). Population codes as in Table 1.

Weak, but significant, genetic structure was detected among *P. sinuosa* populations sampled across the latitudinal gradient based on global $F_{ST}$ (= 0.080), with pairwise $F_{ST}$ ranging from 0.012 to 0.176 (Table S1). A similar level of genetic differentiation was present among populations within each of the three geographic sampling clusters: northern ($F_{ST}$ = 0.076), central ($F_{ST}$ = 0.066); southern ($F_{ST}$ = 0.049). There was also weak but significant genetic differentiation among the 11 sampled populations within Cockburn Sound ($F_{ST}$ = 0.057).

Hierarchical AMOVA's indicated there was significant genetic structure associated with the mesoscale IMCRA bioregions (CWC, LNE), geographical sampling clusters (northern, central, southern) and the six sampling areas (GERO, JBMP, MMP, CSMC, SIMP, NCMP; Table 3). The proportion of variance was highly significant at all hierarchical levels and similar among all *K* population hypotheses tested. Less than 2.0 % of the variation was attributed to differences among regions (*F*rt) and < 5.0 % to difference among populations (*F*sr). Most of the variation was among individuals within populations (~40 %, p > 0.001) or within individuals (~54 %, $p < 0.001$).

**Table 3.** Test of hierarchical Analysis of Molecular Variance (AMOVA) among sampled *P. sinuosa* populations.

| Source of variation | *F*-statistic | d.f. | Sun of squares | Variance component (σ) | Total variation (%) | Phi-statistic (ϕ) | P value |
|---|---|---|---|---|---|---|---|
| **IMCRA mesoscale bioregions (Central west coast, Leeuwin-Naturaliste; n = 2):** | | | | | | | |
| Between bioregions | *F*rt | 1 | 5450.8 | 7.6 | 0.5 | 0.01 | >0.002 |
| Among meadows within bioregions | *F*sr | 26 | 85059.7 | 68.5 | 4.9 | 0.05 | >0.001 |
| Among individuals within meadows | *F*ST | 259 | 487295.9 | 561.5 | 40.2 | 0.43 | >0.001 |
| Within individuals | *F*IS | 287 | 217679.0 | 758.5 | 54.3 | 0.46 | <0.001 |
| Total | *F*IT | 573 | 795485.4 | 1396.1 | 100.0 | | |
| **Geographic clusters (northern, central, southern; n = 3):** | | | | | | | |
| Among clusters | *F*rt | 2 | 11813.6 | 21.5 | 1.5 | 0.02 | >0.001 |
| Among meadows within clusters | *F*sr | 25 | 78696.9 | 61.7 | 4.4 | 0.04 | >0.001 |
| Among individuals within meadows | *F*ST | 259 | 487295.9 | 561.5 | 40.0 | 0.43 | >0.001 |
| Within individuals | *F*IS | 287 | 217679.0 | 758.5 | 54.1 | 0.46 | <0.001 |
| Total | *F*IT | 573 | 795485.4 | 1403.1 | 100.0 | | |
| ***K* = 4 populations (GERO, JBMP, central, NCMP):** | | | | | | | |
| Among populations | *F*rt | 3 | 15948.1 | 26.6 | 1.9 | 0.02 | >0.001 |
| Among meadows within populations | *F*sr | 24 | 74562.4 | 58.9 | 4.2 | 0.04 | >0.001 |
| Among individuals within meadows | *F*ST | 259 | 487295.9 | 561.5 | 40.0 | 0.43 | >0.001 |
| Within individuals | *F*IS | 287 | 217679.0 | 758.5 | 54.0 | 0.46 | <0.001 |
| Total | *F*IT | 573 | 795485.4 | 1405.4 | 100.0 | | |
| ***K* = 5 populations (GERO, JBMP, MMP, CS + SIMP, NCMP):** | | | | | | | |
| Among populations | *F*rt | 4 | 19941.6 | 22.0 | 1.6 | 0.02 | >0.001 |
| Among meadows within populations | *F*sr | 23 | 70568.9 | 57.6 | 4.1 | 0.04 | >0.001 |
| Among individuals within meadows | *F*ST | 259 | 487295.9 | 561.5 | 40.1 | 0.43 | >0.001 |
| Within individuals | *F*IS | 287 | 217679.0 | 758.5 | 54.2 | 0.46 | <0.001 |
| Total | *F*IT | 573 | 795485.4 | 1399.6 | 100.0 | | |
| **Sampling areas (GERO, JBMP, MMP, CS, SIMP, NCMP; n = 6):** | | | | | | | |
| Among sampling areas | *F*rt | 5 | 24270.6 | 20.9 | 1.5 | 0.01 | >0.001 |
| Among meadows within sampling areas | *F*sr | 22 | 66239.9 | 55.4 | 4.0 | 0.04 | >0.001 |
| Among individuals within meadows | *F*ST | 259 | 487295.9 | 561.5 | 40.2 | 0.43 | >0.001 |
| Within individuals | *F*IS | 287 | 217679.0 | 758.5 | 54.3 | 0.46 | <0.001 |
| Total | *F*IT | 573 | 795485.4 | 1396.2 | 100.0 | | |

## 5.4 Contemporary migration among populations

We detected migration among all *P. sinuosa* populations along the WA coastline, with dispersal in a predominantly northerly direction (Figure 6; Table S2). The northern (Geraldton) and southern (Ngari Capes Marine Park) populations, however, had limited connectivity with central populations, as well as low migration among local populations. Maximum migration was 0.44 between the two Geraldton populations and 0.55 among populations within Ngari Capes Marine Park. The longest distance migration (> 0.5) occurred between Geraldton (BSFD) and Becher Point (BEPO) in Shoalwater Islands Marine Park, with a migration of 0.63 over a distance of 428 km. The northern most meadows at Geraldton (BSFD, POMO) were not well-connected with nearest populations to the south at Jurien Bay Marine Park. Significant (0.80) long distance migration southwards was detected between Jurien Bay Marine Park (JUIS) and Owen Anchorage (S4S2W) over 212 km. Significant southward migration (> 0.6) was also observed among populations in Jurien Bay Marine Park (JUIS and BOUL) and Marmion Marine Park (MULL, NOBE), Cockburn Sound (S4S2W, GRDN) and Shoalwater Islands Marine Park (BEPO).

Populations in the central cluster - Marmion Marine Park (MULL, NOBE), northern Cockburn Sound (S4S2W, CAIS, GRDN) and Shoalwater Islands Marine Park (BEPO, WNBR) were well-connected, with predominantly northernly connections. Populations from central to southern Cockburn Sound were poorly connected (GNDP, KWIN). This included Mangles Bay (MANG), Garden Island Settlement (GDNS) and Point Peron (POPE) in the Shoalwater Island Marine Park which all had connectivity < 0.7 with neighbouring populations, and were thus not connected in Figure 6.

The most significant migration (darker lines) occurred within Jurien Bay Marine Park, and within and among the central populations (Figure 6, Marmion Marine Park, Cockburn Sound and Shoalwater Islands Marine Park). High migration was often detected between nearby populations: between Boulanger Island (BOUL) and Jurien Bay (JUIS) in Jurien Bay Marine Park (BOUL-JUIS = 0.82 over 5 km), between Mullaloo (MULL) and North Beach (NOBE) in the Marmion Marine Park (MULL-NOBE = 0.92 and NOBE-MULL = 0.91 over 11 km), between Carnac Island (CAIS), Woodman Point (WOOD) and Owen Anchorage (S4S2W) in Cockburn Sound (CAIS-S4S2W = 0.86 over 7 km; WOOD-S4S2W = 0.86 over 4 km), and between Warnbro Sound (WNBR, WBDP) and Becher Point (BEPO) in the Shoalwater Islands Marine Park (WNBR-BEPO = 1.0 over 7 km; WBDP-BEPO = 0.96 over 4 km). However, high migration also occurred up to 60 km, between Becher Point (BEPO) in the Shoalwater Islands Marine Park and North Beach (NOBE) in Marmion Marine Park, with a migration of 0.8. Strong northward migration was detected from multiple central populations in Shoalwater Islands Marine Park (WBDP, WNBR) and Cockburn Sound (CSWY, GNDP and CAIS) to North Beach (NOBE) and Mullaloo (MULL) in Marmion Marine Park, inferring dispersal distances up to 66 km (WBDP-MULL). North Beach (NOBE) and Mullaloo (MULL) appear to act as a sink for northward migration, while Becher Point (BEPO) primarily receives southwards migration, mostly from Marmion Marine Park. Owen Anchorage (S4S2W) appears to be acting as a ‘sink’ for both southward and northward migration, although northward migration was stronger and more common.

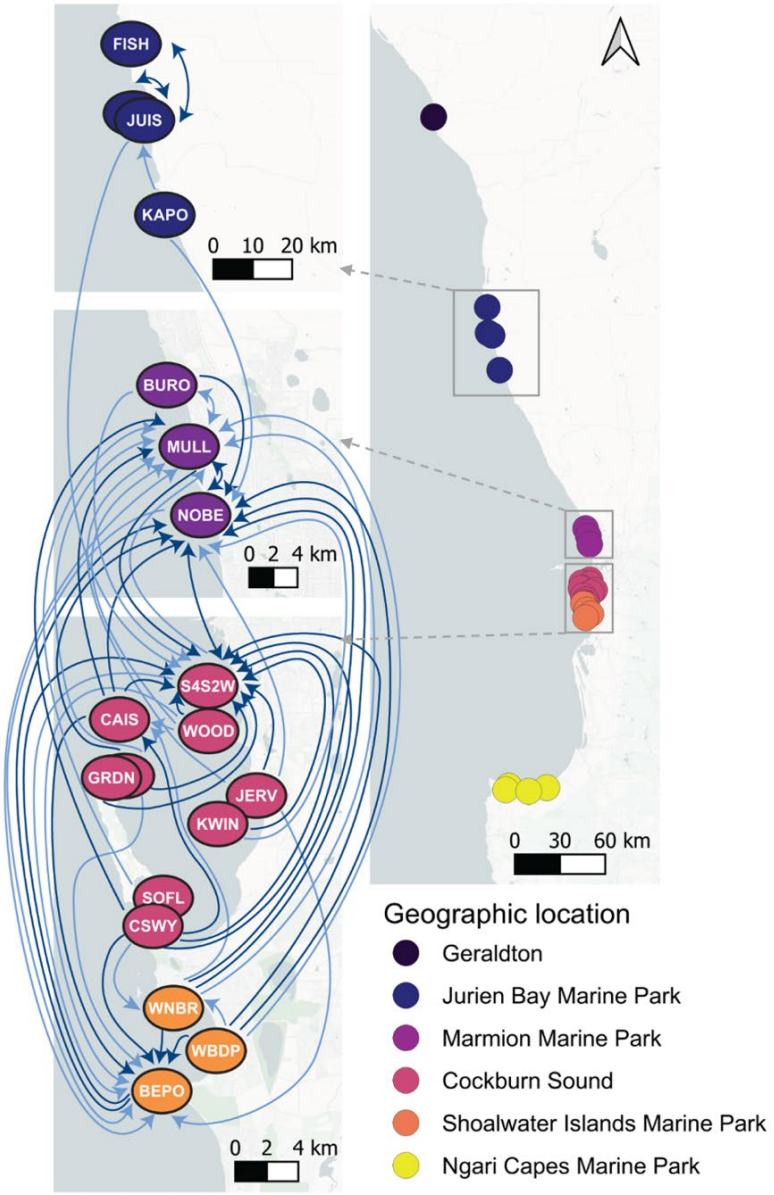


**Figure 6.** Directional migration network among sampled *P. sinuosa* populations. Only populations with significant migration >0.7 are shown in the maps on the left. The map on the right shows all populations that were included in the analysis, with rectangles indicating the locations of the three maps on the left. Populations are indicated by coloured nodes and codes (codes only for populations with migration >0.7) (described in Table 1) and are oriented relative to geographic location. Arrows and lines indicate direction and strength of relative migration rates: dark blue arrows = > 0.80; light blue arrows = 0.7 - 0.79.

A significant Isolation by distance (IBD) relationship was detected among *P. sinuosa* meadows along the WA coastline (Figure 5; Mantel statistic = 0.52, p <0.01; Table S2). Patches of high density indicate that there was greater connectivity among meadows that were geographically closer (< ~30 km, Figure 7). However, moderate to long distance dispersal events also occurred at lower frequencies along the broader continental shelf, suggesting connectivity up to ~200 km. Monmonier barrier analysis identified a significant barrier to dispersal in central Cockburn Sound, where there was low to no connectivity between southern Cockburn Sound populations (Garden Island settlement, Southern Flats, Mangles Bay) with other populations to the north (Jervoise Bay, Kwinana, Woodman Point) (Figure S2).

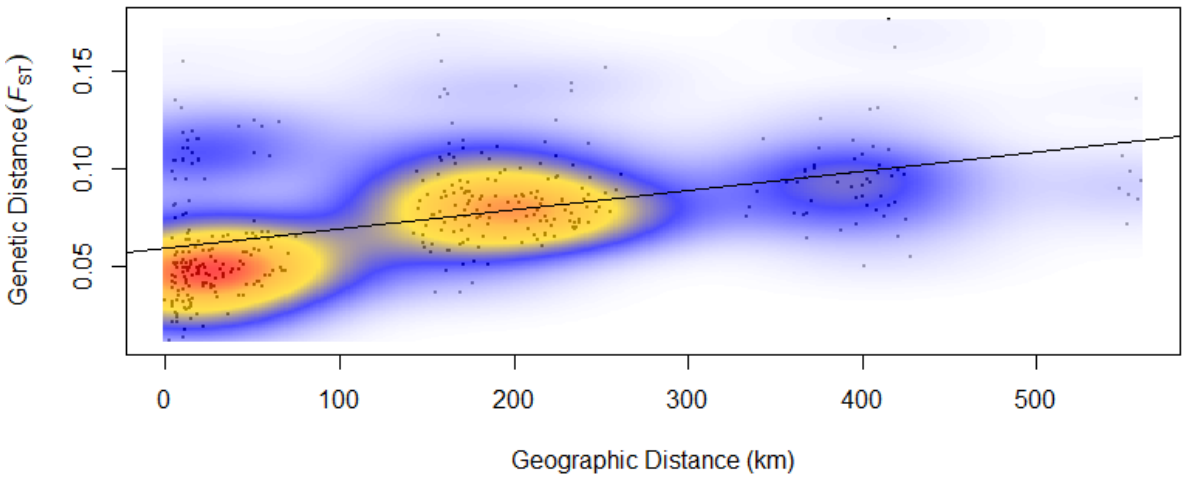


**Figure 7.** Isolation by distance. Heat map of genetic distance ($F_{ST}$) relative to geographic distance (over water >1m depth). Colours represent the relative density of the points; from low (blue) to high (red) density. Pairwise distance is 0 - 560 km.

## 5.5 Genotype by Environment association

Genotype by environment association analyses identified putatively adaptive loci across the eight out of ten environmental variables (Table 4; $SST_{ma}$, $SST_{max}$, $SST_{summer}$, $SST_{min}$, $SST_{winter}$, TAR, kd490, and depth). $SST_{WARMQ}$ and $SST_{COLDQ}$ were dropped from these association tests as they were highly correlated with $SST_{summer}$ and $SST_{winter}$, respectively. A total of 474 out of 7,777 putative adaptive SNP loci were identified across both methods, 368 (4.7 %) using LFMM2 and 247 (3.2 %) using RDA (Table 4, Figure 8). The higher number of loci identified using LFMM2 was likely due to the less conservation nature of the method, despite applying conservative thresholds across multiple environmental. Most environmental associations for univariate LFMM2 and multivariate RDA were associated with mean sea surface temperature in summer ($SST_{summer}$) and mean sea surface temperature in the warmest month ($SST_{max}$; Table 4). $SST_{max}$ exhibited the most unique associations (n = 215) across both methods. Several loci were detected on multiple occasions. 139 out of the 474 (= 29.3%) unique loci were associated with the same environmental variable across both methodologies, with greatest congruence for $SST_{summer}$ (n = 71/163, 43.6 %). Two loci were detected in both methods with different environmental variables, although no gene identification was made. Locus X0041 was significantly associated with mean sea surface temperature of the warmest ($SST_{summer}$; RDA) and coolest month ($SST_{winter}$; LFMM2). Locus X5464 was associated with depth (RDA) and annual range in mean sea surface temperature (TAR; LFMM2).

**Table 4.** Genotype by environment association analyses (GEAs). Number of putative adaptive loci associated with environmental variables using two GEA detection methods: Latent Factor Mixed Model 2 (LFMM2) and Redundancy Analysis (RDA).

| Environmental variable | Abbreviation | No. loci LFMM2 | No. loci RDA | Combined (unique) |
|---|---|---|---|---|
| mean annual sea surface temperature | $SST_{MA}$ (°C) | 115 | 3 | 116 |
| mean sea surface temperature of the warmest month | $SST_{max}$ (°C) | 139 | 97 | 215 |
| mean sea surface temperature of summer | $SST_{summer}$ (°C) | 161 | 73 | 163 |
| mean sea surface temperature of the coolest month | $SST_{min}$ (°C) | 105 | 3 | 105 |
| mean sea surface temperature of winter | $SST_{winter}$ (°C) | 118 | 1 | 118 |
| mean sea surface temperature annual range | TAR (°C) | 113 | 46 | 128 |
| mean water turbidity | kd490 ($m^{-1}$) | 87 | 14 | 93 |
| Depth | Depth (m) | 34 | 10 | 42 |
| **TOTAL (unique)** | | **368** | **247** | **474** |

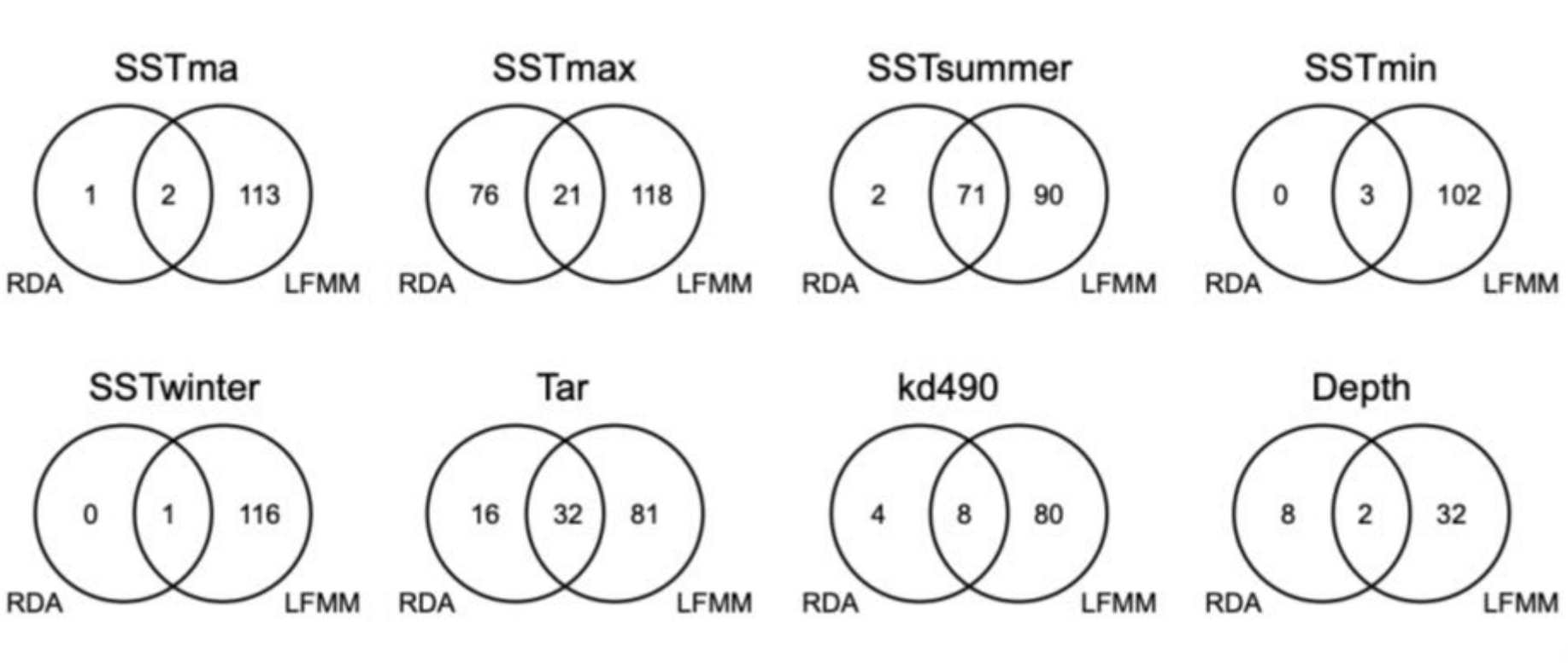


**Figure 8.** Venn diagrams of putatively adaptive loci detected between and among the two genotype by environment association methods (redundancy analysis (RDA) and Latent Factor Mixed Model 2 (LFMM2)) for eight environmental variables: $SST_{ma}$ = mean annual sea surface temperature; $SST_{max}$ = mean maximum sea surface temperature; $SST_{summer}$ = mean austral summer sea surface temperature; $SST_{min}$ = mean minimum sea surface temperature; $SST_{winter}$ = mean Austral winter sea surface temperature; TAR = mean annual range of sea surface temperature; kd490 = turbidity; and depth.

Higher allelic turnover (> 0.25) of putatively adaptive loci in the GDM was found in 33 out of 159 loci (20.8%; Figure 9). The 150 bp *P. sinuosa* sequences were based on partial alignments with the *Arabidopsis thaliana* genome. Of the loci that had a higher turnover across environmental space, locus X5371 exhibited the greatest deviance out of all of the environmental variables and appears to be highly informative in adaptation to warmer sea surface temperatures ($SST_{max}$ > 0.80). Locus X0295 had the highest allelic turnover in four variables ($SST_{ma}$, $SST_{min}$, $SST_{winter}$, Kd490; Table 5). Locus X1496 was observed to have an allelic turnover greater than 0.5 in $SST_{max}$ and $SST_{summer}$.

The locations where seasonal changes in allelic turnover related to temperature (> 0.4) occurred between Jurien Bay Marine Park and Geraldton (Figure 9A-C■: $SST_{max}$ 22.08 – 23.18 °C, $SST_{summer}$ 21.44 – 22.26 °C; $SST_{min}$: 20.20 - 20.35 °C; $SST_{winter}$ 20.02 - 20.30 °C), Perth metropolitan waters and Jurien Bay Marine Park (Figure 9A-E ▲: $SST_{max}$ 21.88 – 22.08 °C; $SST_{summer}$ 21.27 – 21.44 °C; $SST_{min}$: 18.85 - 20.22 °C; $SST_{winter}$ 18.81 - 20.22 °C), and Ngari Capes Marine Park and Perth metropolitan waters (Figure 9A-E ∗ : $SST_{max}$ 20.78 – 21.88 °C; $SST_{summer}$ 20.21 – 21.27 °C; $SST_{min}$: 17.56 – 18.85 °C; $SST_{winter}$ 17.53 – 18.81 °C). For example, for the top loci curves associated with $SST_{ma}$ (Figure 9A), there was a rapid allele frequency change from locations where $SST_{ma}$ is 19 °C up to locations where $SST_{ma}$ is 19.8 °C and above after which allele frequency remained constant. In this case all populations within the Perth metropolitan waters (=Marmion Marine Park, Cockburn Sound, Shoalwater Islands Marine Park), Jurien Bay Marine Park, and Geraldton have a similar allele frequency, whilst Ngari Capes Marine Park was different. In contrast, the bottom curve in Figure 9A shows similar allele frequency from locations with 19 °C up to 20 °C, then the curve increases with increasing temperature. This change occurred at Jurien Bay Marine Park, indicating all locations south of Jurien Bay have a similar allele frequency. These two trends in allelic turnover can be observed in loci for each temperature variable, although the level of turnover differed. There was no latitudinal cline associated with allelic turnover for mean annual range of sea surface temperature (TAR), turbidity (kd490) or depth (Figure 9F-H), although there was an abrupt turnover for locus X6536 for at 4 - 5 m depth (Figure 9H).

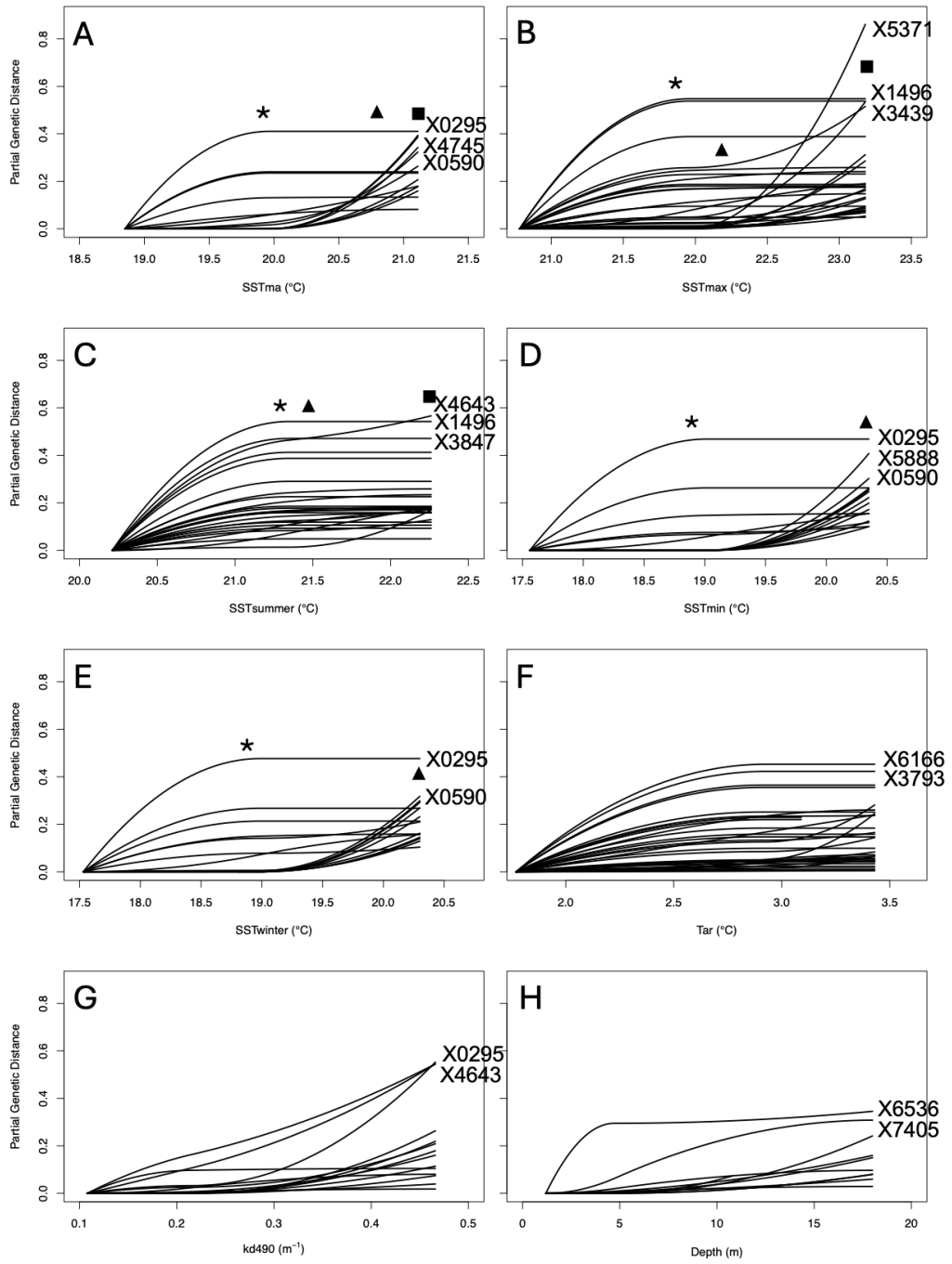


**Figure 9.** Spline plots of allelic turnover across environmental space for (A) mean annual sea surface temperature ($SST_{ma}$), (B) mean sea surface temperature of the warmest month ($SST_{max}$), (C) mean summer sea surface temperature ($SST_{summer}$), (D) mean sea surface temperature of the coolest month ($SST_{min}$), (E) mean winter sea surface temperature ($SST_{winter}$), (F) mean annual range of sea surface temperature (TAR), (G) mean diffuse attenuation coefficient at 490 nm (turbidity; kd490) and (H) depth. Each curve corresponds to a specific locus that has a significant turnover for a particular variable. * = change in allelic turnover differentiates Ngari Capes Marine Park from Perth metropolitan waters populations; ▲ = allelic turnover differentiates Perth metropolitan waters populations from Jurien Bay Marine Park and Geraldton, ■ = allelic turnover differentiates Jurien Bay Marine Park from Geraldton.

Genes were identified for 15 putatively adaptive SNP loci from the *Arabidopsis thaliana* genome (Table 5). Thirteen of the putatively adaptive SNP loci were associated with temperature variables, with four associated with more than one variable. Locus X4745 aligned most significantly with the AT4G20520 protein-coding gene on chromosome 4 in *A. thaliana* (Bit score = 43.7, E value = 0.001). Locus X5371 significantly aligned with an AT Expansin A3 gene on *A. thaliana* chromosome 2 (AT2G37640; Bit score = 40.1, E value = 0.012). Locus X0295 (AT1G11362; Bit score = 38.3, E value = 0.042) was identified as a plant invertase/pectin methyl esterase inhibitor superfamily protein and associated with four environmental variables. Other notable genes identified relate to specific heat shock proteins (X1496, X5888) and metabolic processes associated with plant growth and development (X0590, X3439, X3793, X4643).

**Table 5.** Putatively adaptive genes with loci exhibiting the largest allelic turnover across environmental space and identified against *Arabidopsis thaliana*. AGRF ID = the sequencing fragment identifier provided by AGRF; Locus ID = the sequential locus identifier used by researchers; Chr. = chromosome aligned to *A. thaliana*.

| | | | ***Arabidopsis thaliana* Accession** | | | | |
|---|---|---|---|---|---|---|---|
| **AGRF ID** | **Locus ID** | **Environ. variable** | **Bit score** | **E-value** | **Chr.** | **Position Start/End** | **Gene name and description** |
| 7167 | X0295 | SSTma SSTmin SSTwinter kd490 | 38.3 | 0.042 | 1 | 3824630/ 3825184 | AT1G11362: Plant invertase/pectin methyl esterase inhibitor superfamily protein |
| 112252 | X4745 | SSTma | 43.7 | 0.001 | 4 | 11045912/ 11047716 | AT4G20520: RNA binding/RNA-directed DNA polymerase |
| 12366 | X0590 | SSTma SSTmin SSTwinter | 31.0 | 6.2 | 5 | 5202798/ 5204478 | AT5G15940: NAD(P)-binding Rossmann-fold superfamily protein |
| 151010 | X5371 | SSTmax | 40.1 | 0.012 | 2 | 15788077/ 15789577 | AT2G37640: AT Expansin A3 gene |
| 27226 | X1496 | SSTmax SSTsummer | 31.0 | 6.2 | 5 | 2862719/ 2865359 | AT5G09210: GC-rich sequence DNA binding factor-like protein |
| | | | | | 4 | 6370537/ 6371124 | AT4G10250: ATHSP22 Columbia endomembrane-localised small heat shock protein |
| 67870 | X3439 | SSTmax | 35.6 | 0.15 | 4 | 6024970/ 6026751 | AT4G09520: AHL3 AT hook motif DNA-binding family protein |
| 106437 | X4643 | SSTsummer kd490 | 35.6 | 0.15 | 5 | 20176385/ 20188307 | AT5G49680: conserved among eukaryotes, predicted to be targeted to the secretory pathway |
| 77482 | X3847 | SSTsummer | 32.8 | 1.8 | 5 | 19444313/ 19446970 | AT5G48000: Encodes a member of the CYP708A family of cytochrome P450 enzymes |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 219999 | X5888 | SSTmin | 32.8 | 1.8 | 2 | 10881790/ 10883760 | AT2G25560: DNAJ heat shock N-terminal domain-containing protein |
| 247935 | X6166 | TAR | 35.6 | 0.15 | 3 | 18215788/ 18217848 | AT3G49142: Tetratricopeptide repeat (TPR)-like superfamily protein |
| 76014 | X3793 | TAR | 35.6 | 0.15 | 5 | 15158342/ 15160118 | AT5G38010: UDP-Glycosyltransferase superfamily protein |
| 372110 | X6940 | TAR | 31.0 | 6.2 | 3 | 19925254/ 19926648 | AT3G53780: RHOMBOID-like protein 4 |
| 372110 | X6536 | Depth | 31.9 | 1.8 | 5 | 435322/ 436683 | AT5G02190: Aspartic protease 38, important role in determining cell fate during embryonic development |
| | | | 31.9 | 1.8 | 5 | 1400832/ 1401584 | AT4G03170: AP2/B3-like transcriptional factor family protein |
| 494001 | X7405 | Depth | 31.9 | 1.8 | 3 | 8575268/ 8581001 | AT3G23790: AAE16, AMP-dependent synthetase and ligase family protein involved in fatty acid biosynthesis |
| | | | 31.9 | 1.8 | 1 | 7489385/ 7490554 | AT1G21390: emb2170 |
| | | | 31.9 | 1.8 | 1 | 6207128/ 6209299 | AT1G18040: Cyclin-dependent kinase D1, regulation of cell cycle |
| 38871 | X2057 | Depth | 31.9 | 1.8 | 5 | 23911151/ 23913244 | AT5G59270: L-type lectin receptor kinase 11.2 involved in bacterial defence |
| | | | 31.9 | 1.8 | 3 | 18046527/ 18049295 | AT3G48720: encodes a hydroxycinnamoyl-CoA: v-hydroxy fatty acid transferase involved in cutin synthesis |

# 6 Discussion

A population genomic and genome by environment approach of the IUCN Vulnerable listed seagrass species, *P. sinuosa,* was applied along ~560 km latitudinal gradient of the WA coastline from Geraldton to Geographe Bay. The goal was to inform potential options to build resilience to ocean warming into these populations, particularly in Perth metropolitan waters where urbanisation and further proposed industrial developments may place seagrass meadows under further pressure. Genetic diversity was low, and within populations was similar along the coastline, including a high level of inbreeding detected. The populations were generally genetically well connected via gene flow, with evidence for long distance dispersal predominantly northwards. Significant southwards and northwards migration was detected over smaller spatial scales (~60 km), particularly across Perth metropolitan waters. This evidence of migration supports weak genetic structure among the *P. sinuosa* meadows sampled (global $F_{ST}$ = 0.080) with the most southern region more distinct from those further north. The lack of strong patterns in genetic structure along the coastline, and identification of allelic turnover in putative adaptive loci associated with temperature, provides support for resilience building options, such as translocation through assisted migration or climate adjusted provenancing. Allelic turnover for some of these alleles associated with temperature were detected between Perth metropolitan waters and populations further south, or Perth metropolitan waters and Jurien Bay Marine Park, suggesting these loci may be warm-adapted and good candidates for further studies linked to resilience building.

## 6.1 Population diversity, connectivity, and structure

Similar levels of genetic diversity were present in all *P. sinuosa* populations sampled throughout the latitudinal range, and most of the variation was attributed to within populations. This is in contrast to lower levels of diversity detected in range edge populations including the northern range edge populations for two habitat forming species along the WA coastline, *P. australis* (Sinclair et al., 2023) and the kelp, *Ecklonia radiata* (Wernberg et al., 2018), where genetic diversity (clonal, allelic, heterozygosity) significantly declined at lower latitudes. The highly positive $F_{IS}$ values in all *P. sinuosa* populations were consistent with widespread inbreeding (a result of pollination among closely-related plants within populations), differing from outcrossed *P. australis* populations, possibly assisted via the long inflorescence stems in the latter species (Sinclair et al., 2020). *Posidonia sinuosa* develops fruit on short inflorescence stems which remain within the leaf canopy, compared to *P. australis* where they visibly extend above the leaf canopy (Cambridge and Kuo 1979; Smith and Walker, 2002). The almost complete absence of resampled clones within populations was consistent with a more vertical growth form in *P. sinuosa*, relative to *P. australis* which has extensive horizontal rhizome expansion at tens of metres (e.g. Sinclair et al., 2014; Evans et al., 2014; Sinclair et al., 2023) to > 100 km (Edgeloe et al., 2022). We note that within population sampling distances between *P. sinuosa* shoots were larger than in the *P. australis* studies, thus favouring sampling from different genetic individuals. High clonal diversity highlights the importance of sexual reproduction and dispersal for maintaining seagrass populations (reviewed in Kendrick et al., 2012, 2017). This genetic diversity provides some resilience as populations with a diversity of genotypes may be able to respond to changing or extreme environmental conditions that could mitigate population declines.

We identified a highly connected meta-population over 560 km of coastline. Genetic structure was weaker than expected, as the sister taxon, *P. australis,* sampled over a similar range had much higher differentiation ($F_{ST}$ = 0.268, p < 0.001; Sinclair et al., 2023). We note, however, that these two studies used different genetic markers (SNPs versus microsatellites) with different sampling strategies, although it has been shown that the markers are broadly comparable for assessing population structure (Emanuelli et al., 2013; e.g. Phair et al., 2019). The somewhat contrasting population structure along the WA coastline between persistent *P. australis* and *P. sinuosa* populations is

interesting given the two species have largely overlapping, naturally fragmented ranges, with the ability to disperse fruit via surface currents over 10s – 100s km among meadows (Ruiz-Montoya et al., 2015).

We predicted our study sites would show genetic structure associated with the sampling regions, where *P. sinuosa* generally forms dense meadows in embayments or more protected waters inshore of temperate reefs (northern - Geraldton and Jurien Bay; central – Perth metropolitan waters including Cockburn Sound; southern - Geographe Bay). The weak, but significant genetic structure was in fact detected at all hierarchical levels tested, although it explained < 2 % of the genetic variation at higher levels. Overall structure in *P. sinuosa* more closely resembled the panmictic populations observed in species with pelagic larval dispersal along the WA coastline (e.g. dhufish, *Glaucosoma hebraicum*, Berry et al., 2012; western rock lobster, *Panulirus cygnus,* Kennington et al., 2013), highlighting the important role of the Leeuwin Current, Capes Current and local hydrodynamics play in connecting populations at different spatial scales. Overall, our results indicate that *P. sinuosa* populations along the WA coastline were more genetically connected than *P. australis* meadows. *Posidonia sinuosa* has smaller, narrower fruit (and seeds) relative to *P. australis*, so appear less likely to disperse as far due to windage (Orth, 1999). Buoyant fruits of *P. australis* can disperse 10s to about 100 km among meadows via surface currents and windage (Ruiz-Montoya et al., 2015). These two biological traits (associated pollen and seed dispersal) could lead to less connectivity among *P. sinuosa* populations, although our results do not support this. The more open water sites at Becher Point, Owen Anchorage and Marmion Marine Park show populations were well-connected, although they did not have significantly higher levels of genetic diversity. Thus, local pollination is driving high levels of inbreeding (leading to closely-related genotypes within populations), and occasional recruitment (from dispersing fruit/seeds) has maintained genetic connectivity among populations more broadly, despite successful seedling recruitment known to be a significant bottleneck in seagrasses (Hocking et al., 1980; Statton et al., 2017).

## 6.2 Cockburn Sound area

Genetic structure among *P. sinuosa* populations within the Cockburn Sound area was weak ($F_{ST}$ = 0.057), and similar to the closely related taxon, *P. australis* ($F_{ST}$ = 0.085, Sinclair et al., 2014), although there was no significant north-south pattern in *P. sinuosa*. There was a high level of connectivity among populations across Perth metropolitan waters from Shoalwater Islands Marine Park Marmion Marine Park, over ~70 km of coastline based on migration analysis, with the exception of meadows towards the southern half of Cockburn Sound. This indicates the species has been effective at dispersing fruit across this region over time. A predominantly northward dispersal pattern was consistent with modelling of *P. australis* fruit dispersal using a particle-tracking model developed by Ruiz-Montoya et al. (2015). Ruiz-Montoya et al. (2012) reported 75 % of *P. australis* fruit dehisced within four days of being released, with long distance dispersal events were detected at > 100 km (Ruiz-Montoya et al., 2015). *Posidonia australis* shares a similar life history to *P. sinuosa*, with floating fruit dehisced in late spring to early summer, although *P. sinuosa* fruit (and seeds) are smaller and narrower, relative to *P. australis*, so are maybe less likely to disperse as far due to windage (Orth, 1999).

The most genetically differentiated populations of *P. sinuosa* were Owen Anchorage and Garden Island settlement, different to *P. australis* with Mangles Bay and Carnac Island most distinct(Sinclair et al., 2014, 2018). Mangles Bay had the lowest genetic diversity for *P. australis* (Sinclair et al., 2014), but this was not the case for *P. sinuosa* where all meadows had similar levels of genetic diversity. Mangles Bay is a sheltered site at the southern end of Cockburn Sound where water movement is restricted (Steedman and Craig, 1983) and a low probability of connectivity was detected, into/out of southern Cockburn Sound, as was the case with *P. australis* (Ruiz-Montoya et al., 2015; Sinclair et al.,

2018). Low to no connectivity, combined with the high epiphytic algal cover and long-term decline of seagrass in Mangles Bay (Webster et al., 2024) may reduce the likelihood of healthy *Posidonia* meadows in the future. Mangles Bay is a potential site for resilience building interventions where short-term mitigation efforts, such as the translocation of genotypes to increase genomic diversity could be employed and be successful, though long-term monitoring, quantification, and validation will be required.

## 6.3 Local adaptation

Plants are sessile organisms, so the need to adapt to changing environments is critical. Thirteen of the putatively adaptive loci detected in *P. sinuosa,* were associated with *A. thaliana* annotated genes related to temperature, consistent with temperature being one of the most important factors associated with climate change in the marine environment (Duarte et al., 2018). Some of these genes come from large ancient families present across the plant kingdom, for example, the *AT-hook motif* gene family (AT4G09520; Zhang et al., 2022), the plant invertase/Pectin methylesterase inhibitor superfamily (AT1G11362; Coculo and Lionetti, 2022) and the Cytochromes P450 (AT5G48000; Hansen et al., 2021). This work contributes to an increasing number of studies exploring a genetic basis of local adaptation in seagrasses (e.g. Lauritano et al. 2015; Phair et al., 2019; Nguyen et al., 2023; Nimbs et al., 2024; Sotka et al., 2024). Our results provide evidence to justify exploring the potential for resilience building options in southern *P. sinousa* populations to future-proof against ocean-warming and heatwaves. Putatively adaptive loci (< 4.7 %) were detected in *P. sinuosa*, despite high levels of gene flow across the seascape that can counteract selection. It is not uncommon for adaptation of populations within a species to their local environments to occur with high gene flow (Savolainen et al, 2007; Tigano and Friesen, 2016). Empirical studies are needed to determine if the genetic variation is truly adaptive and provide insights into adaptation and potential drivers of selection (Rellstab et al., 2015).

Physiological assessments of *P. sinuosa* along this latitudinal gradient have identified higher thermal optima in Geraldton and Jurien Bay Marine Park populations relative to Cockburn Sound (Said et al., 2024), supporting inferences from the genome by environment analysis. The thermal optimum for *P. sinuosa* populations between Geraldton and Ngari Capes Marine Park varied by ~4 °C ($T_{opt}$ = 26.5 – 30.7 °C; Said et al., 2024). Interestingly, Cockburn Sound (Perth metropolitan waters) had the lowest optimum temperature ($T_{opt}$ = 26.5 °C), and was the location of a significant barrier to gene flow and allelic turnover. The thermal optima from Said et al. (2024) were warmer than the published thermal optima for *P. sinuosa* from Marmion Marine Park almost 25 years earlier ($T_{opt}$ = 18 – 23 °C; Masini and Manning,1997). However, 23 °C was the maximum temperature tested by Masini and Manning (1997), while Said et al. (2024) generated thermal performance curves for *P. sinuosa* plants between 15 – 40 °C. The thermal optimum temperatures from Said et al. (2024) were also higher than current mean sea surface temperatures of the warmest month and mean sea surface temperature for summer, suggesting that populations may be able to tolerate future projected temperatures up to a point, but are unlikely to tolerate current projections which equate to +3.2 °C by 2100 (IPCC, 2023). The additional temperature associated with extreme marine heat waves (e.g. +2 - 4 °C Wernberg et al., 2013) would likely lead to breakdown of cellular processes, such as photosynthesis, ATP synthesis, and respiration, leading to canopy loss and death (e.g. Rassmusson et al., 2020). A mesocosm experiment conducted using *P. australis* seedlings collected from Woodman Point in Cockburn Sound, showed that exposing dehisced seedlings to thermal priming of 26 °C (exposure to 5 °C above local spring temperatures) prior to planting did not improve survival or growth rates of seedlings growing in warmer conditions (Whale et al., 2024b). This result suggests that thermal priming may not be a useful strategy to assist with climate change adaptation in Australian *Posidonia spp*., highlighting the importance of incorporating remaining natural seagrass meadows that are already warm-adapted into resilience building strategies. This could be through transplantation of seeds or vegetative units.

Further research is required to explore these opportunities. Of relevance is the impact of warming on seed production in *Posidonia.* Little is known about the triggers of floral development (leading to reproduction) in *Posidonia spp.*, although it coincides with cooler temperatures and shortening day length in the Austral autumn (McComb et al., 1981). A negative impact of warmer ocean temperatures on flowering and seed production is potentially reduced opportunities for seed-based restoration, and less seed production could also have implications for genetic connectivity among the naturally fragmented *P. sinuosa* populations along the WA coastline, with reduced connectivity (reviewed in Kendrick et al., 2017).

The lack of annotated seagrass genomes created challenges for gene coding regions and gene function identification. However, coding regions and gene functions associated with temperature, turbidity and depth were identified, suggesting that environmental adaptation can be inferred for non-model organisms (seagrasses), even when compared to the unrelated model organism *A. thaliana.* Locus X0295 (AT1G11362) was identified as a plant invertase/pectin methyl esterase inhibitor which belongs to a large protein superfamily with distinct enzymatic activities in carbohydrate metabolism (Cucolo and Lionetti, 2022), yet it was associated with temperature and turbidity variables. There is limited work on adaptation in seagrasses, but gene expression profiles in the mediterranean seagrass *Posidonia oceanica* suggested locally adapted genotypes were linked to a depth-light-temperature relationship (Proccacini et al., 2017). Two loci with allelic turnover, locus X5888 ($SST_{min}$) between Perth metropolitan waters and Jurien Bay Marine Park and locus X1496 ($SST_{max}$, $SST_{summer}$) between Ngari Capes Marine Park and Perth metropolitan waters, were identified as heat shock proteins. The temperature differences associated with these allelic turnovers were less than 1.4 °C. Small plant heat shock proteins are ubiquitous, acting as molecular chaperones to protect other proteins from stress-induced damage (Waters and Vierling, 2020; e.g. Lauritano et al., 2015). They are also sensitive to minor changes and Bergman et al. (2010) suggest they are suitable as early-warning bio-indicators of seagrass health.

There are many physiological processes associated with temperature adaptation in plants (Allen et al., 2010), that are likely determined by loci of small effect to mitigate the risk of extinction (Gomulkiewicz et al., 2010). We suggest that these processes will be complex in marine systems, as multiple genes were identified in temperature regulation in *P. sinuosa* to maintain plant function and growth and development. The loci detected in this study may be a small part in the overall adaptation in the *P. sinuosa* genome. The detection of these loci provides inferences of environmental adaptation, and further experimental evidence will be required to validate this relationship. We note that the large (4-5 km pixel resolution) for environmental data was unlikely to pick up fine scale variation, particularly associated with turbidity (kd490) or depth (m). Depth (as a proxy for light) is an import factor for seagrass growth and persistence, and the depth range sampled here (< 20 m) was well within the normal range for seagrass meadows (in the absence of disturbance events).

Resilience building options such as assisted (gene) migration to supplement cool-origin populations with individuals of warmer-origin is promising but there are potential downsides to consider. A major concern is that this activity could lead to outbreeding depression of maladapted alleles in the cool-origin populations due to translocating individuals from the warmer origin (Broadhurst et al., 2008; Breed et al., 2018). It is likely that any benefits of assisted migration would outweigh any negative impacts into the recipient population, since both neutral and adaptive genomic diversity would be enhanced. *In situ* common garden experiments using potential donor populations can determine whether assisted gene migration would be viable. The assisted migration future-proofing strategy could be deployed between populations exhibiting similar genomic characteristics, such as Jurien Bay Marine Park and Cockburn Sound, to value-add to existing restoration approaches, such as 'Seeds for Snapper' (Sinclair et al., 2021) or develop new programs to supplement genetic diversity and future-proof less well-connected populations in southern Cockburn Sound.

# 7 Conclusions/recommendations

Overall, we highlight the importance of using genomic tools in determining genetic diversity, connectivity and assessment of putative adaptation in seagrass meadows. Genomic data can inform decision-making for conservation and restoration efforts by characterising gene flow among meadows, and detecting where, or which populations are genetically distinct. Here, we found similar levels of genetic diversity and inbreeding among all sampled *Posidonia sinuosa* populations along the WA coastline, with evidence of some weak population structure. We highlight high connectivity among central populations, but also evidence of migration among three geographic clusters. Most putatively adaptive genetic markers detected were associated with temperature variables that may be important for this species and may also indicate the presence of local adaptation in warmer populations. We suggest that translocations of warmer-adapted genotypes between populations that have exhibited historic connectivity would be a viable strategy for future conservation to build resilience in *Posidonia sinuosa.*

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# 10 Appendices

**Table S1. Genetic and geographic distance.** Pairwise genetic ($F_{ST}$, lower triangular matrix) and geographic over-water distance (km, upper triangular matrix) among sampled *P. sinuosa* populations (population codes described in Table 1). All pairwise $F_{ST}$ values were significantly greater than zero ($p < 0.05$). Shading indicates lower (light blue) to higher (dark blue) differentation.

| Site | BSFD | POMO | FISH | BOUL | JUIS | KAPO | BURO | MULL | NOBE | S4S2W | CAIS | WOOD | GNDP | GRDN | JERV | KWIN | GDNS | SOFL | CSWY | MANG | POPE | WNBR | WBDP | BEPO | ODUN | VAWO | DUBO | MBRD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BSFD | | 4 | 166 | 185 | 190 | 218 | 361 | 366 | 371 | 397 | 400 | 401 | 403 | 403 | 410 | 408 | 409 | 413 | 415 | 419 | 419 | 421 | 425 | 428 | 552 | 560 | 553 | 558 |
| POMO | 0.022 | | 163 | 182 | 186 | 215 | 357 | 363 | 367 | 394 | 396 | 397 | 400 | 400 | 406 | 405 | 405 | 409 | 411 | 415 | 415 | 417 | 421 | 424 | 548 | 557 | 549 | 554 |
| FISH | 0.105 | 0.107 | | 19 | 24 | 52 | 195 | 201 | 205 | 232 | 234 | 235 | 238 | 238 | 244 | 243 | 243 | 247 | 249 | 253 | 253 | 255 | 259 | 262 | 386 | 395 | 388 | 393 |
| BOUL | 0.095 | 0.108 | 0.047 | | 5 | 33 | 175 | 181 | 186 | 212 | 214 | 216 | 218 | 218 | 225 | 223 | 224 | 228 | 230 | 234 | 233 | 236 | 240 | 243 | 367 | 376 | 368 | 373 |
| JUIS | 0.093 | 0.105 | 0.042 | 0.052 | | 33 | 175 | 181 | 186 | 212 | 214 | 215 | 218 | 218 | 224 | 223 | 224 | 228 | 230 | 233 | 233 | 235 | 239 | 242 | 367 | 375 | 368 | 373 |
| KAPO | 0.076 | 0.103 | 0.065 | 0.045 | 0.065 | | 143 | 148 | 153 | 179 | 182 | 183 | 185 | 185 | 192 | 190 | 191 | 195 | 197 | 201 | 201 | 203 | 207 | 210 | 334 | 343 | 335 | 340 |
| BURO | 0.077 | 0.093 | 0.099 | 0.088 | 0.088 | 0.067 | | 10 | 16 | 43 | 47 | 47 | 51 | 51 | 56 | 54 | 56 | 59 | 61 | 65 | 65 | 67 | 71 | 75 | 219 | 218 | 222 | 220 |
| MULL | 0.076 | 0.093 | 0.086 | 0.076 | 0.084 | 0.066 | 0.054 | | 11 | 38 | 42 | 42 | 45 | 45 | 50 | 49 | 51 | 54 | 56 | 59 | 60 | 62 | 66 | 69 | 214 | 213 | 216 | 215 |
| NOBE | 0.083 | 0.095 | 0.087 | 0.081 | 0.075 | 0.077 | 0.045 | 0.029 | | 28 | 33 | 32 | 36 | 36 | 41 | 39 | 42 | 45 | 47 | 51 | 51 | 53 | 57 | 60 | 205 | 204 | 208 | 206 |
| S4S2W | 0.071 | 0.091 | 0.073 | 0.076 | 0.066 | 0.079 | 0.053 | 0.047 | 0.040 | | 7 | 4 | 10 | 10 | 13 | 11 | 14 | 17 | 19 | 23 | 23 | 26 | 30 | 33 | 177 | 176 | 180 | 179 |
| CAIS | 0.092 | 0.105 | 0.102 | 0.078 | 0.089 | 0.086 | 0.067 | 0.049 | 0.046 | 0.052 | | 6 | 4 | 4 | 13 | 10 | 10 | 14 | 15 | 19 | 19 | 22 | 26 | 29 | 172 | 171 | 175 | 174 |
| WOOD | 0.051 | 0.085 | 0.073 | 0.084 | 0.073 | 0.065 | 0.057 | 0.035 | 0.049 | 0.031 | 0.041 | | 7 | 7 | 9 | 7 | 10 | 14 | 15 | 19 | 19 | 22 | 26 | 29 | 174 | 172 | 176 | 175 |
| GNDP | 0.081 | 0.086 | 0.074 | 0.067 | 0.067 | 0.051 | 0.041 | 0.035 | 0.025 | 0.014 | 0.045 | 0.025 | | 0 | 12 | 7 | 6 | 10 | 12 | 15 | 16 | 18 | 22 | 25 | 169 | 167 | 171 | 170 |
| GRDN | 0.094 | 0.107 | 0.073 | 0.067 | 0.066 | 0.072 | 0.073 | 0.058 | 0.066 | 0.047 | 0.064 | 0.058 | 0.033 | | 12 | 7 | 6 | 10 | 12 | 15 | 16 | 18 | 22 | 25 | 169 | 167 | 171 | 170 |
| JERV | 0.102 | 0.111 | 0.096 | 0.096 | 0.096 | 0.098 | 0.067 | 0.066 | 0.063 | 0.048 | 0.067 | 0.052 | 0.042 | 0.077 | | 6 | 10 | 12 | 14 | 17 | 18 | 20 | 24 | 27 | 172 | 171 | 174 | 173 |
| KWIN | 0.079 | 0.093 | 0.073 | 0.074 | 0.066 | 0.086 | 0.058 | 0.056 | 0.048 | 0.017 | 0.057 | 0.026 | 0.024 | 0.050 | 0.056 | | 5 | 6 | 8 | 12 | 13 | 14 | 18 | 22 | 166 | 165 | 169 | 167 |
| GDNS | 0.131 | 0.130 | 0.113 | 0.125 | 0.106 | 0.111 | 0.122 | 0.108 | 0.121 | 0.084 | 0.118 | 0.077 | 0.095 | 0.075 | 0.110 | 0.082 | | 4 | 6 | 10 | 10 | 12 | 16 | 19 | 163 | 162 | 166 | 165 |
| SOFL | 0.075 | 0.097 | 0.078 | 0.084 | 0.084 | 0.064 | 0.068 | 0.042 | 0.051 | 0.053 | 0.049 | 0.033 | 0.032 | 0.049 | 0.063 | 0.051 | 0.099 | | 2 | 6 | 6 | 8 | 12 | 15 | 160 | 159 | 163 | 161 |
| CSWY | 0.081 | 0.086 | 0.081 | 0.079 | 0.079 | 0.082 | 0.060 | 0.040 | 0.040 | 0.046 | 0.029 | 0.031 | 0.028 | 0.043 | 0.058 | 0.037 | 0.082 | 0.012 | | 4 | 5 | 6 | 10 | 14 | 158 | 157 | 161 | 159 |
| MANG | 0.083 | 0.110 | 0.088 | 0.070 | 0.077 | 0.073 | 0.066 | 0.064 | 0.059 | 0.049 | 0.063 | 0.049 | 0.023 | 0.052 | 0.060 | 0.052 | 0.105 | 0.044 | 0.042 | | 9 | 7 | 11 | 14 | 159 | 158 | 162 | 160 |
| POPE | 0.162 | 0.176 | 0.152 | 0.140 | 0.144 | 0.142 | 0.124 | 0.107 | 0.124 | 0.095 | 0.115 | 0.105 | 0.108 | 0.119 | 0.115 | 0.115 | 0.155 | 0.135 | 0.104 | 0.131 | | 6 | 10 | 13 | 158 | 157 | 160 | 159 |
| WNBR | 0.066 | 0.096 | 0.078 | 0.070 | 0.069 | 0.082 | 0.048 | 0.048 | 0.042 | 0.041 | 0.042 | 0.040 | 0.025 | 0.046 | 0.050 | 0.040 | 0.094 | 0.035 | 0.027 | 0.033 | 0.104 | | 4 | 7 | 153 | 151 | 155 | 154 |
| WBDP | 0.074 | 0.099 | 0.089 | 0.084 | 0.090 | 0.076 | 0.057 | 0.049 | 0.046 | 0.034 | 0.055 | 0.039 | 0.019 | 0.058 | 0.047 | 0.049 | 0.103 | 0.050 | 0.038 | 0.044 | 0.104 | 0.030 | | 4 | 149 | 148 | 152 | 150 |
| BEPO | 0.055 | 0.097 | 0.097 | 0.087 | 0.081 | 0.087 | 0.066 | 0.048 | 0.045 | 0.051 | 0.052 | 0.037 | 0.045 | 0.058 | 0.065 | 0.051 | 0.104 | 0.051 | 0.029 | 0.063 | 0.110 | 0.022 | 0.032 | | 145 | 144 | 148 | 146 |
| ODUN | 0.072 | 0.090 | 0.093 | 0.099 | 0.099 | 0.076 | 0.074 | 0.064 | 0.062 | 0.053 | 0.079 | 0.064 | 0.037 | 0.094 | 0.087 | 0.068 | 0.113 | 0.073 | 0.078 | 0.068 | 0.137 | 0.061 | 0.059 | 0.080 | | 27 | 4 | 13 |
| VAWO | 0.093 | 0.136 | 0.115 | 0.125 | 0.111 | 0.115 | 0.114 | 0.085 | 0.100 | 0.086 | 0.114 | 0.076 | 0.081 | 0.103 | 0.111 | 0.091 | 0.138 | 0.104 | 0.097 | 0.106 | 0.169 | 0.086 | 0.094 | 0.099 | 0.059 | | 29 | 14 |
| DUBO | 0.087 | 0.107 | 0.069 | 0.077 | 0.064 | 0.080 | 0.071 | 0.060 | 0.069 | 0.060 | 0.084 | 0.041 | 0.051 | 0.062 | 0.082 | 0.062 | 0.088 | 0.058 | 0.048 | 0.058 | 0.141 | 0.036 | 0.057 | 0.062 | 0.039 | 0.044 | | 16 |
| MBRD | 0.084 | 0.099 | 0.098 | 0.101 | 0.100 | 0.088 | 0.095 | 0.082 | 0.086 | 0.075 | 0.093 | 0.071 | 0.068 | 0.090 | 0.088 | 0.074 | 0.123 | 0.086 | 0.085 | 0.089 | 0.155 | 0.076 | 0.071 | 0.083 | 0.036 | 0.068 | 0.034 | |

**Table S2**. **Directional migration.** Directional migration among *P. sinuosa* populations following the *divMigrate* analysis using genetic distance ($G_{ST}$). Population codes described in Table 1. Populations are orientated in a north to south direction, with the direction of migration from source (left column) and receiving populations (top row). Cell colour indicates the strength of the migration: light blue = 0.50 - 0.59, mid blue = 0.60 - 0.69, royal blue = 0.70 - 0.79, dark blue > 0.80. All migration values were significant after 1000 bootstraps ($p < 0.05$).

| Site | BSFD | POMO | FISH | BOUL | JUIS | KAPO | BURO | MULL | NOBE | S4S2W | CAIS | WOOD | GNDP | GRDN | JERV | KWIN | GDNS | SOFL | CSWY | MANG | POPE | WNBR | WBDP | BEPO | ODUN | VAWO | DUBO | MBRD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BSFD | | 0.44 | 0.34 | 0.44 | 0.49 | 0.38 | 0.37 | 0.54 | 0.54 | 0.54 | 0.47 | 0.33 | 0.34 | 0.45 | 0.42 | 0.37 | 0.29 | 0.25 | 0.41 | 0.25 | 0.33 | 0.47 | 0.41 | 0.63 | 0.39 | 0.26 | 0.28 | 0.35 |
| POMO | 0.28 | | 0.36 | 0.46 | 0.56 | 0.40 | 0.36 | 0.55 | 0.57 | 0.54 | 0.44 | 0.30 | 0.34 | 0.47 | 0.42 | 0.37 | 0.31 | 0.25 | 0.42 | 0.24 | 0.32 | 0.43 | 0.42 | 0.54 | 0.42 | 0.25 | 0.28 | 0.34 |
| FISH | 0.21 | 0.30 | | 0.64 | 0.89 | 0.44 | 0.36 | 0.59 | 0.55 | 0.66 | 0.47 | 0.39 | 0.37 | 0.56 | 0.45 | 0.40 | 0.34 | 0.28 | 0.43 | 0.27 | 0.38 | 0.45 | 0.41 | 0.53 | 0.39 | 0.27 | 0.34 | 0.35 |
| BOUL | 0.22 | 0.30 | 0.51 | | 0.82 | 0.46 | 0.38 | 0.62 | 0.60 | 0.70 | 0.53 | 0.34 | 0.36 | 0.60 | 0.46 | 0.43 | 0.33 | 0.26 | 0.42 | 0.29 | 0.42 | 0.51 | 0.40 | 0.56 | 0.40 | 0.26 | 0.32 | 0.35 |
| JUIS | 0.22 | 0.32 | 0.53 | 0.64 | | 0.43 | 0.41 | 0.65 | 0.69 | 0.80 | 0.57 | 0.34 | 0.40 | 0.66 | 0.50 | 0.47 | 0.33 | 0.26 | 0.47 | 0.27 | 0.42 | 0.52 | 0.46 | 0.65 | 0.43 | 0.27 | 0.35 | 0.34 |
| KAPO | 0.24 | 0.35 | 0.46 | 0.65 | 0.73 | | 0.44 | 0.67 | 0.70 | 0.69 | 0.59 | 0.37 | 0.42 | 0.59 | 0.54 | 0.43 | 0.34 | 0.29 | 0.52 | 0.29 | 0.40 | 0.53 | 0.51 | 0.66 | 0.46 | 0.27 | 0.32 | 0.37 |
| BURO | 0.25 | 0.33 | 0.41 | 0.57 | 0.61 | 0.48 | | 0.74 | 0.85 | 0.78 | 0.58 | 0.39 | 0.43 | 0.59 | 0.60 | 0.46 | 0.38 | 0.30 | 0.53 | 0.29 | 0.43 | 0.59 | 0.55 | 0.68 | 0.49 | 0.30 | 0.33 | 0.38 |
| MULL | 0.22 | 0.31 | 0.40 | 0.54 | 0.62 | 0.40 | 0.43 | | 0.92 | 0.85 | 0.69 | 0.39 | 0.43 | 0.64 | 0.60 | 0.49 | 0.32 | 0.31 | 0.56 | 0.30 | 0.48 | 0.57 | 0.53 | 0.78 | 0.50 | 0.28 | 0.34 | 0.35 |
| NOBE | 0.22 | 0.30 | 0.39 | 0.54 | 0.64 | 0.44 | 0.48 | 0.91 | | 0.87 | 0.68 | 0.36 | 0.46 | 0.61 | 0.59 | 0.51 | 0.30 | 0.28 | 0.57 | 0.29 | 0.44 | 0.60 | 0.53 | 0.79 | 0.50 | 0.28 | 0.32 | 0.34 |
| S4S2W | 0.23 | 0.32 | 0.42 | 0.50 | 0.70 | 0.37 | 0.42 | 0.75 | 0.88 | | 0.67 | 0.39 | 0.50 | 0.66 | 0.67 | 0.61 | 0.35 | 0.27 | 0.51 | 0.29 | 0.53 | 0.59 | 0.54 | 0.74 | 0.55 | 0.28 | 0.32 | 0.37 |
| CAIS | 0.24 | 0.33 | 0.38 | 0.56 | 0.62 | 0.44 | 0.45 | 0.86 | 0.88 | 0.86 | | 0.40 | 0.46 | 0.68 | 0.63 | 0.51 | 0.32 | 0.32 | 0.68 | 0.32 | 0.53 | 0.65 | 0.53 | 0.82 | 0.47 | 0.29 | 0.32 | 0.36 |
| WOOD | 0.24 | 0.31 | 0.44 | 0.51 | 0.62 | 0.40 | 0.45 | 0.78 | 0.67 | 0.86 | 0.70 | | 0.44 | 0.59 | 0.62 | 0.53 | 0.39 | 0.31 | 0.63 | 0.31 | 0.46 | 0.65 | 0.55 | 0.77 | 0.47 | 0.30 | 0.35 | 0.39 |
| GNDP | 0.23 | 0.33 | 0.43 | 0.51 | 0.65 | 0.44 | 0.45 | 0.76 | 0.82 | 0.93 | 0.67 | 0.40 | | 0.64 | 0.62 | 0.54 | 0.33 | 0.29 | 0.58 | 0.32 | 0.45 | 0.67 | 0.59 | 0.74 | 0.55 | 0.28 | 0.34 | 0.40 |
| GRDN | 0.20 | 0.28 | 0.43 | 0.50 | 0.68 | 0.38 | 0.36 | 0.68 | 0.61 | 0.81 | 0.54 | 0.32 | 0.40 | | 0.48 | 0.45 | 0.35 | 0.27 | 0.49 | 0.29 | 0.45 | 0.52 | 0.44 | 0.65 | 0.38 | 0.26 | 0.31 | 0.31 |
| JERV | 0.22 | 0.30 | 0.39 | 0.53 | 0.63 | 0.40 | 0.44 | 0.73 | 0.74 | 0.86 | 0.63 | 0.37 | 0.44 | 0.62 | | 0.51 | 0.33 | 0.28 | 0.55 | 0.30 | 0.50 | 0.59 | 0.57 | 0.74 | 0.47 | 0.27 | 0.31 | 0.37 |
| KWIN | 0.22 | 0.30 | 0.40 | 0.48 | 0.61 | 0.36 | 0.40 | 0.67 | 0.73 | 0.90 | 0.57 | 0.37 | 0.43 | 0.60 | 0.57 | | 0.34 | 0.27 | 0.53 | 0.28 | 0.45 | 0.54 | 0.46 | 0.68 | 0.45 | 0.27 | 0.31 | 0.35 |
| GDNS | 0.19 | 0.26 | 0.39 | 0.44 | 0.57 | 0.39 | 0.33 | 0.55 | 0.44 | 0.66 | 0.42 | 0.35 | 0.32 | 0.56 | 0.45 | 0.37 | | 0.27 | 0.42 | 0.26 | 0.39 | 0.42 | 0.39 | 0.51 | 0.36 | 0.25 | 0.30 | 0.31 |
| SOFL | 0.22 | 0.31 | 0.45 | 0.45 | 0.60 | 0.44 | 0.42 | 0.74 | 0.59 | 0.68 | 0.62 | 0.41 | 0.40 | 0.59 | 0.57 | 0.42 | 0.39 | | 0.67 | 0.32 | 0.36 | 0.56 | 0.55 | 0.65 | 0.40 | 0.27 | 0.34 | 0.35 |
| CSWY | 0.23 | 0.33 | 0.41 | 0.52 | 0.63 | 0.43 | 0.46 | 0.85 | 0.81 | 0.83 | 0.83 | 0.42 | 0.44 | 0.66 | 0.63 | 0.52 | 0.34 | 0.36 | | 0.33 | 0.48 | 0.71 | 0.55 | 0.85 | 0.46 | 0.28 | 0.34 | 0.35 |
| MANG | 0.24 | 0.29 | 0.38 | 0.49 | 0.55 | 0.39 | 0.40 | 0.68 | 0.64 | 0.68 | 0.64 | 0.39 | 0.43 | 0.56 | 0.56 | 0.43 | 0.32 | 0.30 | 0.55 | | 0.41 | 0.64 | 0.50 | 0.64 | 0.46 | 0.26 | 0.32 | 0.35 |
| POPE | 0.18 | 0.25 | 0.33 | 0.41 | 0.45 | 0.30 | 0.33 | 0.62 | 0.54 | 0.66 | 0.50 | 0.33 | 0.34 | 0.44 | 0.48 | 0.40 | 0.29 | 0.23 | 0.42 | 0.25 | | 0.47 | 0.40 | 0.61 | 0.38 | 0.23 | 0.26 | 0.27 |
| WNBR | 0.25 | 0.32 | 0.43 | 0.59 | 0.69 | 0.45 | 0.46 | 0.77 | 0.87 | 0.91 | 0.73 | 0.41 | 0.53 | 0.68 | 0.63 | 0.52 | 0.33 | 0.31 | 0.67 | 0.34 | 0.48 | | 0.61 | 1.00 | 0.50 | 0.29 | 0.38 | 0.37 |
| WBDP | 0.24 | 0.34 | 0.41 | 0.53 | 0.66 | 0.46 | 0.49 | 0.79 | 0.90 | 0.90 | 0.66 | 0.42 | 0.53 | 0.68 | 0.67 | 0.54 | 0.37 | 0.33 | 0.60 | 0.32 | 0.49 | 0.71 | | 0.96 | 0.56 | 0.28 | 0.36 | 0.40 |
| BEPO | 0.24 | 0.31 | 0.36 | 0.50 | 0.58 | 0.40 | 0.40 | 0.76 | 0.80 | 0.81 | 0.62 | 0.39 | 0.41 | 0.62 | 0.56 | 0.48 | 0.33 | 0.29 | 0.59 | 0.28 | 0.46 | 0.64 | 0.56 | | 0.45 | 0.27 | 0.31 | 0.34 |
| ODUN | 0.21 | 0.30 | 0.36 | 0.43 | 0.51 | 0.38 | 0.38 | 0.58 | 0.65 | 0.64 | 0.50 | 0.32 | 0.40 | 0.45 | 0.47 | 0.41 | 0.28 | 0.25 | 0.42 | 0.25 | 0.37 | 0.49 | 0.46 | 0.57 | | 0.30 | 0.34 | 0.41 |
| VAWO | 0.20 | 0.27 | 0.33 | 0.39 | 0.45 | 0.35 | 0.37 | 0.52 | 0.51 | 0.55 | 0.42 | 0.34 | 0.33 | 0.40 | 0.44 | 0.36 | 0.29 | 0.25 | 0.40 | 0.23 | 0.33 | 0.45 | 0.40 | 0.51 | 0.45 | | 0.35 | 0.40 |
| DUBO | 0.20 | 0.28 | 0.39 | 0.44 | 0.56 | 0.34 | 0.35 | 0.55 | 0.54 | 0.56 | 0.47 | 0.33 | 0.37 | 0.46 | 0.42 | 0.37 | 0.30 | 0.26 | 0.44 | 0.25 | 0.35 | 0.52 | 0.41 | 0.55 | 0.48 | 0.30 | | 0.39 |
| MBRD | 0.22 | 0.30 | 0.35 | 0.43 | 0.52 | 0.39 | 0.37 | 0.54 | 0.56 | 0.56 | 0.49 | 0.32 | 0.36 | 0.44 | 0.45 | 0.37 | 0.30 | 0.25 | 0.43 | 0.26 | 0.33 | 0.47 | 0.43 | 0.55 | 0.55 | 0.31 | 0.36 | |

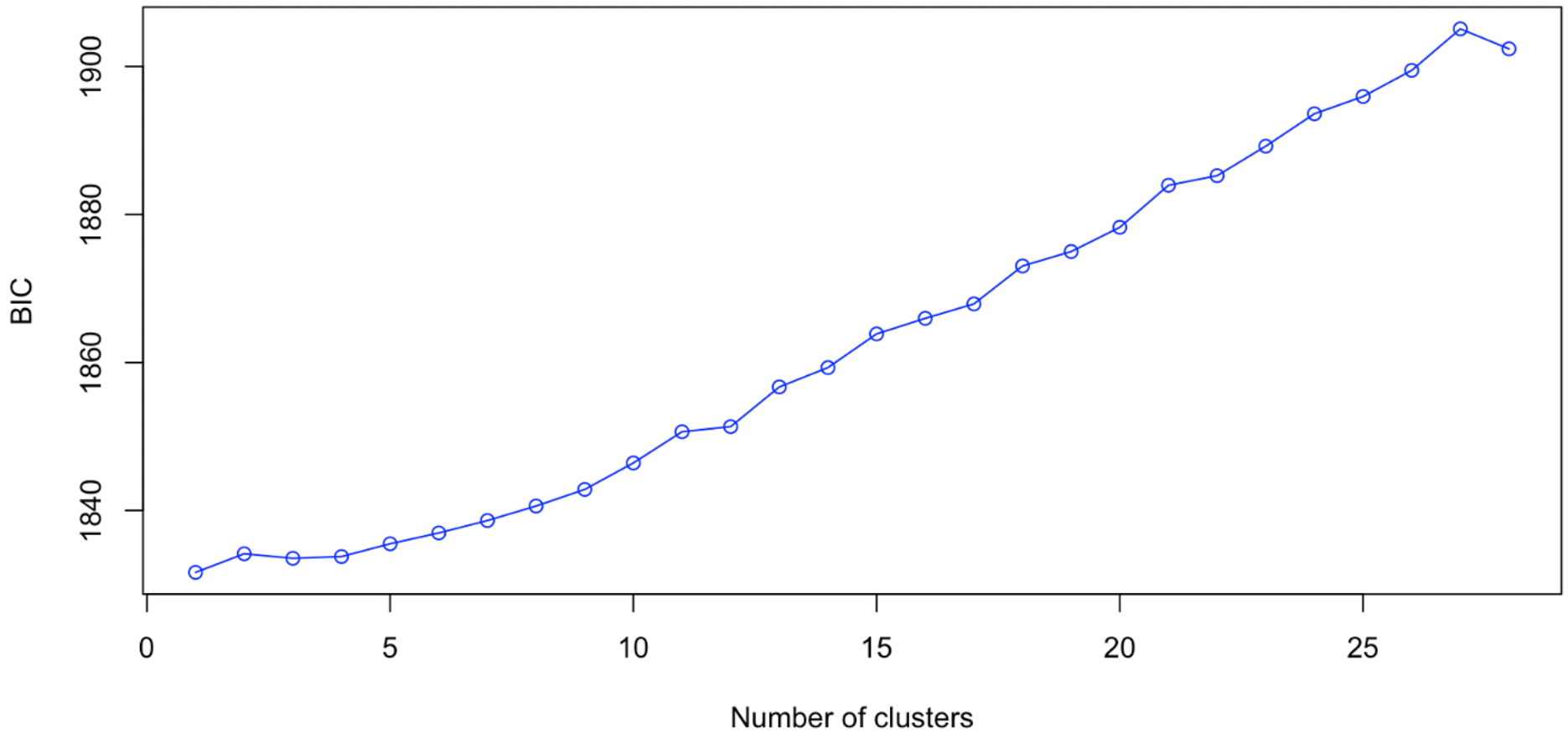


**Figure S1**. **Bayesian Information Criterion (BIC).** Values for the BIC for *K* = 1 to 28 clusters. We have inferred an optimal value of *K* = 4 populations based on the 'best' BIC score, as indicated by an elbow in the curve of BIC values.

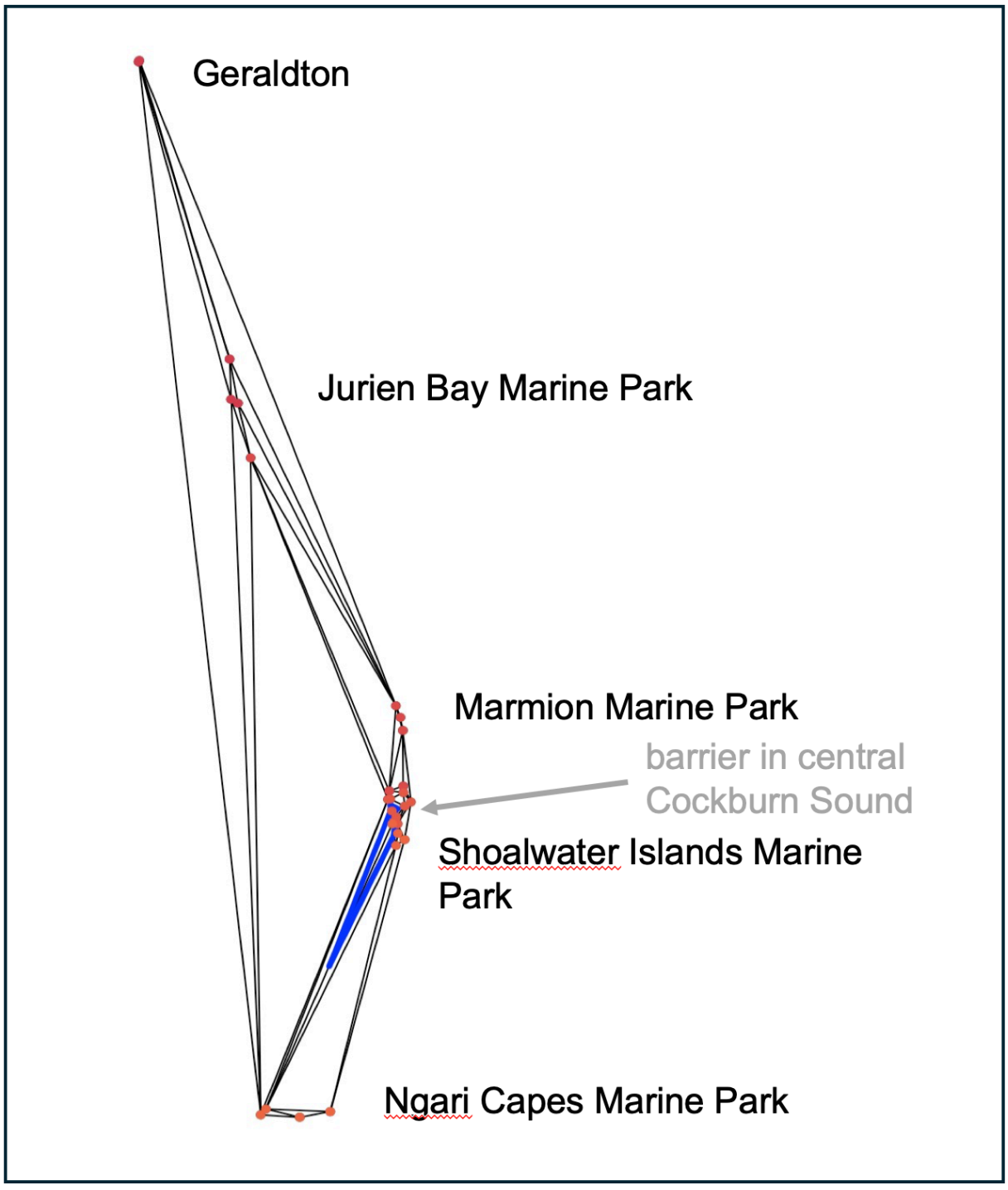


**Figure S2**. **Monmonier's barrier.** A genetic barrier to gene flow was identified within Cockburn Sound.

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