Extensive polyploid clonality was a successful strategy for seagrass to expand into a newly submerged environment

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Polyploidy has the potential to allow organisms to outcompete their diploid progenitor(s) and occupy new environments. Shark Bay, Western Australia, is a World Heritage Area dominated by temperate seagrass meadows including Poseidon’s ribbon weed, Posidonia australis. This seagrass is at the northern extent of its natural geographic range and experiences extremes in temperature and salinity. Our genomic and cytogenetic assessments of 10 meadows identified geographically restricted, diploid clones (2n = 20) in a single location, and a single widespread, high-heterozygosity, polyploid clone (2n = 40) in all other locations. The polyploid clone spanned at least 180 km, making it the largest known example of a clone in any environment on earth. Whole-genome duplication through polyploidy, combined with clonality, may have provided the mechanism for P. australis to expand into new habitats and adapt to new environments that became increasingly stressful for its diploid progenitor(s). The new polyploid clone probably formed in shallow waters after the inundation of Shark Bay less than 8500 years ago and subsequently expanded via vegetative growth into newly submerged habitats.

1. Introduction

Whole-genome duplication through polyploidy is a widely repeated mechanism of significant diversification throughout the evolutionary history of flowering plants [1–6]. Phylogenomic analyses now suggest all angiosperms have been through at least one round of polyploidization [7]. Past polyploid events appear to be associated with periods of significant changes in global climate [5], including deglaciation of terrestrial environments since the Last Glacial Maximum (e.g. [8,9]). Terrestrial polyploids are widespread globally, but more frequent at higher latitudes and in extreme environments [10]. They are also frequently found in habitats that contrast to the habitat of their diploid progenitor(s), indicating polyploidy is associated with evolutionary success in terms of their ability to occupy new environmental niches [11–13].

In a global review of terrestrial ecosystems, specific environmental and life-history attributes were highlighted by [10] that facilitate the establishment of new polyploid lineages. The attributes provide polyploids with sufficient time (usually perennial species with vegetative growth) and space (new environments with low species richness) to outcompete their diploid progenitor(s). Polyploidy has often been regarded as an ‘evolutionary dead end’, yet...
the process can create ‘hopeful monsters’ by conferring a rapid increase in genetic diversity that can facilitate an adaptive advantage over diploid progenitor(s), especially under environmentally stressful conditions [5,6,14,15]. The success of genome duplication depends strongly on morphological, physiological and ecological attributes of the new polyploid, with ploidy-related changes also influencing species interactions through gene expression and epigenetic processes (reviewed in [5,16]). Has polyploidy also played a role in diversification and expansion of marine angiosperms—seagrasses?

Seagrasses are a polyphyletic group which evolved through at least three independent ‘return to the sea’ events [17] in the early Cretaceous [4]. Seagrasses now inhabit marine coastlines and estuaries globally, except for Antarctica [18]. They reproduce sexually through flowering and seed production, and clonally through vegetative growth via horizontal rhizome extension [19]. Most seagrass species have broad geographical distributions with wide-ranging levels of genetic diversity (e.g. [20,21]), and some meadows have expansive, long-lived clones (e.g. [22,23]). Additional alleles at co-dominant genetic markers (3+ alleles) have been reported in several seagrass species [24,25], including Posidonia angustata, Posidonia australis, in Shark Bay [26,27], and are indicative of polyploidy. Variation in chromosome number among populations has been reported in several seagrass species [28,29] including Cymodocea angustata in Shark Bay, Western Australia [29]. The possibility of hybridization was first identified in the genus Zostera [30], and more recent studies indicate that phenomenon is probably more widespread in seagrasses [31–35].

Seagrasses recolonized the Australian Continental shelf with rising sea levels following the Last Glacial Maximum [36], and this included the marine transgression at Shark Bay (figure 1) [38]. A period of rapid sea-level rise during the Holocene (1–2 cm year⁻¹; see [39] and references therein) created extensive inundation and new habitat for benthic marine species, including temperate seagrasses. Expanding seagrass meadows trap sediments which ultimately control environmental gradients through the development of the Faure Sill and Woomaree Seagrass Bank [38], creating increasingly extreme environments for seagrasses and other marine species to inhabit. The metahaline and hypersaline shallow waters within Shark Bay now experience temporal and spatial fluctuations in temperature and salinity in phosphorus-limited waters [40–42]. Here, we use a genotype-by-sequencing approach to (i) assess population genetic diversity and structure of P. australis across the environmental gradient within Shark Bay and (ii) use karyotyping and flow cytometry to determine the presence of polyploidy.

2. Material and methods

(a) Shark Bay and temperate seagrasses

The UNESCO World Heritage Site of Shark Bay, or Gathaaagudu to the traditional custodians, the Malgana Peoples, is home to one of the largest continuous seagrass meadows in the world and creates habitat for a biodiversity marine environment including 12 species of seagrass (figure 1) [43]. A horizontal salinity gradient varies with tidal wave propagation across the shallow bays and sills diurnally, seasonally and varies by gulf [44]. A combination of minimal freshwater input, poor-flushing and high evaporation (approx. 2000 mm) which exceeds precipitation (approx. 200 mm) [37,45] maintains a steep gradient in the eastern gulf. The clear, shallow waters across most of the Bay mean that seagrass meadows are exposed to saturated light levels (greater than 3000 µmol m⁻² s⁻¹), experience a large annual range in temperature, typically 17–26°C although large annual salinity ranges can exceed 30°C in summer [42], and a salinity range between 35 (oceanic) and 64 psu [43]. Large, perennial, seagrass meadows of Amphibolis antarctica and Posidonia australis dominate much of this marine ecosystem [43], creating an ideal location in which to study evolutionary change and adaptation.

Shark Bay is situated at the temperate–tropical interface meaning it is exposed to temperate and tropical extremes of climate change and extreme weather events, such as marine heatwaves and cyclones. The west coast of Australia was exposed to an unprecedented heatwave in the summer of 2010–2011, which impacted both terrestrial and marine ecosystems [46], with sea surface temperatures greater than 3°C above long-term averages in Shark Bay [47]. Most areal loss of seagrass meadows was associated with Amphibolis antarctica, where extensive defoliation resulted in local extinction of meadows [48]. A total estimated area of 1310 km² of dense seagrass meadows disappeared between 2010 and 2014 and consisted almost entirely of temperate species [37]. The approximately 200 km² of Posidonia australis meadows were also impacted, although some natural recovery has occurred where shoot densities have returned to pre-heatwave levels in some locations [49].

(b) Genetic sampling and laboratory protocols

Adult shoot samples of P. australis were collected via SCUBA in 2012 and 2019 from 10 meadows across the geographic range within Shark Bay (figure 1; electronic supplementary material, table S1). Randomized shoot sampling was standardized across sites (within a 50 m diameter area) following [50]. Shoot meristem (non-photosynthetic) tissue was processed and frozen prior to DNA extraction, as described in [51]. Local environmental conditions measured in situ at the time of sampling include depth (m), water temperature (°C), salinity (practical salinity units, psu) and pH.

Genomic DNA was extracted from frozen shoot meristems using a Qiagen DNeasy Plant Pro Kit (Qiagen, Germany) for 144 samples from 10 sampled meadows (12–14 per meadow). Manufacturer protocols were used with the following modifications to improve DNA quality and quantity recovered: half a frozen shoot meristem (approx. 20 mm, approximately 0.25 g) was ground in a mortar and pestle to a fine powder in liquid nitrogen. A buffer mix of 900 µl of CD1 and 100 µl of PS solution was added to the ground sample and left to defrost. The defrosted sample was transferred into a 2 ml microcentrifuge tube containing one ceramic bead and 3 µl of Rnaise. The tubes were placed on a shaker for 10 min, followed by centrifugation at 16 128 RCF for 2 min. The supernatant was transferred into a clean labelled 1.5 ml collection tube. Four hundred microliter of CD2 was added to the supernatant, vortexed for 5 s and placed on ice for 5 min. The final genomic DNA was suspended in 50 µl of EB buffer, which was soaked on the spin column membrane for 30 min. The genomic DNA samples were stored at −20°C. Genomic DNA quantity was measured using the dsDNA broad range Qubit 3.0 Fluorometer (ThermoFisher Scientific, Australia) and the quality was assessed using the Lab Chip GX Touch 24 (PerkinElmer) with HT DNA gDNA reagents.

Library preparation followed the protocol for ddRAD-seq in [52]. Fourteen samples were genotyped from each meadow, with four technical replicates included. Samples were randomly assigned across three libraries, with no two pairs of replicates occurring in the same library. Pooled libraries were sequenced on a HighSeqX10 sequencing machine as 2 × 150 bp paired-end reads (KCCG Sequencing Laboratory, Garvan Institute, New South Wales). Raw reads were processed following the pipeline
detailed in [52]. In brief, a de novo ddRAD loci assembly and identification of single-nucleotide polymorphisms (SNPs) was performed using the denovo_map pipeline in STACKS v. 2.52 [53]. A minimum distance of three nucleotides was used to identify a stack (-m), a maximum of three nucleotides were permitted between stacks (-M) and a maximum of three mismatches were permitted between loci of different individuals during catalogue construction (-n) (electronic supplementary material, table S2). A total of 133 out of 144 samples remained after the m3-M3-n3 pipelining was completed. We used read depth to estimate a new statistic, $H_{\text{ind}}/H_E$ [54] to identify individuals with unexpected ploidy and hybrid status. The expected value of $H_{\text{ind}}/H_E$ is the same across all loci in a dataset, regardless of read depth or allele frequency. The $H_{\text{ind}}/H_E$ statistic was calculated for all samples, where a $H_{\text{ind}}/H_E$ of 0.50 is expected for diploids, 0.75 for triploids, and 1.00 for tetraploids. The statistic was estimated using PoxRAD v. 1.5 (available at https://github.com/lvclark/polyRAD).

(c) Genomic diversity and structure

Sequencing error was assessed by comparing the percentage of non-identical SNP alleles across the four pairs of technical replicates. An error rate cut-off was based on the maximum difference between the four pairs of technical replicates. Percentage similarity was determined as a measure of the variation among SNP profiles within each of the 10 sampled meadows. Technical replicates were removed prior to all subsequent analyses. Clonal richness ($R = (\text{MLG} - 1)/(N - 1)$) [55] was assessed after applying an error rate estimated from the technical replicates using the R package Poppr [56], with values close to zero indicating high levels of clonality and 1 indicating all samples were from different plants. Diversity statistics were calculated based on all SNPs and estimated using Stacks: populations within the pipeline (https://github.com/ascheben/RAD_analysis_workflow#Diversity-analysis-protocol [52]). These diversity statistics included number of private alleles in the population (private, alleles that occur only in a single sample location), mean frequency of the most common allele at each locus ($P$), observed ($H_o$) and expected heterozygosity ($H_e$), inbreeding coefficient ($F_{IS}$) and nucleotide diversity ($\pi$). A cladogram was generated to visualize the relationship among all individuals using (SNPRelate 1.28.0 [57]).

An assessment of population structure was conducted using the whole dataset ($n = 10$ meadows, 18 021 SNPs). Identification of the extent of admixture among sampled $P. australis$ meadows estimated ancestry proportions using an approach based on sparse non-negative matrix factorization (sNMF) [58].
sNMF program assumes that genetic data originates from the admixture of K parental populations, where K is unknown [58]. An estimate of ancestry proportions for each sample was computed. The number of distinct genetic clusters (K) was determined, based on 10 iterations per K value for K = 1 to 10 for the complete dataset, and for nine high heterozygosity meadows K = 1 to 9. Cross-entropy plots were generated to visualize the optimal number of K ancestral populations. A hierarchical analysis of molecular variation (AMOVA) was performed to partition genetic diversity by ploidy and within and among high heterozygosity meadow using all SNPs using Poppr. Variance components were computed at multiple levels to test for significance between ploidy, among meadows and within meadows, with significance based on 9999 permutations.

3. Results

(a) Raw sequence filtering, error rate and coverage

A total of 133 samples including four technical replicates from 10 meadows were sampled across Shark Bay and remained after the removal of 11 samples which did not pass the filtering parameters (missingness greater than 90% on a read depth of 5 and a minimum minor allele filtering; MAF = 0.05; electronic supplementary material, table S2). The complete SNP dataset consisted of 18 021 biallelic SNPs after filtering. The number of SNP differences between each of the four pairs of technical replicates ranged between 355 and 439 (out of 15 625–16 660 SNPs allowing for missing data), which equated to a maximum difference of 2.8%. All mismatches between SNP loci occurred where one individual was homozygous and one was heterozygous, except three SNPs, which were homozygous for alternate alleles. Technical replicates were removed for all subsequent statistical and diversity estimates, and we applied a maximum error rate of 2.8% to estimate clonal richness. The distribution of depth coverage was similar across all samples (H_{ind}/H_{E} greater than 0.8), except those from Guischenault Point where H_{ind}/H_{E} less than 0.5 (electronic supplementary material, figure S1a). The overall weighted mean H_{ind}/H_{E} was 0.95 when including Guischenault Point individuals and 1.0 when excluding Guischenault Point individuals (electronic supplementary material, figure S1b).

(b) Genetic diversity and population genetic structure

Diversity statistics were remarkably similar for nine out of 10 sampled meadows (table 1). Almost identical multi-locus SNP profiles (=multi-locus genotypes) were identified across these nine meadows which spanned the entire 200 km² of mapped P. australis meadows in Shark Bay, over a wide salinity range (33.9–48.8 psu; electronic supplementary material, table S1). One widespread multi-locus genotype was shared among seven out of nine meadows in Shark Bay, with an average multi-locus genotype frequency of 0.224. Almost identical multi-locus genotypes were identified from Northern Territory meadows (sites 1 and 6), a southwest multi-locus genotype was from Nanga Bay and White Island (sites 4 and 5), and a low-frequency multi-locus genotype (two samples) was unique to Fowlers Camp (site 3). The nine meadows were highly heterozygous (mean H_{E} = 0.891 ± 0.078 s.d.), with high-nucleotide diversity (π = 0.477 ± 0.005 s.d.) and a high percentage SNP identity (mean identity = 0.998 ± 0.008 s.d.).

Diversity statistics for the Guischenault Point meadow (site 7) were very different to all other meadows (table 1). Eight unique multi-locus genotypes were identified from the reproductive meadow at Guischenault Point, after the 2.8% error was applied (clonal diversity, R = 0.58). Observed heterozygosity was 0.246 and in Hardy–Weinberg equilibrium, showing no evidence of inbreeding (F_{Is} = 0.016). Nucleotide diversity was moderate (π = 0.241), with 64 to 89% alleles shared among the eight multi-locus genotypes. This meadow had a high number of private alleles (n = 207).
A cladogram showed two distinct clades, one containing all samples from Guischenault Point (site 7) and a second containing all samples from the high heterozygosity meadows (all sites, except 7), and including the adjacent meadow to Guischenault Point at Herald Bight (site 6; figure 2n). The Guischenault Point clade contained all eight multi-locus genotypes, including one which was resampled on six occasions. The second clade contained multi-locus genotypes from all high heterozygosity meadows (nodes 1–4 indicated above the 2.8% error, figure 2n). Population admixture analysis supported K = 2 clusters (figure 2b; electronic supplementary material, figure S2a) for the complete dataset, and is consistent with two distinct clades in the cladogram. No significant structure was detected among the nine high heterozygosity meadows (K = 1; electronic supplementary material, figure S2b). The hierarchical AMOVA partitioned a significant amount of variation between Guischenault Point and all high heterozygosity meadows (between ploidy F_{ST}: 85.4%, p = 0.001; table 2). There was very little variation among meadows (F_{ST}: 1.1%, p = 0.001) relative to within meadows (F_{SC}: 13.5%, p = 0.001). Similar amounts of variation were attributed to variation among (F_{ST}: 46.8%, p = 0.001) relative to within (F_{SC}: 47.3%, p = 0.001) high heterozygosity meadows.

### Table 1. Population diversity statistics. Diversity statistics for 10 sampled *P. australis* meadows based on 18 021 SNPs: N = number of samples sequenced; unique profile = number of unique multi-locus SNP profiles; R = clonal diversity; private = number of private alleles; p = frequency of the most common allele at each locus; $H_o$ (%) = observed heterozygosity; $H_e$ (%) = expected heterozygosity; $F_{IS}$ = inbreeding coefficient; $\pi$ = nucleotide diversity; SNP identity = proportion of shared SNPs (±s.d.).

| pop | meadow | abbrev. | n   | unique profile | R   | private | $H_o$ (%) | $H_e$ (%) | $F_{IS}$ | $\pi$ | SNP identity (±s.d.) |
|-----|--------|---------|-----|----------------|-----|---------|-----------|-----------|----------|------|---------------------|
| 1   | Dirk Hartog | DH     | 14  | 1              | 0.00| 87      | 0.547     | 90.5      | 46.2     | -0.814| 0.482   | 0.960 ± 0.004       |
| 2   | Middle Bluff | MB*    | 12  | 1              | 0.00| 0      | 0.555     | 88.5      | 45.4     | -0.787| 0.475   | 0.959 ± 0.006       |
| 3   | Fowlers Camp | FC     | 12  | 2              | 0.09| 1      | 0.558     | 88.0      | 45.3     | -0.783| 0.473   | 0.953 ± 0.008       |
| 4   | Nanga Bay    | NB     | 14  | 2              | 0.08| 0      | 0.551     | 89.4      | 46.2     | -0.806| 0.478   | 0.944 ± 0.016       |
| 5   | White Island | WH     | 13  | 1              | 0.00| 0      | 0.542     | 89.5      | 46.6     | -0.821| 0.486   | 0.966 ± 0.003       |
| 6   | Herald Bight | HB     | 14  | 2              | 0.08| 0      | 0.551     | 89.6      | 46.1     | -0.806| 0.479   | 0.949 ± 0.019       |
| 7   | Guischenault Point | GU* | 13  | 8              | 0.58| 207     | 0.819     | 24.6      | 23.0     | 0.016| 0.241   | 0.735 ± 0.129       |
| 8   | Monkey Mia   | MM     | 13  | 1              | 0.00| 14     | 0.556     | 88.5      | 45.4     | -0.797| 0.472   | 0.960 ± 0.004       |
| 9   | Dubaut Point | DP     | 12  | 1              | 0.00| 0      | 0.551     | 89.6      | 45.7     | -0.801| 0.478   | 0.966 ± 0.005       |
| 10  | Faure sill   | FI     | 12  | 1              | 0.00| 24     | 0.556     | 88.6      | 45.1     | -0.793| 0.472   | 0.970 ± 0.002       |
| average over nine high heterozygosity meadows: | 116 | 4      | 0.02| 1            | —   | 0.552     | 89.1      | 45.8     | -0.801| 0.477   | 0.958 ± 0.008       |

*Ploidy determined.

(c) Ploidy and genome size

The karyotype of somatic chromosomes for plants from Guischenault Point indicated they were diploid (2n = 2x = 20; table 3 and figure 2n). Karyotypes from shoots in the high heterozygosity meadow at Middle Bluff were tetraploid (2n = 4x = 40). The genome size was estimated at 2C value of 4.56 pg ± 0.030 s.e. for the 2n = 20 karyotype from Guischenault Point, while the 2n = 40 karyotype from Middle Bluff had a 2C value of 7.89 pg ± 0.049 s.e. The polyploid genome was significantly larger at approximately 1.7x the diploid genome size (t-test; p < 0.001). The relative length and form percentage of chromosomes was significantly longer in diploid compared to polyploid karyotypes (relative length = 10.0 µm and 5.0 µm, t-test, p < 0.001; form percentage = 4.07 and 1.86, t-test, p < 0.001; electronic supplementary material, table S3). No significant differences were observed for the remaining six chromosome parameters.

### 4. Discussion

We identified that *P. australis* meadows sampled across Shark Bay, Western Australia, consisted of a single polyploid clone spanning more than 180 km in fragmented, near-shore meadows. This makes it the most widespread known clone on earth. There was only one diploid meadow (Guischenault Point) among the 10 locations sampled. Whole-genome duplication associated with polyploidy appears to have enabled the *P. australis* clone to occupy new habitat and/or outcompete the diploid progenitor(s) within Shark Bay during rapid changes in environmental conditions following the Last Glacial Maximum. Shark Bay contains large areas of sandy sediment and is sheltered from oceanic swells. The shallow, sheltered, environment is ideal for clonal growth and vegetatively spreading meadows. Over millennia, shallow coastal banks and sills from the biological capture of carbonate sediments have resulted in more extreme conditions throughout Shark Bay, with hypersalinity, extremely high light levels and wide temperature fluctuations in a phosphorus-limited system [38,40,41,43]. Our findings suggest a significant new example of polyploidy as a successful evolutionary strategy that enabled an advantage over diploid progenitor(s) and access to new, disturbed or harsher habitats as they developed [5,6,14]. The observed widespread distribution of the polyploid clone (greater than 180 km) is consistent with vegetative expansion following occupation of habitat created by inundation associated with a period of rapid sea-level rise approximately 8500 years ago [38]. The single *P. australis* clone exceeds that of an ancient diploid *Posidonia oceanica* clone discovered in the western Mediterranean that
Individual seagrass clones may persist almost indefinitely if left undisturbed, as they rely on vegetative, horizontal rhizome expansion, rather than sexual reproduction [25]. Our multiple lines of evidence are consistent with extensive polyploid meadows across Shark Bay. High heterozygosity probably confers a fitness advantage (heterosis) and may mask the effects of deleterious mutations. The highly negative $F_{IS}$ values are the result of a whole-genome duplication event between different *Posidonia* lineages. The $H_{nad}/H_E$ statistic for high heterozygosity samples was 1.0, where individual $H_{nad}/H_E$ values were 3 to 4 times those of diploid individuals from Guischenault Point, and consistent with tetraploidy. The traditional estimation of population diversity statistics was compromised by clonality (low sample size as a result of resampling the same widespread clone). However, a direct comparison showed microsatellite loci identified 11 multi-locus genotypes (out of 28 shoot samples) at Guischenault Point and six (out of 27 shoot samples) at Fowlers Camp [27], compared to eight and two respectively, using 18,021 SNPs (this study), raising the question of whether SNPs are the best marker to study clonality.

The genome size of the polyploids was less than double ($1.7 \times$) that of the diploid individuals. Two explanations are plausible. This could be an older autoploid event in which there has been a significant loss of duplicated genes (rediploidization), or hybridization leading to allopolyploidy occurred in which the alternate progenitor had a smaller genome size than *P. australis*. All eight Australian *Posidonia* species have a somatic chromosome number $2n = 20$ [64],

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**Figure 2.** Synthesis of genomic diversity and structure among *Posidonia* meadows. (a) Phylogram showing the relationship among *Posidonia* samples, based on 18,021 SNPs. Broken line indicates the 2.8% cut-off for SNP calling error, as defined by technical replicates. Karyotypes are indicated along the branches, Guischenault Point clade (light blue closed circle) $2n = 20$, all other sites are $2n = 40$; clade 1 (southwest) Nanga Bay (black closed circle), White Island (brown closed circle); clade 2 (northern) Dirk Hartog Island (black closed circle), Herald Bight (purple closed circle); clade 3 (widespread) Middle Bluff (yellow closed circle), Fowlers Camp (green closed circle), Nanga Bay (black closed circle), Herald Bight (purple closed circle), Monkey Mia (grey closed circle), Dubaut Point (brown closed circle) and Faure sill (blue closed circle); clade 4 low-frequency genotype at Fowlers Camp (green closed circle). (b) Admixture results for optimal $K$ ancestral populations, where $K = 2$. Each bar corresponds to an individual, with shared colour indicating genetic homogeneity. See table 1 for abbreviations.
along with the most basal member of the genus from the Mediterranean Sea, *P. oceanica* ([65]). The genome size for *P. oceanica* (6.2 pg [28]) is considerably larger than *P. australis*, while the genome sizes for other Australian *Posidonia* species are currently unknown. Consequently, differentiating between these two competing hypotheses is challenging. However, based on the currently available evidence, it is less likely that autopolyploidy in edge of range meadows would lead to such a significant increase in genetic diversity. We propose that polyploidy as a result of hybridization between *P. australis* and an as yet unidentified *Posidonia* spp. was the most likely pathway. *Posidonia coriacea* and *P. angustifolia* would seem likely contenders, as they are also known from the Shark Bay region ([43]).

We showed that a single widespread polyploid clone spanned at least 180 km, from White Island to the Faure sill near L’Haridon Bight in the eastern gulf of Shark Bay. Horizontal vegetative expansion of the clone means the genetic signature of polyploidy was retained spatially and temporally. However, clones become fragmented as they age, so genetically identical ramets can be no longer physically connected through rhizomes. Thus, a direct measurement of clone size and age can be challenging ([66]). We used the total estimated area of *P. australis* meadows in Shark Bay (200 km² pre 2010/11 heatwave [43]), divided by a conservative range in average annual rhizome extension of 0.15−0.35 m year−¹ based on *P. australis* from Oyster Harbour [67] and raised to an exponent of 2.5 to account for complex non-linear rhizome branching [68] to estimate the time required for *P. australis* to reach its current range within Shark Bay. The derived estimate was a maximum of 4500 years old, a date which corresponds to the Holocene high stand for the region (up to 2.0 m above current sea level; [69]). The same calculations applied to the largest *P. oceanica* clone in the western Mediterranean Sea [22], which has a growth rate closer to 0.04 m year−¹, put it at approximately 7900 years old. Similar calculations for a *Thalassia testudinum* clone indicate its predicted age was less than 6000 years [23].

Global records of large clones across four seagrass families (summarized in the electronic supplementary material, table S4) suggests they are effective at tracking sea-level changes. The one shared difference between these large, old clones (*P. oceanica* in the western Mediterranean [22], *Zostera marina* in the Baltic Sea [24,70], *Thalassia testudinum* in the Indian River Lagoon, Florida [23]) and our results here is that these previous studies showed no evidence for polyploidy, nor do they currently occur in extreme environments. Our data further support a hypothesis and evidence based on 900 angiosperm species, that polyploid clones reproduce mostly through vegetative, or clonal growth, whereas diploids prefer sexual reproduction [71]. Localized mass flowering events, however, have been observed at multiple locations across the range of polyploid *P. australis* clone including at Fowlers Camp, Denham, Big Lagoon, Dubaut Point (unpublished observations by the authors) and Red Hill Bay and Useless Loop in the western gulf (most with three or more microsatellite alleles; [26,27], Sinclair et al. unpublished data). Fruit containing viable embryos have been collected from only two locations in Shark Bay (Guischenault Point and Red Hill Bay), to date, and both have much lower outcrossing rates than higher latitude meadows [27]. Additional microsatellite alleles were also observed in 38% of embryos genotyped from the diploid maternal plants at Guischenault Point, suggesting that unreduced pollen from the nearby polyploid clone was pollinating flowers on diploid plants (i.e. polyploid pollen backcrossing to diploid *P. australis*). Further, we note that

### Table 2. Hierarchical AMOVA. Variance among *P. australis* meadows based on all 18 021 SNPs with 999 permutations.

| source of variation | statistic | d.f. | sum sq | mean sq | sigma | % | p-value |
|---------------------|-----------|------|--------|---------|-------|----|---------|
| all sampled meadows (n = 10): |            |      |        |         |       |    |         |
| between ploidy       | $F_{SR}$  | 1    | 28957.1| 28957.1 | 1218.4| 85.4| <0.001 |
| among meadows        | $F_{ST}$  | 1    | 1097.5 | 1097.5  | 15.8  | 1.1 | 0.001  |
| within meadows       | $F_{IS}$  | 126  | 24234.4| 192.3   | 192.3 | 13.5| 0.001  |
| total                | $F_{IT}$  | 128  | 54289.0| 424.1   | 1426.6| 100.0|         |
| high heterozygosity meadows (n = 9): |            |      |        |         |       |    |         |
| between gulf s       | $F_{SR}$  | 1    | 1166.8 | 1166.8  | 6.9   | 5.9 | 0.122  |
| among meadows        | $F_{ST}$  | 7    | 5383.1 | 769.0   | 55.4  | 46.8| 0.001  |
| within meadows       | $F_{IS}$  | 107  | 5991.0 | 56.0    | 56.0  | 47.3| 0.001  |
| total                | $F_{IT}$  | 115  | 12540.8| 109.1   | 118.3 | 100.0|         |

### Table 3. Ploidy and genome size for *P. australis* samples from two meadows.

| population          | ploidy level | 2n | 2C-value (pg ± s.e.) | 1C-value (pg) | 1C-value (pg) | holoploid genome size (Mbp) | monoploid genome size (Mbp) |
|---------------------|--------------|----|----------------------|---------------|---------------|-----------------------------|-----------------------------|
| Guischenault Point (GU) | $2x$         | 20 | 4.56 ± 0.030         | 2.28          | 2.28          | 2229.84                     | 2229.84                     |
| Middle Bluff (MB)    | $4x$         | 40 | 7.89 ± 0.049         | 3.94          | 1.97          | 3853.32                     | 1926.66                     |
several stigmas examined from the flowering polyploid were deformed and unlikely to produce viable fruit. The widespread polyploid clone may be at least partially sterile, thus explaining the seedling recruitment bottleneck in Shark Bay [72]. Together, these observations are consistent with seagrass having the capacity for extensive clonal expansion in relatively stable locations, with a virtual absence of sexual reproduction.

Maximum sequence variation among our 116 polyploid Posidonia SNP profiles was 4%, of which 2.8% of this variation was attributed to genotyping errors determined by our technical replicate data. We then infer the residual 1.2% variation may represent an accumulation of somatic mutations in different parts of a single widespread clone, creating spatially arranged subclones. Somatic mutations are common in plants [73], however, their frequency in seagrass clones remains unclear, with only one example documented in Zostera marina [24,70]. No such mutations were reported as a source of variation in P. oceanica [22] or Thalassia testudinum [23] clones that occupy tens of kilometres. Our approach to sampling, as in these previous studies (i.e. [22,23]), was not designed to target physically connected ramets or quantify somatic mutations, nevertheless they provide a plausible explanation for variation among the subclones identified in this study.

Whole-genome duplication through polyploidy can be a particularly effective mechanism to increase diversity at a species range edge, where populations are often small, low in genetic diversity, and living at physiological limits [74]. Here, the polyploid probably had an advantage over diploids because (i) stressful conditions promoted polyploid formation, (ii) conditions were unsuitable for diploid progenitors to grow and (iii) polyploid had an increased capacity for genetic change through the larger genome and consequently adapted faster to changing environments [6]. Posidonia australis meadows in Shark Bay are at the northern extent of their distribution. The widespread polyploid clone experiences an annual temperature change from 17°C in winter to 30°C in summer and 20 psu variation in salinity over its geographic range relative to oceanic conditions for winter to 30°C in summer and 20 psu variation in salinity [43]. The Shark Bay findings are consistent with a broad range of (natural and synthesized) terrestrial polyploids that have shown increased tolerance to stress relative to their diploid progenitors (reviewed in [15]). The polyploid P. australis clone also showed a capacity to recover from an extreme climate event via vegetative growth [49]. The proposed superiority of this polyploid clone over diploid P. australis suggests vegetative material from the polyploid is best for the restoration of degraded meadows in Shark Bay. Exactly how the polyploid clone varies its response to local environmental conditions is unknown and the subject of further research, but its relative abundance suggests that it has evolved a resilience to variable and often extreme conditions that enable it to persist now and into the future.

Ethics. Permission to conduct research on Gathaaugu, Malgana Land and Sea Country, was provided by the Malaga Aboriginal Corporation. All plant collections were made under valid scientific collecting permits issued by the Western Australian Department of Biodiversity, Conservation, and Attractions (SW019452, CE005885).

Data accessibility. The SNP data files (with and without technical replicates) are available at https://doi.org/10.5061/dryad.0cfwn4wp [75]. Electronic supplementary material is available online [76].

Authors’ contributions. J.M.E.: data curation, formal analysis, funding acquisition, methodology, project administration, software, validation, visualization, writing—original draft and writing—review and editing; A.A.S.-E.: data curation, formal analysis, methodology, project administration, resources, software, supervision, validation and writing—review and editing; P.E.B.: data curation, formal analysis, methodology, resources, software, validation, visualization, writing—review and editing; S.M.: conceptualization, data curation, formal analysis, methodology, project administration, resources, software, visualization, writing—original draft and writing—review and editing; M.F.B.: funding acquisition, investigation, writing—original draft and writing—review and editing; S.L.K.: funding acquisition, investigation, writing and reviewing—review and editing; G.A.K.: conceptualization, investigation, methodology, project administration, resources, supervision, validation and writing—review and editing; P.E.B.: conceptualization, funding acquisition, investigation, project administration, resources and writing—review and editing; A.A.S.-E.: data curation, formal analysis, methodology, project administration, resources, supervision, visualization, writing—original draft and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. Ohno S. 1970 Evolution by gene duplication. Berlin, Germany: Springer-Verlag.
2. Lynch M. 2007 The frailty of adaptive hypotheses for the origins of organismal complexity. Proc. Natl Acad. Sci. USA 104, 8597–8604. (doi:10.1073/pnas.0702207104)
3. De Bodt S, Maere S, Van de Peer Y. 2005 Genome duplication and the origin of angiosperms. Trends Ecol. Evol. 20, 591–597. (doi:10.1016/j.tree.2005.07.008)
4. Du ZY, Wang QF, China Phylogeny C. 2016 Phylogenetic tree of vascular plants reveals the origins of aquatic angiosperms. J. Syst. Evol. 54, 342–348. (doi:10.1111/jse.12182)
5. Van de Peer Y, Mizrachi E, Marchal K. 2017 The frailty of adaptive hypotheses for the origins of organismal complexity. Proc. Natl Acad. Sci. USA 104, 8597–8604. (doi:10.1073/pnas.0702207104)
6. De Bodt S, Maere S, Van de Peer Y. 2005 Genome duplication and the origin of angiosperms. Trends Ecol. Evol. 20, 591–597. (doi:10.1016/j.tree.2005.07.008)
7. Jiao Y et al. 2011 Ancestral polyploidy in seed plants and angiosperms. Nature 473, 97–100. (doi:10.1038/nature09916)
10. Rice A, Šmarda P, Novosoklov M, Drovi M, Glick L, Sabath N, Meini S, Belmaker J, Mayrose I. 2019 The global biogeography of polyploid plants. *Nat. Ecol. Evol.* 3, 265–273. (doi:10.1038/s41559-018-0787-9)

11. Hegarty MJ, Hiscock SJ. 2008 Genomic clues to the evolutionary success of polyploid plants. *Curr. Biol.* 18, R435–R444. (doi:10.1016/j.cub.2007.08.060)

12. Mable BK. 2013 Polyploids and hybrids in changing environments: winners or losers in the struggle for adaptation? *Hereditas* 110, 95–96. (doi:10.1038/hdy.2012.105)

13. Alix K, Gérard PR, Schwarzacher T, Heslop-Harrison JS. 2017 Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. *Ann. Bot.* 120, 183–194. (doi:10.1093/aob/mcx079)

14. Madlung A. 2013 Polyploidy and its effect on evolutionary success: old questions revisited with new tools. *Hereditas* 110, 99–104. (doi:10.1038/hdy.2012.79)

15. Bomblies K. 2020 When everything changes at once: finding a new normal after genome duplication. *Proc. R. Soc. B* 287, 20202154. (doi:10.1098/rspb.2020.2154)

16. te Beest M, Le Roux JJ, Richardson DM, Bysting AK, Suda J, Kube M, van Dijk KJ, Kendrick GA, Feng M, Hernawan UE, van Dijk KJ, Kendrick GA, Lavery PS, Lowe R, Puotinen M, Breed MF, Kendrick GA. 2020 Variation in reproductive effort, genetic diversity and mating systems across *Posidonia australis* seagrass meadows in Western Australia. *AoB PLANTS* 12, plaa038. (doi:10.1093/aobpla/plaa038)

17. Les DH, Cleland MA, Waycott M. 2007 Vicariance patterns in the Mediterranean Sea: east-west cleavage and low connectivity of edge-of-range *Halodule wrightii* populations. *Aquat. Bot.* 161, 103161. (doi:10.1016/j.aquabot.2019.103161)

18. Sinclair EA, Statton J, Hovey RK, Anthony JM, Dixon KW, Kendrick GA. 2016 Reproduction at the extremes: pseudovivipary and genetic mosaicism in *Posidonia australis* Hook (Posidoniaceae). *Ann. Bot.* 117, 237–247. (doi:10.1093/aob/mcv162)

19. Sinclair EA, Edgell JM, Anthony JM, Statton J, Breed MF, Kendrick GA. 2020 Variation in reproductive effort, genetic diversity and mating systems across *Posidonia australis* seagrass meadows in Western Australia. *AoB PLANTS* 12, plaa038. (doi:10.1093/aobpla/plaa038)

20. Koce JD, Vilhar B, Bohanech B, Dermastia M. 2003 Genome size of Adriatic seagrasses. *Aquat. Bot.* 77, 17–25. (doi:10.1016/S0304-4797(03)00072-X)

21. Kuo J. 2006 Taxonomy and biogeography of seagrasses. In *Seagrasses: biology, ecology and conservation* (eds AW Larkum, RJ Orth, G. Fox). Springer.

22. Short FT et al. 2011 Extinction risk assessment of the world’s seagrass species. *Biol. Cons.* 144, 1961–1971. (doi:10.1016/j.biocon.2011.04.010)

23. den Hartog CKJ. 1970 The seagrasses of the world. Amsterdam, The Netherlands: North-Holland Publishing Company.

24. Auma-Haard S, Migliaccio M, Diaz-Almela E, Teixeira M, van den N, Almela P, Procaccini G, Duarte CM, Serrao EA. 2007 Vicariance patterns in the Mediterranean Sea: east-west cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. *J. Biogeogr.* 34, 963–976. (doi:10.1111/j.1365-2699.2006.01671.x)

25. Hemawas U, van Dijk KJ, Kendrick GA, Feng M, Biffen E, Lavery PS, McMahon KM. 2017 Historical processes and contemporary current drives control genetic structure in the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. *Med. Ecol. 26*, 1008–1021. (doi:10.1111/mec.13966)

26. Auma-Haard S, Duarte CM, Diaz-Almela P, Mari S, Serrao EA. 2012 Implications of extreme life span in clonal organisms: Millenarian clones in meadows of the threatened seagrass *Posidonia oceanica*. *Plas ONE* 7, e04054. (doi:10.1371/journal. pone.0030454)

27. Bricker E, Calladine A, Virstein R, Waycott M. 2018 Mega clonality in an aquatic plant: a potential survival strategy in a changing environment. *Front. Plant Sci.* 9, 435. (doi:10.3389/fpls.2018.00435)

28. Reusch TBI, Boström C. 2011 Widespread genetic mosaicism in the marine angiosperm Zostera marina is correlated with donor reproduction. *Ecol. Evol.* 25, 899–913. (doi:10.1002/ece3.1099-00436-0)

29. Di Giangtonio G, Blum L, McGlathery KJ, Van Dijk KJ, Waycott M. 2020 Genetic mosaicism and population connectivity of edge-of-range *Halodule wrightii* populations. *Aquat. Bot.* 161, 103161. (doi:10.1016/j.aquabot.2019.103161)

30. Sinclair EA, Statton J, Hovey RK, Anthony JM, Dixon KW, Kendrick GA. 2016 Reproduction at the extremes: pseudovivipary and genetic mosaicism in *Posidonia australis* Hook (Posidoniaceae). *Ann. Bot.* 117, 237–247. (doi:10.1093/aob/mcv162)

31. Sinclair EA, Edgell JM, Anthony JM, Statton J, Breed MF, Kendrick GA. 2020 Variation in reproductive effort, genetic diversity and mating systems across *Posidonia australis* seagrass meadows in Western Australia. *AoB PLANTS* 12, plaa038. (doi:10.1093/aobpla/plaa038)

32. Koce JD, Vilhar B, Bohanech B, Dermastia M. 2003 Genome size of Adriatic seagrasses. *Aquat. Bot.* 77, 17–25. (doi:10.1016/S0304-4797(03)00072-X)

33. Kuo J. 2006 Taxonomy and biogeography of seagrasses. In *Seagrasses: biology, ecology and conservation* (eds AW Larkum, RJ Orth, G. Fox). Springer.

34. Coyer IA, Miller KA, Engel CR, Veldink J, Cabello- Pasini A, Starn WT, Olsen JL. 2008 Eelgrass meadows in the California Channel Islands and adjacent coastal reef reveal a mosaic of two variable, evidence for introgression and variable clonality. *Ann. Bot.* 101, 73–87. (doi:10.1093/aob/mcm288)

35. Ito Y, Ohi-Toma T, Murata J, Tanaka N. 2010 Hybridization and polyplody of an aquatic plant, *Rupia* (Ruppiaceae), inferred from plastid and nuclear DNA phylogenies. *Amer. J. Bot.* 97, 1156–1167. (doi:10.1669/000363644153689988)

36. Ito Y, Tanaka N. 2011 Hybridization in a tropical seagrass genus, *Halodule* (Cymodoceaceae), inferred from plastid and nuclear DNA phylogenies. *Telopea* 13, 219–231. (doi:10.7751/telopea20116015)

37. Sinclair EA, Cambridge ML, Kendrick GA. 2019 First report of hybridisation in the marine plant genus *Posidonia*. *Aquat. Bot.* 156, 10–13. (doi:10.1016/j.aquabot.2019.03.004)

38. Liu SYV, Kumara TP, Hsu CH. 2020 Genetic identification and hybridization in the seagrass genus *Halophila* (Glycophytaeae) in Sri Lankan waters. *PeerJ* 8, e10027. (doi:10.7717/peerj.10027)

39. Brooke BP, Nichol SL, Huang Z, Beaman RJ, Waycott M. 2017 Disturbance is an important driver of clonal richness in tropical seagrasses. *Front. Plant Sci.* 8, 2026. (doi:10.3389/fpls.2017.02026)

40. Sinclair EA, Krauss SL, Anthony JM, Hovey RK, Kendrick GA. 2014 The interaction of environment and genetic diversity within meadows of the
seagrass *Posidonia australis* (Posidoniaceae). *Mar. Ecol. Prog. Ser.* **506**, 87–98. doi:10.3354/meps10812

52. Severn-Ellis AA, Scheben A, Neik TX, Saad NSH, Pradhan A, Batley J. 2020 Genotyping for species identification and diversity assessment using double-digest restriction site-associated DNA sequencing (ddRAD-seq). In Legume genomics: methods and protocols (methods in molecular biology) (eds M Jain, R Garg), pp. 159–187. New York, NY: Humana Press.

53. Rochette NC, Rivera-Colon AG, Catchen JM. 2019 Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. *Mol. Ecol.* **28**, 4737–4754. doi:10.1111/mec.15253

54. Clark LV, Mays W, Lipka AE, Sacks EL. 2022 A population-level statistic for assessing Mendelian behavior of genotyping-by-sequencing data from highly duplicated genomes. *BMC Bioinf.* **23**, 101. (doi:10.1186/s12859-022-04635-9)

55. Dorken ME, Eckert CG. 2001 Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *J. Ecol.* **89**, 339–350. (doi:10.1046/j.1365-2745.2001.00558.x)

56. Kamvrás ZH, Tabima JF, Grunwald NJ. 2014 Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2**, e281. (doi:10.7717/peerj.281)

57. Zheng X, Levine D, Shen J, Gogarten S, Laurie C, Weir BS. 2012 A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* **28**, 3326–3328. (doi:10.1093/bioinformatics/bts606)

58. Frichot E, Mathieu F, Trouillon T, Bouchard G, François O. 2014 Fast and efficient estimation of individual ancestry coefficients. *Genetics* **196**, 973–983. (doi:10.1534/genetics.113.160572)

59. Loureiro J, Rodriguez E, Doyle J, Santos C. 2007 Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Ann. Bot.* **100**, 875–888. doi:10.1093/abo/mcm152

60. Nowicka A, Sliwinska E, Grzebelus D, Baranski R, Doležel J, Bartol J, Voglmayr H, Greilhuber J. 2003 Nuclear DNA content and genome size of trout and human. *Cytometry Part A* **51**, 127–128. (doi:10.1002/cyto.a.10013)

61. Doležel J, Doležel š, Frichot E, Mathieu F, Trouillon T, Bouchard G, Simon PW, Nothnagel T, Grzebelus E. 2016 Nuclear DNA content variation within the genus *Daucus* (Apiaceae) determined by flow cytometry. *Sci. Hort.* **209**, 132–138. (doi:10.1016/j.scienta.2016.06.023)

62. Abdolmalaki Z, Mirzaghadari G, Mason AS, Badaeva K. 1990 IdeoKar: an ideogram constructing and karyotype analyzing software. *Cytologia* **68**, 31–35. (doi:10.1080/000877114.2014.998526)

63. Kuo J, James SH, Kirkman H, den Hartog CKJ. 1990 Chromosome numbers and their systematic implications in Australian marine angiosperms of the *Posidoniaceae*. *Plant Syst. Evol.* **171**, 199–204. (doi:10.1007/BF00940605)

64. de Witte LC, Stöcklin J. 2010 Longevity of clonal seagrasses. *Ethol. Ecol. Evol.* **20**, 459–475. (doi:10.1080/06952740.2007.998526)

65. de Witte LC, Stöcklin J. 2010 Longevity of clonal seagrasses. *Ethol. Ecol. Evol.* **20**, 459–475. (doi:10.1080/06952740.2007.998526)

66. Kuo J, James SH, Kirkman H, den Hartog CKJ. 1990 Chromosome numbers and their systematic implications in Australian marine angiosperms of the *Posidoniaceae*. *Plant Syst. Evol.* **171**, 199–204. (doi:10.1007/BF00940605)

67. Nowicka A, Sliwinska E, Grzebelus D, Baranski R, Doležel J, Bartol J, Voglmayr H, Greilhuber J. 2003 Nuclear DNA content and genome size of trout and human. *Cytometry Part A* **51**, 127–128. (doi:10.1002/cyto.a.10013)

68. Abdolmalaki Z, Mirzaghadari G, Mason AS, Badaeva K. 1990 IdeoKar: an ideogram constructing and karyotype analyzing software. *Cytologia* **68**, 31–35. (doi:10.1080/000877114.2014.998526)

69. Kuo J, James SH, Kirkman H, den Hartog CKJ. 1990 Chromosome numbers and their systematic implications in Australian marine angiosperms of the *Posidoniaceae*. *Plant Syst. Evol.* **171**, 199–204. (doi:10.1007/BF00940605)

70. Yu L, Bostrom C, Franzenburg S, Bayer T, Dagan T, Reusch TBH. 2020 Somatic genetic drift and multilevel selection in a clonal seagrass. *Nat. Ecol. Evol.* **4**, 952–962. (doi:10.1038/s41559-020-1196-4)

71. Herben T, Suda J, Kliměčová J. 2017 Polyploid species rely on vegetative reproduction more than diploids: a re-examination of the old hypothesis. *Ann. Bot.* **120**, 341–349. (doi:10.1093/aob/mcx009)

72. Statton J, Ruiz-Montoya L, Orth RJ, Dixon KW, Kendrick GA. 2017 Identifying critical recruitment bottlenecks limiting seedling establishment in a degraded seagrass ecosystem. *Sci. Rep.* **7**, 14786. (doi:10.1038/s41598-017-13833-y)

73. Scheon DJ, Schultz ST. 2019 Somatic mutation and evolution in plants. *Ann. Rev. Ecol. Evol. Syst.* **50**, 49–73. (doi:10.1146/annurev-ecolsys-110218-024955)

74. Eckert CG, Samis KE, Lougheed SC. 2008 Genetic variation across species’ geographical ranges: the central-marginal hypothesis and beyond. *Mol. Ecol.* **17**, 1170–1188. (doi:10.1111/j.1365-294X.2007.03659.x)

75. Edgloe JM, Severn-Ellis AA, Bayer PE, Mehravi S, Breed MF, Krauss SL, Batley J, Kendrick GA, Sinclair EA. 2022 Data from: Extensive polyploid clonality was a successful strategy for seagrass to expand into a newly submerged environment. Dryad Digital Repository. (https://doi.org/10.5061/dryad.of5gnnwp4p)

76. Edgloe JM, Severn-Ellis AA, Bayer PE, Mehravi S, Breed MF, Krauss SL, Batley J, Kendrick GA, Sinclair EA. 2022 Extensive polyploid clonality was a successful strategy for seagrass to expand into a newly submerged environment. Dryad Digital Repository. (https://doi.org/10.5061/dryad.of5gnnwp4p)