Coral Reefs
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REPORT

Population and clonal structure of *Acropora* cf. *hyacinthus* to inform coral restoration practices on the Great Barrier Reef

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Abstract A key goal of coral restoration is to re-establish self-sustaining coral populations and ensure resilience to future stressors, which requires that genetic diversity is maximised. However, coral genetic and genotypic (clonal) diversity is variable across reef sites via success of sexual recruitment, and cryptic species diversity can complicate breeding efforts. Assessing genotypic and genetic diversity of colonies to be used in restoration is therefore critical to avoid founder, inbreeding or outbreeding effects. Considering recent efforts to upscale coral propagation on the Great Barrier Reef (GBR), we examined species, population and clonal structure of a commonly out-planted tabular *Acropora* species—*Acropora hyacinthus* (Dana, 1864). A total of 189 colonies were sampled from six reef systems throughout the northern-central GBR and genotyped using an *Acropora*-specific Affymetrix microarray, which resulted in 1387 variant sites that passed quality control. Cryptic species were readily resolved and all sampled *A. hyacinthus* colonies represented unique genotypes within sites at three reefs. At reefs that contained multi-ramet genets (clonal genotypes), the mean and maximum between-ramet distances were 0.68 and 1.99 m, respectively. Therefore, sampling colonies > 2 m apart increases the likelihood these colonies represent distinct genets. Such a sampling design therefore maximises genotypic diversity when sourcing colonies for propagation and out-planting. Based on these variant sites, we found no between-reef genetic divergence based on locality. Furthermore, through unintentional sampling of non-target tabular Acroporid species, we show how this genotyping method may be used for resolving taxonomic uncertainty as well as population dynamics.

Keywords Genotypic diversity · Clonality · Population structure · Colony selection · Restoration

Introduction

Coral reefs worldwide are facing unprecedented loss of coral from global and local stressors (Hughes et al. 2017; Heron et al. 2018). Reef management agencies are considering more proactive management methods to safeguard vital reef resources (Reef Authority 2021) spanning diverse approaches from coral propagation to human-assisted larval settlement (Hein et al. 2021; Suggett and van Oppen 2022). For example, the central management agency for the Great Barrier Reef (GBR), the Great Barrier Reef Marine Park Authority (Reef Authority), has developed approaches for coral restoration via local stakeholder activity (Reef Authority 2021; Howlett et al. 2022). Success of such efforts, however, requires new knowledge and the tailoring of solutions to ensure they are locally effective (Quigley et al. 2022; Shaver et al. 2022; Suggett and van Oppen et al. 2022). Key considerations include scalability, economic viability, social licencing (e.g. McAfee et al. 2021), ecological impact and

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ecosystem resilience to future change (Camp 2022). For the latter point, maintaining genetic diversity is crucial for ecosystem resilience (Baums et al. 2019; Shaver et al. 2022), and thus, care must be taken to avoid unintentional losses of genetic and genotypic diversity in coral restoration projects (e.g. Baums 2008; Bayraktarov et al. 2019; Suggett et al. 2019).

In the last two decades, coral restoration has been adopted worldwide (Boström-Einarsson et al. 2020) but remains a relatively new concept on the GBR (McLeod et al. 2022). Propagation methods based on asexual and sexual coral reproduction have been recently implemented on the GBR; for example, propagation and out-planting of coral fragments (Howlett et al. 2021, 2022, 2023), and larval reseeding using novel settlement devices (Randall et al. 2021). Whilst these various efforts have shown increasing promise to increase coral cover within reef sites, none are yet able to demonstrate retained or improved local genetic and/or species diversity in restored areas. Propagation and out-planting of just a few genets as donor colonies carry the risk of reduced sexual reproduction of the restored population (Baums 2008; Baums et al. 2019). Out-planting of closely related juveniles reared from gametes of just a few parents carries the risk of inbreeding (Baums et al. 2022)—as well as potential community-wide effects such as biotic homogenisation and reductions in species diversity (Holl et al. 2022). Many reef or coral restoration programmes fundamentally lack the information and means to ensure the maintenance of diversity at both inter-species community (Vardi et al. 2021) and indeed intra-species genetic levels (Baums et al. 2019).

Recent advances in genomic testing have revealed small-scale adaptation and cryptic diversity amongst coral populations (Adam et al. 2022; Matias et al. 2022; Thomas et al. 2020). Historically, given the planktonic life stage of many coral species, coral populations were assumed open with high levels of gene flow, thus rendering small-scale adaptation unlikely (Warner 1997; Hellberg 2007). However, increasingly cost-effective genotyping methods that assay genetic diversity across the genome have resolved cryptic diversity and population structure in both coral hosts and their associated microbial communities (e.g. van Oppen et al. 2011a; Feldman et al. 2022; Matias et al. 2022). On the GBR, studies on a limited number of broadcast spawning coral species have shown latitudinal and across-shelf differentiation between populations, owing to oceanographic restrictions in larval dispersal, temporal differences in spawning and allopatric divergence (*Acropora millepora*, van Oppen et al. 2011b; *Acropora kenti* (previously *Acropora tenuis* on the GBR; Bridge et al. 2023), Matias et al. 2022). However, knowledge of genetic and genotypic population structure remains lacking for most GBR coral species, including those now commonly propagated (Howlett et al. 2022)—knowledge that is vital to guide more effective restoration and potentially increase reef resilience (van Oppen et al. 2011a; Baums et al. 2019).

Utilisation of genomic techniques within coral restoration practices, and during the project design phase (Shaver et al. 2022), is important to maximise the adaptation potential of coral populations (Parkinson et al. 2020; Quigley et al. 2019; Caruso et al. 2021; Baums et al. 2019). At the most fundamental level, genotypic identification and tracking are required for restoration of potentially clonal coral populations to ensure genetic diversity is maintained (Baums et al. 2019; Parkinson et al. 2020; Kitchen et al. 2020). In response, a novel 30 K single-nucleotide polymorphism (SNP) genotyping array was recently developed to determine coral population structure and ancestry of Caribbean Acroporids necessary for restoration planning (Kitchen et al. 2020). This array was developed using SNPs identified from *Acropora cervicornis* and *Acropora palmata* (Kitchen et al. 2020) and tested on a limited number of Pacific *Acropora* species samples. It has not yet been applied to guide restoration of the immense diversity of *Acropora* species in the Indo-Pacific (e.g. Suggett et al. 2019; Boström-Einarsson et al. 2020; Howlett et al. 2022).

On the GBR, tabular *Acropora* species are known drivers of ecosystem recovery following disturbances (Ortiz et al. 2021), but they are also disproportionately more vulnerable to temperature extremes and resulting bleaching events (Baird & Marshall 2002; Linares et al. 2011). Delicate colony structure of tabular *Acropora*—as with other growth morphs within this genus—results in regular fragmentation via physical disturbance and hence presumably increased clonal proliferation (Baums et al. 2006). However, historical studies have shown higher rates of larval recruitment as opposed to recruitment by fragmentation for tabular colonies identified as *Acropora hyacinthus* (Wallace 1985). Whilst increased prevalence of asexual fragmentation and clonal growth can prevent species extinction in times of lower sexual recruitment, unknowingly propagating colonies of the same genet and out-planting them in close proximity will ultimately reduce chances of successful outcrossing (Baums et al. 2006, 2019). Considering recent efforts to upscale coral restoration practices on the northern-central GBR (Howlett et al. 2022; McLeod et al. 2022), we therefore examined the population and clonal structure of a commonly out-planted tabular *Acropora* species—*A. hyacinthus*—within the region. Specifically, we obtained baseline knowledge of clonal dynamics to inform colony selection for propagation and out-planting. We apply the novel genotyping microarray, previously developed for Caribbean *Acropora* species and their algal symbionts (Kitchen et al. 2020). In addition to genotypic diversity, the microarray resolved species-level diversity within the current *A. hyacinthus* species complex and, therefore, this genotyping method can operate as a rapid

approach to genotyping closely related tabular *Acropora* taxa for which genomic resources have not yet been developed.

Materials and methods

Sample collection

A total of 189 colonies of tabular *Acropora* were sampled across six reef systems throughout the northern-central GBR, over an extent of 304 km, (Fig. 1; Table 1) between August 2020 and September 2021. During sampling, we specifically targeted colonies of *A. hyacinthus* (Dana, 1846) based on morphology as described in Wallace et al. (2012).

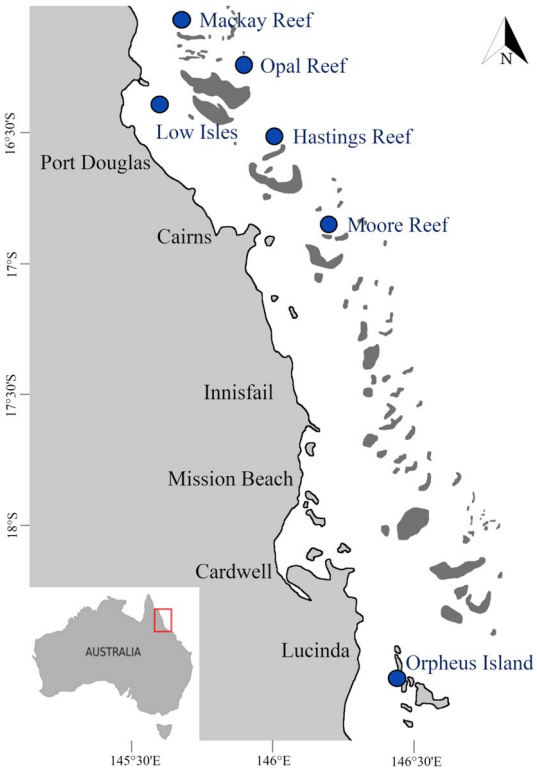

Fig. 1 Sampling sites for *Acropora hyacinthus* on the northern-central Great Barrier Reef

Sampling method was dependant on visual density of tabular *Acropora* on the reef crest. Sampling sites on Opal and Moore Reef were visually dominated by tabular *Acropora* (Fig. 2), and therefore, two complimentary sampling methods were employed: "systematic" and "random": (i) For the "systematic" sampling design, a central point was chosen. A 25 m measuring tape and compass were used to sample and note the coordinates of every *A. hyacinthus* colony within the 25 m radius sampling area. (ii) In the case of the "random" sampling design (Baums et al. 2006), a sequence of numbers was first generated via the "random number" generating function within Microsoft Excel (Microsoft Corporation 2018) limited between 0 and 5, 0 and 10, and 0 and 15. These were paired with randomly generated compass bearings between 10° and 360°, with increments of 10°, to create coordinates within three nested sampling areas with radii of 5m, 10m and 15m. Once at the sampling site, a central point was chosen on the reef crest with a visibly high tabular *Acropora* population at least 30 m distance from the previously sampled ares. A transect tape was then used to measure each pre-determined distance from the central point along each corresponding bearing by compass, thereby allowing a random location to be reached within the nested sampling area. If an *A. hyacinthus* colony was within 0.5 m of the coordinate, a 2-cm fragment was collected from the growing edge of the colony using wire cutters and placed into a pre-labelled, sealed plastic bag for transport. If an *A. hyacinthus* colony was not within 0.5 m of the coordinate, the next coordinate was used. This process was repeated until eight samples were taken from each of the three nested sampling areas (Fig. S.1; adapted from Baums 2006). For both sampling methods, the same colony was never sampled more than once.

The "systematic" sampling design was conducted at all sites. At Opal and Moore Reefs, where densities of tabular

Table 1 Sample sizes and sampling design of *Acropora hyacinthus* colonies at reef sites within the northern-central Great Barrier Reef. Where colony density was high, sampling was conducted using a random sampling method (Moore Reef, Opal Reef)

Reef	Site latitude and longitude	Sampling design	Site ID	No. sampled colonies (*n*)
Orpheus Island	18°39′34"S 146°30′09"E	Systematic	FI	26
Moore Reef	16°50′54"S 146°13′40"E	Random	RM	19
		Systematic	MO	32
Hastings Reef	16°30′02"S 146°00′50"E	Systematic	HA	14
Low Isles	16°23′19"S 145°33′43"E	Systematic	LI	3
Opal Reef	16°12′20"S 145°53′54"E	Random	BL	10
		Systematic	OP	31
Mackay Reef	16°02′24"S 145°38′50"E	Systematic	AN	28

At sites with lower colony density, a systematic design was used. ID = identification

Fig. 2 Tabular *Acropora* dominated reef crest at sampling site on Moore Reef. At this site, complimentary "random" (adapted from Baums 2006) and "systematic" sampling designs were employed based on high visual density of tabular *Acropora* species. Photograph by Matt Curnock

Acropora colonies were visually high, the "random" sampling design was also performed.

At Opal Reef, two colonies of *A. millepora* were sampled to act as an internal control within analyses of population structure. These colonies were located > 10 m apart from each other and from within the "random" sampling area for *A. hyacinthus*. Because *A. millepora* and *A. hyacinthus* are not part of the same species complex, *A. millepora* was an appropriate choice for an outgroup (Wallace et al. 2012). At Moore Reef, one *A. hyacinthus* colony was sampled four times within the "random" sampling area to act as a control for later analyses of within-site relatedness and genet identity.

Colony size and morphology

Given that larger colonies would likely fragment more readily and thus have increased clonal proliferation, we measured size frequency distributions within sites. Scaled top-down photographs were taken of each sampled colony and analysed within ImageJ2 to measure surface area (SA; length × width) (Rueden et al. 2017). Length was the longest horizontal measurement possible on the upper side of the colony and width was the corresponding perpendicular measurement. Colony size could not be determined in cases where a colony was too shallow for a top-down scaled photograph to be acquired ($n = 8$ at Mackay Reef); these samples were not included in the size frequency calculations. Images were also used to check species identification.

Species resolution

After initial sampling—and in consulting with rapidly developing *Acropora* taxonomic literature (e.g. Sheets et al. 2018; Suggett et al. 2019)—it was apparent that not all colonies corresponded with previous descriptions of *A. hyacinthus* (Wallace 1999). Specimens were independently identified based on comparisons of in situ colony images to *Acropora* type specimens and original descriptions, with the use of open nomenclature qualifiers to indicate levels of uncertainty in taxonomic assessments, as outlined in Cowman et al. (2020). Of the 189 sampled colonies, 156 were identified as *A.* cf. *hyacinthus*, because of their morphological resemblance to the type of *Acropora hyacinthus* from Fiji (Fig. 3). A further 20 colonies, mostly from Opal Reef and all with similar morphological features—notably distinct due to their compact branchlet formations—could not be identified as any of the nominal *Acropora* species and could potentially be of an undescribed species of tabular *Acropora*, henceforth referred to as *Acropora* aff. *hyacinthus* (Fig. 3). Finally, 13 colonies had morphologies that most closely correlated to previously described *Acropora pectinata* which has a type described for a locality of the Torres Straits and GBR (Veron & Wallace 1984). *A. pectinata* is currently deemed synonymous with *A. hyacinthus* (Wallace & Wolstenholme 1998) (Fig. 3). These initial species identifications were based on morphology alone and the results of genomic analyses were not consulted at this time. As such, we postulated that the genetic data (described in Sect. 2.5) would verify the identification of these different tabular *Acropora* species based on morphology.

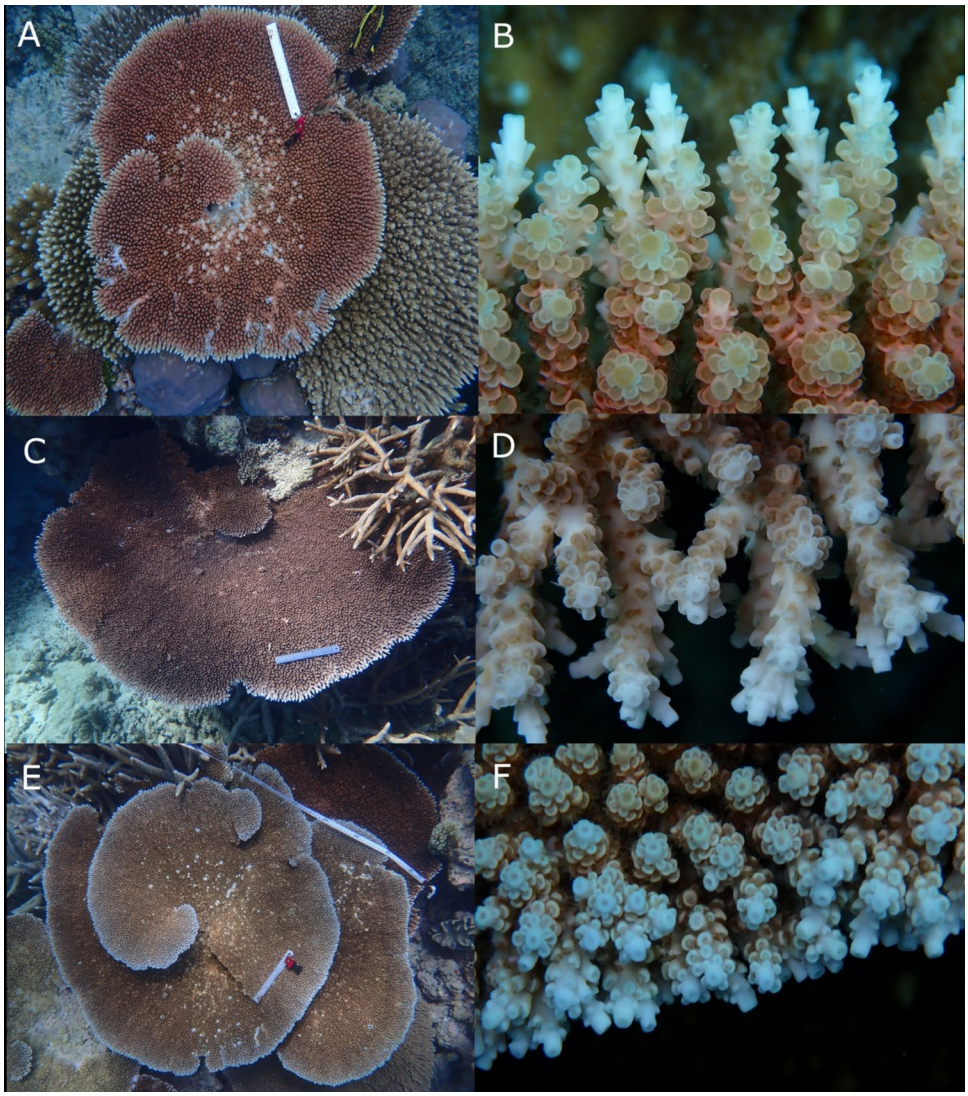

Fig. 3 Images representing colonies of each species cluster (see Fig. 4). **a** Example of *Acropora* cf. *hyacinthus* showing whole colony morphology (centre frame) with 15 cm ruler for scale; **b** macro-photograph of *Acropora* cf. *hyacinthus* showing corallites at the growing edge of the same colony; **c** example of *Acropora pectinata* showing whole colony morphology (centre frame) with 15 cm ruler for scale; **d** macro-photograph of *Acropora pectinata* showing the growing edge of the same colony; **e** example of *Acropora* aff. *hyacinthus* showing whole colony morphology (centre frame) with 15 cm ruler for scale; **f** macro-photograph of *Acropora* aff. *hyacinthus* showing the growing edge of the same colony. Photographs A–B and E–F taken at Opal Reef, and photographs C–D taken at Moore Reef, central Great Barrier Reef

Sample preservation and DNA extraction

Following in-water collection, samples were further fragmented using sterile wire cutters and preserved in 100% molecular-grade ethanol. All samples were subsequently stored in the dark at 4 °C until DNA extraction.

A subsample of 10 mg was taken from each sample for DNA extractions, as follows. Homogenisation was conducted by flash freezing using liquid N_2 and crushing using a metal pestle within 2-mL sterile micro-centrifuge tubes. Once at room temperature, DNA was extracted from the resulting solution using the DNeasy Blood and Tissue kit (Qiagen) following manufacturer's protocols with a final elution volume of 100 µL in nuclease-free water. Quality checks were conducted on the DNA via a NanoDrop spectrophotometer and DNA was subsequently stored at − 30 °C until sequencing.

Genotyping and data analyses

Samples were genotyped by Ramaciotti Centre for Genomics using the Applied Biosystems Axiom Coral Genotyping Array 550,961 (Kitchen et al. 2020). Due to the high specificity of markers within the array, developed from symbiont-free gametes of *Acropora palmata*, the presence of any symbiont DNA did not interfere with the host genotyping process (Kitchen et al. 2020). Raw CEL files were analysed using the Applied Biosystems Axiom Analysis Suite (v5.1.1.1). No dish-quality (DQC) threshold was enforced (set to > 0.005) because the analysed species were not the target species that the microarray was designed for. Quality check (QC) call rate and average call rate for passing samples was set to ≥ 70. All samples passed QC and passing samples had an average call rate of 98% and a filtered call rate of 99.316%. The resulting SNP data was exported in

VCF format and further processed with bcftools (Danecek et al. 2021). The VCF file was filtered using the "Best and Recommended" list of probes from the Analysis Suite. The default output of the Axiom microarray is given in genomic coordinates for *Acropora digitifera* because of its initial design. As such, coordinates were lifted to the *A.* cf. *hyacinthus* reference genome (López-Nandam et al. 2023, NCBI assembly accession GCA_020536085.1) by mapping probe sequences to the reference using *bwa-mem* (Li 2013) and updating the contig ID to that of the chromosomes of *A.* cf. *hyacinthus.* However, this reference genome is from a sample collected in Palau, which does not match the locality type of *A. hyacinthus* (Dana 1846; López-Nandam et al. 2023). Only primary alignments with MAPQ > 3 were preserved for downstream analyses. SNP positions were updated using the position of the "N" in the probe sequence. Probe sequences containing indels were included in downstream analyses, but exact coordinates of SNPs may be imprecise for these markers.

To evaluate kinship across the dataset and remove clones from population structure analyses, SNPs were filtered using bcftools (Danecek et al. 2021), and only biallelic sites with minor allele frequency > 0.01, missingness < 0.8 and linkage disequilibrium $R^2 < 0.2$ were preserved for kinship estimation. Kinship was estimated using the 1,916 remaining sites and the KING-robust estimator (Manichaikul et al. 2010) as implemented in PLINK2 (Chang et al. 2015). For samples exhibiting pairwise kinship > 0.1, the sample with the least amount of missing data was preserved for structure analyses. Replicates of the same colony (taken from Moore Reef) were included in the plate to confirm the reliability of kinship estimation. The coefficient of 0.354 is the geometric mean of first-degree kin (0.25) and monozygotic twins (0.5), and is the standard cut-off for distinguishing the two levels of relatedness. As such, a cut-off of 0.354 was used to assign ramets to genets. For ramets of the same genet, physical distance (m) between ramets was calculated. In the remainder of the analyses, only one ramet per genet was utilised.

To assess species and population structure, we utilised both principal components analysis (PCA) and STRUCTURE methods. For PCAs, bcftools was used to filter the initial VCF as mentioned above, with the additional filter of removing related individuals. The remaining number of sites was 1903 for the species-level dataset and PLINK2 was used to perform PCAs with this dataset. The density of SNPs across the *A.* cf. *hyacinthus* chromosomes used in this analysis are shown in Fig. S.2 in bins of 50Kbp. With the exception of a more stringent minor allele frequency filter of > 0.05, the same procedure was followed for an analysis focusing solely on the largest species cluster, corresponding with *A.* cf. *hyacinthus*. For this dataset, 1055 sites remained for analysis. STRUCTURE analyses were conducted on all the same datasets to further assess genetic groupings (K) at the species and population level (Pritchard et al. 2000). VCF files were converted to STRUCTURE format and replicate simulations from $K = 1$ to $K = 8$ were run and then visualised within StructureSelector (100,000 replications and 100,000 burn-in; Li and Liu 2018). Because the microarray was designed for Caribbean *Acropora*, we acknowledge the presence of ascertainment bias when applying the microarray to Indo-Pacific species. The bias is due to the fact that markers were chosen to be polymorphic in Caribbean species. This does not affect the ability of the array to identify genets and determine species assignments (Lachance and Tischkoff 2013) but possibly reduces the power of the array to reliably detect within-species population structure. We therefore expect that the power of the array to detect significant population structure in Indo-Pacific Acroporids is low and provide the following analyses with this caveat in mind. For the analysis focusing on *A.* cf. *hyacinthus*, STRUCTURE was run with a location prior as population structure was assumed to be weak or absent based on PCAs. The most likely value of K was evaluated using Clumpak (Kopelman et al. 2015) within StructureSelector and based on the ΔK method (Evanno and Goudet 2005) and Puechmaille method (Puechmaille 2016).

For the largest species cluster, associated with *A.* cf. *hyacinthus*, we estimated effective migrations surfaces using FEEMS (Marcus et al. 2021). To generate input data for FEEMS, *A.* cf. *hyacinthus* samples were extracted from the dataset using PLINK2. As Low Isles was only represented by a single individual in the *A.* cf. *hyacinthus* dataset, the site was removed from the FEEMS analysis. Linked SNPs were pruned using the indep-pairwise function (500 kb, $R^2 < 0.2$) and unlinked SNPs were exported as binary BED format and loaded into FEEMS. FEEMS cross-validation was performed with lambda values ranging from 10^{-10} to 7 and the final migration surface estimate was obtained using the lambda value with the lowest cross-validation error (lambda = 7). Finally, F_{ST} was estimated between reefs within *A.* cf. *hyacinthus* using *StAMPP* (Pembleton et al. 2013) with 100 bootstrap replicates. F_{ST} was also estimated between putative species identified via PCAs and STRUCTURE analyses using *StAMPP.*

A Spearman's Rank correlation analysis was conducted between the total number of multi-ramet genets per reef and average colony SA to determine the relationship, if any, between clonality and colony size. This statistical analysis was conducted using R software (R Core Team 2021).

Results

Species and population structure between reefs

Four clusters of samples were resolved based on the PCA and STRUCTURE analyses of SNP markers (Fig. 4a).

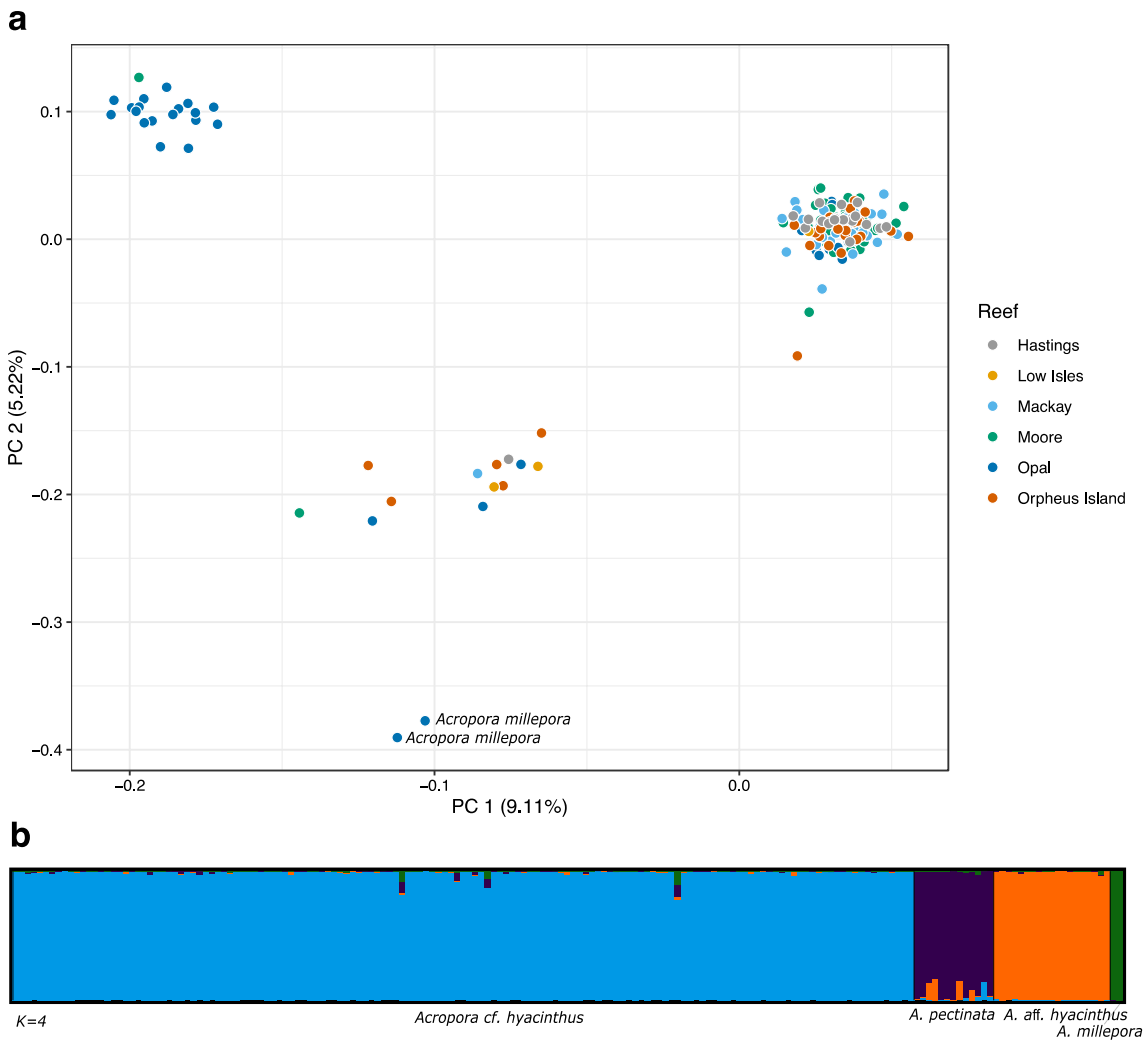

Fig. 4 **a** Principal component analysis showing genetic divergence of tabular Acroporid colonies sampled throughout six reef sites across the northern-central Great Barrier Reef. Two *Acropora millepora* colonies were sampled to act as known controls for the genotyping array. Three clusters were identified conforming with colonies identified as (top-right) *Acropora* cf. *hyacinthus*, (bottom-centre) *Acropora pectinata* and (top-left) *Acropora* aff. *hyacinthus* based on image analysis; **b** structure population assignments for tabular *Acropora* colonies sampled throughout the central Great Barrier Reef. Vertical columns represent individual samples and the associated probability of assignment to $K=4$ genetic clusters. Here, genetically differentiated populations (colours) were assigned to species identified based on morphology and genetic differentiation: *Acropora* cf. *hyacinthus* (light blue), *Acropora* aff. *hyacinthus* (orange), *Acropora pectinata* (dark purple) and *Acropora millepora* (green; outgroup)

For the PCA, the first and second principal components explained 9.11 and 5.22% of the variation in the data, respectively, and the resulting clusters aligned well with the results of morphological analysis. One small cluster included the two *A. millepora* samples. All remaining clusters aligned with the three taxa identified via image analysis after the original sampling (Fig. 3). The largest cluster (top-right Fig. 4a; $n=154$) were all *A.* cf. *hyacinthus* sampled across all reefs. The second largest cluster (top-left; Fig. 4a; $n=20$) containing samples mostly from Opal Reef were all visually identified as *A.* aff. *hyacinthus*. The smallest cluster (bottom-centre; Fig. 4a; $n=13$) was comprised of all samples previously identified visually as *A. pectinata*. Likewise, the STRUCTURE analysis suggested four genetic clusters (Fig. 4b; *K* values 1–8 are shown in Fig. S.5) corresponding with the three identified tabular *Acropora* taxa and *A. millepora*. Pairwise F_{ST} values suggest that the greatest genetic differentiation for the three tabular *Acropora* species was between *A.* cf. *hyacinthus* and *A.* aff. *hyacinthus* ($F_{ST}=0.198$, Table 2). Similar differentiation was found between *A. pectinata* and *A.* aff. *hyacinthus* with comparably lower pairwise differentiation between *A.* cf. *hyacinthus* and *A. pectinata* ($F_{ST}=0.130$). All F_{ST} values at the species level were statistically significant ($p<0.001$).

Sampling of *A. pectinata* and *A.* aff. *hyacinthus* was unintentional and, therefore, it is possible sampling efforts did

Table 2 Fixation index (F_{ST}) values between tabular *Acropora* species clusters sampled throughout the central Great Barrier Reef, confirmed by morphology, structure analysis and principal component analysis

	Acropora cf. *hyacinthus*	*Acropora* aff. *hyacinthus*	*Acropora pectinata*	*Acropora millepora*
Acropora cf. *hyacinthus*	–	0.198	0.130	0.247
Acropora aff. *hyacinthus*	0	–	0.193	0.338
Acropora pectinata	0	0	–	0.240
Acropora millepora	0	0	0	–

Above the diagonal is Weir and Cockerham F_{ST} and below the diagonal is the associated p value (100 bootstraps), both obtained from StAMPP (Pembleton et al. 2013). All F_{ST} values are significant ($p < 0.001$)

not capture colonies representative of the sampling sites. Consequently, differentiation of *A. pectinata* and *A.* aff. *hyacinthus* based on locality was dismissed for this study. However, in the case of *A.* cf. *hyacinthus*, there appeared to be no differentiation in populations based on geographical distance between reefs (Fig. 4a). However, given that these analyses included 1903 SNPs that were initially designed for Caribbean species of *Acropora*, it is likely that we were simply unable to detect weak population structure given our genotyping method and resulting dataset size.

Within-site relatedness and pairwise comparisons

All replicate samples taken from the same *A.* cf. *hyacinthus* colony on Moore Reef had kinship estimates of > 0.486, corresponding with the "monozygotic twin" designation of KING-robust (Manichaikul et al. 2010). For pairwise comparisons between replicates, genotype data varied only at between 4 and 13 SNPs. Within the largest PCA cluster containing all *A.* cf. *hyacinthus* individuals (Fig. 4a), five multi-ramet genets were detected. Two, 3-ramet genets and one, 2-ramet genet were detected at Hastings Reef with a maximum distance between two ramets of the same genet of > 0.51 m. One, 2-ramet genet was detected at Moore Reef in the random sampling set with a between-ramet distance of 1.99 m. One, additional 2-ramet genet was detected at Moore in the systematic sampling set with a between-ramet distance of 0.3 m. Across the remainder of the dataset, only one additional 2-ramet genet was detected in the distinct cluster associated with *A.* aff. *hyacinthus* individuals sampled systematically at Opal Reef; this pair had a between-ramet distance of 1.1 m. Summary of clonality across the full dataset is given in Table 3. Pairwise comparisons between ramets of the same genet varied at between 1 and 24 SNPs, potentially due to unshared somatic mutations between ramets. By means of contrast, pairwise comparisons between the ten least related pairs of samples within the cluster associated with *A.* cf. *hyacinthus* varied at between 487 and 602 SNPs. Collectively this analysis suggests relatively high genotypic diversity and rates of sexual recruitment, thus supporting historical studies on tabular *Acropora* taxa (Wallace 1985; Baums 2006).

Table 3 Summary table highlighting clones identified within *Acropora* cf. *hyacinthus* and *A.* aff. *hyacinthus* and the distances between ramets of the same genet

Genet	No. of ramets	Location ID	Avg distance between ramets (m)	Max distance between ramets (m)
1	2	HA	0.20	0.20
2	3	HA	0.13	0.20
3	3	HA	0.35	0.51
4	2	RM	1.99	1.99
5	2	OP	1.10	1.10
6	2	MO	0.30	0.30

Rerunning PCA and structure analyses using only the non-clonal *A.* cf. *hyacinthus* samples further confirmed lack of discernible population structure across the sampled reefs (Figs. S.4; S.5).

Colony size frequency distributions

Overall, sites with larger tabular *Acropora* colonies within the sample set did not correspond with sites containing multi-ramet genets (Fig. 5). Specifically, mean colony surface area (SA) was highest at Opal Reef (4444.2 ± 1164.2 cm^2; mean ± SE) and Orpheus Island (3426.8 ± 738.9 cm^2), whilst Low Isles had the lowest (460.9 ± 137.5 cm^2). Mackay, Moore and Hastings Reef had similar measures of colony SA (2371 ± 250.4 cm^2, 2856.2 ± 373.5 cm^2 and 1665 ± 321 cm^2, respectively). Whilst Hastings Reef had the most multi-ramet genets, Orpheus Island had none, and Opal Reef had only one. Consequently, there was no statistically significant correlation between the size frequency and observed clonality within sampling sites (Spearman's Rank correlation; $p = 0.862$).

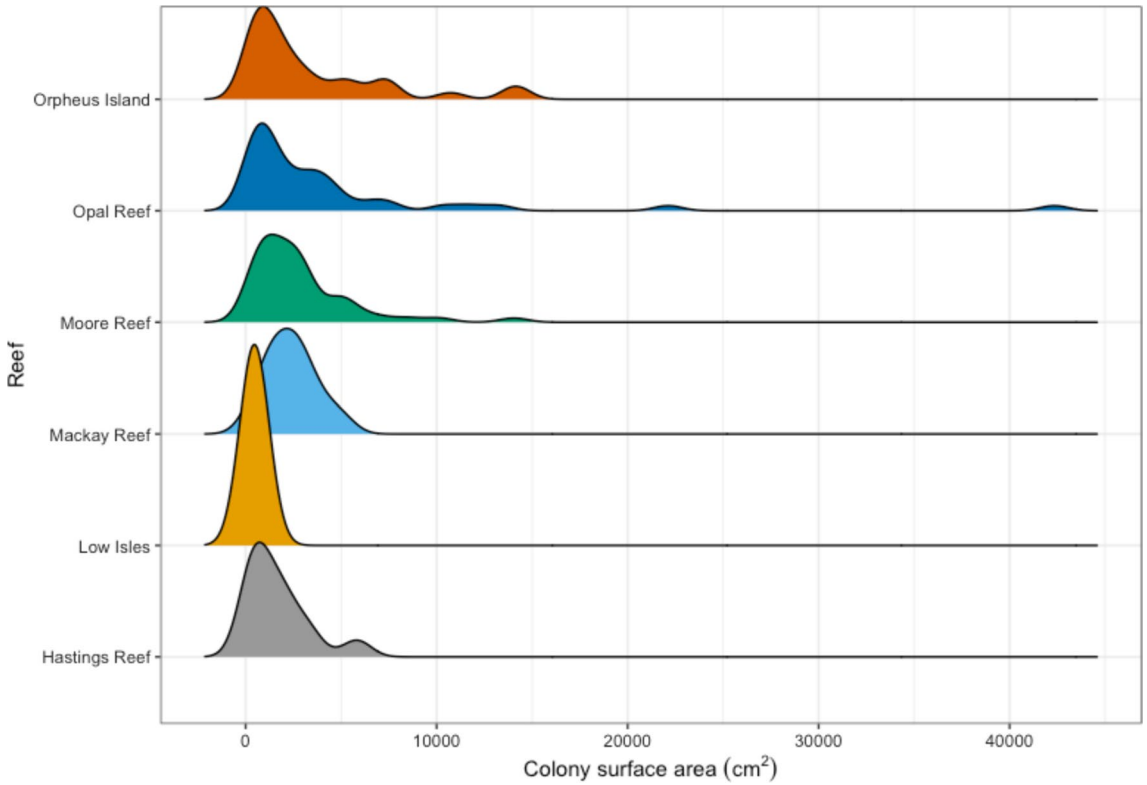

Fig. 5 Size frequency plot showing the proportions of sampled tabular *Acropora* colonies according to surface area (cm^2) within each sampled reef on the central Great Barrier Reef

Effective migration and connectivity between reefs in *Acropora* cf. *hyacinthus*

As expected given the ascertainment bias of the array with respect to within-species population genetic analyses, no prominent barriers to migration were detected in the FEEMS analysis (Fig. S.7). Lambda values higher than 7 did not converge and lambda = 7 was the value with the lowest cross-validation error. No biases in migration rates across the spatial grid were detected at this scale. STRUCTURE runs for *A.* cf. *hyacinthus* also highlight a lack of differentiation at this scale (Fig. S.7). The Puechmaille method (Puechmaille 2016) suggested $K = 2$, whilst ΔK method (Evanno et al. 2005) suggested $K = 6$. STRUCTURE plots for $K = 1$–8 are available in Fig. S.7. Bootstrapped F_{ST} values between sites in *A.* cf. *hyacinthus* further corroborate the finding of no significant differentiation between sites (Table 4). Significant pairwise F_{ST} values were only found between Opal and Hastings Reefs ($p = 0.01$); however, these pairwise F_{ST} values were still comparatively low ($F_{ST} = 0.004$), highlighting a lack of population differentiation within the species at this spatial scale.

Table 4 Fixation index (F_{ST}) values between sites of *Acropora* cf. *hyacinthus*. Above the diagonal is Weir and Cockerham F_{ST} and below the diagonal is the associated p value (100 bootstraps), both obtained from StAMPP (Pembleton et al. 2013).

	Opal	Moore	Mackay	Orpheus	Hastings
Opal	–	− 0.0002	0.0004	− 0.0004	**0.0040**
Moore	0.58	–	− 0.0003	− 0.0009	0.0021
Mackay	0.45	0.59	–	0.0004	0.0011
Orpheus	0.66	0.85	0.43	–	0.0013
Hastings	**0.01**	0.06	0.26	0.25	–

Bolded values indicate significance at $p < 0.05$

Discussion

Due to recent upscaling of coral propagation activities on the GBR, some high-value reef sites have shown an increase in proportions of commonly out-planted taxa (Howlett et al 2022; Roper et al. 2022; Howlett et al. 2023). Given the accelerating increase in this type of activity on the GBR, determining coral population structure is essential to understanding the effects of colony selection and propagation on

genetic diversity (Baums et al. 2019; Shaver et al. 2022). Here, we show that, through cost-effective analyses of SNP markers (25.69 USD per sample in 2021) previously developed for Caribbean *Acropora* species, we can gain information to inform colony selection for propagation in the Indo-Pacific (Kitchen et al. 2020). Specifically, we found evidence of high genotypic diversity for commonly outplanted tabular *Acropora* (notably *A.* cf. *hyacinthus*) within the northern-central GBR. Furthermore, through unintentional sampling of multiple tabular *Acropora* species within our study, we have highlighted the challenges with tabular species taxonomy within the region, which to date has not been easily resolved. In turn we propose the use of a novel genotyping microarray (Kitchen et al. 2020) as a potential solution for rapid species resolution on the GBR.

For our most sampled taxa, *A.* cf. *hyacinthus*, we were unable to discern any population structure based on geographical locality, which may be due to the limitations of applying an array developed for the Caribbean to the Indo-Pacific and the resulting small number of polymorphic markers. It is likely that the lower genomic resolution obtained through the genotyping method itself may have limited resolution of population structure due to SNP ascertainment bias (Lachance and Tishkoff 2013). Population structure could be resolved via shallow genome sequencing or reduced representation sequencing as these methodologies do not rely on predesigned probe sequences (Kitchen et al. 2020; Cooke et al. 2020). However, achieving higher genomic resolution ultimately comes with added costs for data storage and analysis expertise, limiting its widespread adoption in coral restoration projects (Baums et al. 2022). The development of a Pacific Acroporid species microarray may be warranted. Until such a time, it would be useful to benchmark results from the Caribbean genotyping array against genomic sequencing dataset from the GBR (e.g. for *A. kenti*). This could further determine the validity of the SNP microarray for use in restoration within the Indo-Pacific.

Application of the SNP approach here appeared to robustly resolve clonal structure for *A.* cf. *hyacinthus* throughout the region. Overall, populations of *A.* cf. *hyacinthus* within the sampled sites were highly genotypically diverse, where each ramet was genetically unique at half of the sampled reefs and no site contained more than three multi-ramet genets (Baums et al. 2006). As such, sexual recruitment would seem relatively high for this species, given similar trends observed in the Caribbean for other broadcast spawners. High genotypic diversity is typical of well-connected reef sites (whereas genotypically depauperate sites are associated with isolated populations—*A. palmata*; Baums et al. 2006). Observed trends for clonal structure of *A.* cf. *hyacinthus* are therefore indicative of an open population and high gene flow within this GBR region. Conversely, previous studies showed comparatively high clonality for *A. millepora* and *Acropora valida* on the GBR, demonstrating inter-species differences in rates of asexual reproduction and genotypic diversity (Ayre and Hughes 2000). Importantly for *A.* cf. *hyacinthus*, at those sites containing multi-ramet genets (and where ramets were no more than 2 m apart), collection of fragments from colonies > 2m apart can ensure propagation of unique genotypes within restoration projects (Baums 2008; Baums et al. 2019). Such knowledge is essential for ensuring retention of genetic diversity within sites targeted for restoration activity on the GBR (Baums et al. 2019; Canty et al. 2021). Further study on clonality for other commonly propagated species is therefore warranted, given the applicability of these studies to inform guided sampling of source colonies.

Retention of species diversity within restoration activities is key for ensuring resilience and maintenance of reef communities (Quigley et al. 2022; Shaver et al. 2022). As a result of the unintentional sampling of multiple tabular *Acropora* species throughout this study, use of the novel genotyping microarray shows promise in taxonomic resolution of species. Here, we resolved genetic divergence between three clusters visually identified as *A.* cf. *hyacinthus*, *A. pectinata* and *A.* aff. *hyacinthus*, suggestive of three unique species. This finding aligns with other studies that have resolved cryptic species within the *A. hyacinthus* species complex (Ladner and Palumbi 2012; Cornwell et al. 2021). The current lack of taxonomic resolution for these species impairs past and future studies on population structure for *A.* cf. *hyacinthus* and resolving this is key to understanding genetic and species diversity on the GBR, and throughout the Indo-Pacific (Sheets et al. 2018; Holl et al. 2022; Matias et al. 2022). Unintentionally pooling individuals of different species within molecular analyses overestimates genetic diversity and leads to incorrect assumptions of gene flow and population differentiation (Sheets et al. 2018). Rapid species identification is essential for effective reef restoration, thus emphasising the need for a relatively low cost genomic-based approach that can consistently inform practitioners what species are present (and/or can be targeted) for propagation.

Conclusion

Guided selection of coral colonies for further propagation is a key aspect in upscaling restoration projects to ensure sufficient genetic and phenotypic variation (Baums et al. 2019; Quigley et al. 2022). Thus, determining population structure and delineating clonality within source populations of propagated species provides critical information pertaining to colony selection (Baums 2008; Matias et al. 2022). Here, we found that population structure of *A.* cf. *hyacinthus* within the northern-central GBR region

is relatively weak, as assayed with the microarray, and that sites contain high genotypic diversity—consistent with high levels of sexual recruitment. Additionally, our findings add to the growing number of studies highlighting taxonomic uncertainty and underestimations of species diversity on the GBR (Cowman et al. 2020; Holl et al. 2022; Matias et al. 2022). The true scope of evolutionarily relevant diversity for the tabular *Acropora* complex needs to be resolved to ensure the maintenance of species diversity within future restoration projects (Holl et al. 2022; Matias et al. 2022). The 30K SNP genotyping array approach is a suitable tool for resolving taxonomic uncertainty as well as population dynamics, and represents a cost-effective alternative for source material selection in coral restoration. Given the highlighted potential of this genotyping method, we recommend further exploration of its utility across diverse reef geographies and restoration contexts.

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Data availability The data that supports the findings of this study is available from GitHub (https://github.com/lornahowlett/Howlett-et-al-2024-Coral-Reefs) and raw CEL files are available upon request.

Declarations

Conflict of interest The authors declare no financial interests/personal relationships which may be considered potential competing interests.

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