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Genetic Diversity of Sexually Propagated Corals Is Maintained From the Aquarium to the Reef

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ABSTRACT

Amidst global reef declines, large-scale coral aquaculture is being developed to support reef intervention. Genetic diversity underpins population resilience and therefore it is critical that aquaculture methods maintain diversity. However, it remains unclear how genetic diversity of coral progeny is shaped by (1) parental genetic composition, (2) winnowing during aquaculture grow-out, and (3) field deployment. We utilised single nucleotide polymorphisms to examine genetic diversity dynamics in two coral progeny cohorts produced from 5 and 14 parents, with standardised gamete input per parent. Cohorts were sampled over 1 month of aquarium rearing, and for the 14-parent cohort, again after 2 years of field deployment. Parentage analysis confirmed that all parents contributed genetic material to surviving offspring at each cohort's end-point. However, per-parent contributions differed significantly, indicating skewed reproductive success despite equal gamete inputs. Temporal and parent-pool differences in heterozygosity (H_o) were observed during larval stages. At 12 h post-fertilisation, H_o was highest in the 5-parent offspring cohort, and lowest in the 14-parent cohort, reflecting high and low parental genetic dispersion among parents, respectively. By 84 h post-fertilisation, H_o decreased in 5-parent and increased in 14-parent cohorts, suggesting early development purges genetic defects from outbreeding or inbreeding depression, respectively. Importantly, H_o was similar among cohorts and did not decline after 7 days in either cohort, nor after 2 years in the 14-parent cohort, suggesting no evidence of a genetic bottleneck after settlement competency. These findings highlight that parental genetic diversity, rather than the number of parents contributing per se, is crucial to maintain genetic diversity in coral aquaculture, with implications for restoration.

1 | Introduction

In response to rapid declines in global coral reef resilience, there is an emerging focus on developing scalable and sustainable coral aquaculture methods to produce corals for reef restoration efforts (Boström-Einarsson et al. 2020; McLeod et al. 2022; van Oppen et al. 2017). Increasingly, restoration approaches harness the mass gamete production of broadcast spawning corals, with gametes collected as floating wild spawn slicks in situ (Cruz and Harrison 2017; Heyward et al. 2002) or harvested from adult colonies in captivity (Edwards and Gomez 2007). Wild-collected

or aquaculture-produced coral embryos are subsequently reared in nurseries and delivered back to the reef as swimming larvae ('larval seeding'), settled spat ('coral seeding') or established juveniles ('out-planting').

A key goal of ecological restoration is to maintain or increase standing genetic diversity within the target species' population (Kardos et al. 2021). Potential inadvertent outcomes from use of aquaculture-produced individuals include the loss of genetic diversity due to small founder populations (Grant et al. 2017) and rapid adaptation to captivity (Christie et al. 2012). As genetic

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diversity underpins coral adaptation and resilience to disturbances, including thermal stress associated with a warming climate (Palumbi et al. 2014), failing to address these factors could negatively impact the recipient populations in the longer term (Baums et al. 2022; Shearer et al. 2009). Therefore, the potential for coral aquaculture efforts to support reef restoration interventions depends on the ability to create a viable supply of genetically diverse offspring.

The genetic diversity of any sexually produced offspring largely derives from the genetic diversity of parent individuals, genetic recombination during meiosis, and fertilisation of the resultant gametes (Smith et al. 2019). In corals, fertilisation compatibility differs between parental genotype pairs (Baums et al. 2013; Fogarty et al. 2012; Koch et al. 2022; Miller et al. 2018) depending on sperm morphology and egg size, as well as other prezygotic isolation mechanisms, leading to deviations from expected parental contributions regardless of initial gamete ratios (Baums et al. 2013). For example, intraspecific sperm competition has led to elimination of some genotypes within bulk fertilisation trials for acroporids (Hagedorn et al. 2021; López-Nandam et al. 2022). Despite this, most scalable coral aquaculture currently relies on bulk fertilisation cultures, without quantification or standardisation of gamete input from each parent (Chamberland et al. 2017; Cruz and Harrison 2017; López-Nandam et al. 2022; Randall et al. 2021; Severati et al. 2024). Hence, parental contribution biases in coral breeding programs remain poorly explored.

Following fertilisation, genetic diversity can decline due to a variety of winnowing processes (Babcock 1985; Babcock and Mundy 1996; Loya 1976; Wilson and Harrison 2005), skewing the representation of certain parental genotypes. Corals display a type III survival curve (Graham et al. 2008), undergoing high mortality in early life stages, which can contribute to a genetic bottleneck if mortality is genetically driven. In captive environments, even with careful culture techniques and aquarium maintenance, the proportion of coral larvae that successfully settle on surfaces can be below 50% of the initial egg count (Miller et al. 2021; Pollock et al. 2017; Randall et al. 2021; Ritson-Williams et al. 2016), with large proportions of settled spat (up to 90%) dying before metamorphosis (Pollock et al. 2017; Severati et al. 2024). In the wild, pre-competency mortality is similarly high (Babcock 1985; Penin et al. 2010; Wilson and Harrison 2005). Throughout these winnowing processes, genetic diversity can be eroded through mortality and selection. However, it is currently unknown if high early life stage mortality in captivity is driven by aquarium-imposed selective pressures different to pressures in the wild, thus potentially reducing deployed individuals' genetic diversity and fitness for on-reef conditions.

Investigating genetic variation between parents and their sexually produced offspring in captive environments will inform critical understanding of the potential genetic bottlenecks within coral breeding and aquaculture programs. Currently, there is little understanding of how the broodstock size (i.e., the number of parents) influences genetic diversity of offspring populations, despite this being highlighted as a critical knowledge gap and research priority (Banaszak et al. 2023). Indeed, previous work found that after a 2-year field deployment of corals derived from a 4-parent spawn mix, ~95% of surviving offspring were derived

from only one parental cross (López-Nandam et al. 2022). As a result, the vast majority of surviving colonies were full siblings, an outcome that could severely limit genetic diversity and future adaptation to climate warming. Many proposed restoration efforts will rely on large scale aquaculture production of offspring (McLeod et al. 2022), though these processes will always, by necessity, use a limited pool of parental stock compared to a natural population. Current best practices emphasise collecting gametes from a large number of parents, based on the assumption that a higher number of contributors leads to greater genetic diversity in the offspring (Banaszak et al. 2023). However, this assumption has not been empirically tested. Managing genetic diversity in any breeding program is critical and, as such, understanding how parental broodstock numbers and their genetic diversity affect diversity of offspring provides valuable and actionable intelligence for optimising aquaculture operations.

Here, we investigate the genetic diversity of sexually propagated corals through different life stages in relation to the number of contributing parent colonies. Using two batches of offspring produced with five or fourteen parent corals, genetic diversity of broodstock and offspring was compared across aquaria-based rearing of larvae and recruits over 1 month, with assessment of the 14-parent offspring cohort continuing up to 2 years after field deployment on seeding devices (Smith et al. 2025).

2 | Materials and Methods

2.1 | Coral Spawning, Culture Maintenance, and Deployment

Twenty gravid colonies of *Acropora kenti* (sensu Bridge et al. 2023), a simultaneous hermaphrodite, were collected from Yunbenun (Magnetic Island) on the central inshore Great Barrier Reef on 11 October 2021 and transferred to the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS; Townsville, QLD, Australia; under GBRMPA permit G19/42897.1). Colonies were maintained in outdoor aquaria and monitored in isolated tanks for the onset of spawning. Overview of the experimental design is illustrated in Figure 1. On 23 October 2021, five colonies spawned and their gametes cross fertilised (this offspring population is hereafter termed '5-parent cohort'; see Supplementary Table 1 for contributed colonies). On the following night, 24 October 2021, fourteen colonies spawned and their gametes cross fertilised ('14-parent cohort').

After spawning, gamete bundles were collected from each isolated parent colony, and eggs were separated from sperm using a 100- μ m filter, then rinsed multiple times with 0.4- μ m filtered seawater (FSW) to remove residual sperm. Eggs were retained in 500 mL of filtered seawater, and sperm retained in a 50-mL tube. A 1-mL subsample of sperm was used to quantify sperm concentration via Computer-Assisted Semen Analysis (CASA; Zuchowicz et al. 2021). Three replicate 100- μ L subsamples of eggs were counted per parent colony to calculate total eggs available for fertilisation (Supplementary Table 2). Prior to fertilisation, quantities of eggs and sperm were standardised such that each parent contributed the approximate same number of eggs and sperm to each of three triplicate bulk cultures (Supplementary Table 2). Eggs and sperm volumes for each

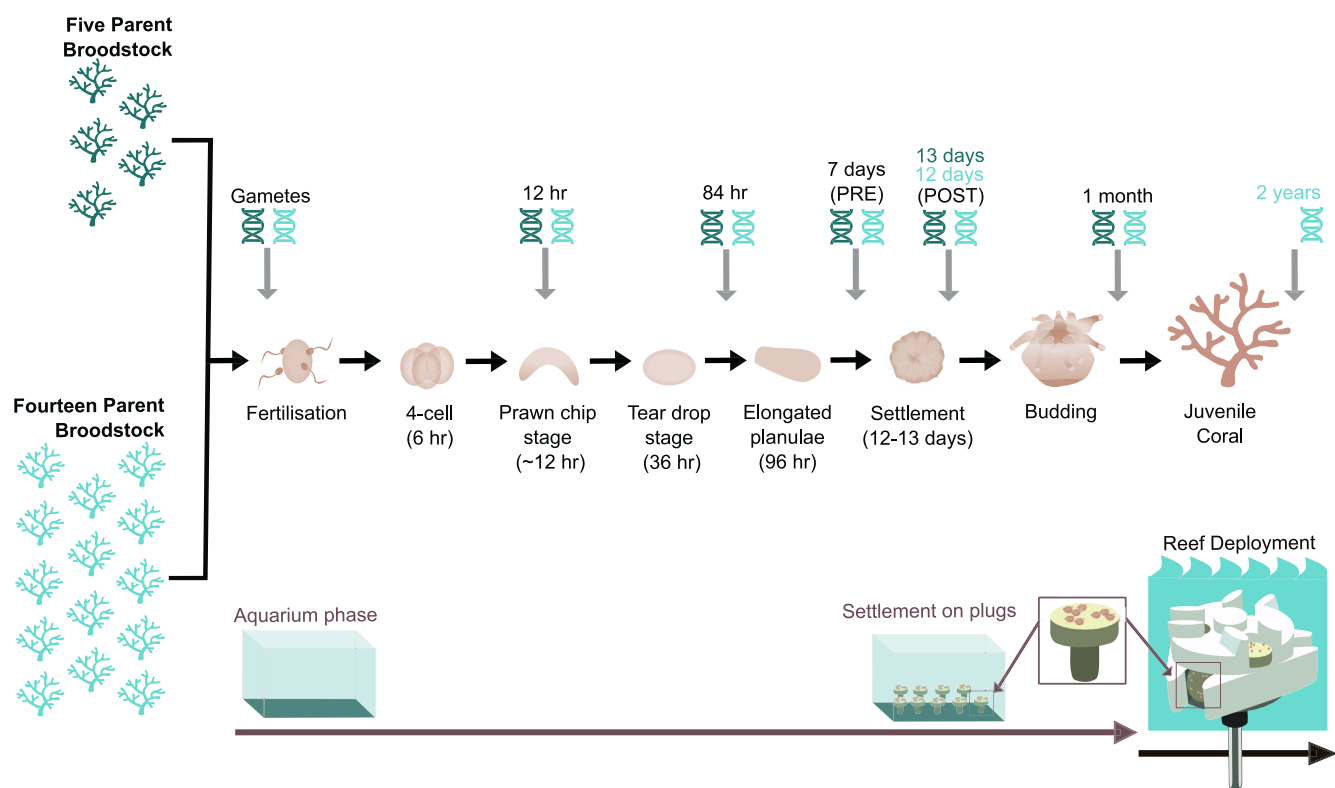


FIGURE 1 | Schematic overview of the experimental design and sampling workflow tracking the genetic diversity of the offspring produced by two broodstock cohorts (5-parent (light blue) and 14-parent (dark green), noting two parent colonies were included in both cohorts due to spawning behaviour (i.e., spawned on consecutive nights)) through key developmental stages. DNA symbols indicate each sampling timepoint, with arrows indicating time of sampling relative to time of fertilisation. Sperm was collected on each night of spawning and preserved to genotype parents, developing embryos were collected at 12 and 84 h after fertilisation, before settlement (7 days post spawning), post-settlement (13 days post spawning of 5-parent cohort; 12 days post spawning of 14-parents), and one-month post-spawning. Juvenile offspring of the 14-parent cohort were sampled after two-years of reef deployment on coral seeded devices. Sample sizes are indicated in Table 1.

TABLE 1 | Sample size (*n*) of tissue samples and number of loci before filtering (A), retained after filtering for (B) analysis of μH_e , H_o , and F_{IS} values, (C) principal component analysis (PCA), and (D) parentage analysis; grouped by 5-parent cohort and 14-parent cohort.

Sampling timepoint (time since spawning)	(A) Sample size for raw data		(B) Sample size for H_e , H_o , F_{IS}		(C) Sample size for PCA		(D) Sample size for parentage assignment	
	5-Parent Cohort	14-Parent Cohort	5-Parent Cohort	14-Parent Cohort	5-Parent Cohort	14-Parent Cohort	5-Parent Cohort	14-Parent Cohort
Sperm	5	14	5	14	5	14	5	14
12 h	72	60	68	49	68	55	67	56
84 h	63	57	49	54	49	56	49	56
7 days (Pre-settlement)	57	59	56	55	56	57	56	57
12–13 days (Post-settlement)	30	30	30	30	30	30	30	30
1 month	30	30	29	30	29	30	29	30
2 years	—	114	—	78	—	81	—	82
Total	257	364	237	310	237	323	236	325
Number of loci	34,282	34,282	4355	4355	4355	4355	625	519

parent were measured and combined in three replicate sterile 30-L tanks of FSW and left to fertilise for 1 h with no aeration or water flow, with occasional stirring to facilitate homogeneous sperm-egg encounters. After 1 h, fertilised embryos were transferred by gentle pipetting into three replicate 60-L tanks of filtered seawater to rinse off excess sperm before finally being transferred by pipette into three replicate 500-L tanks with FSW, aeration and low water flow rate.

Larval settlement competency assays were completed daily for each culture, beginning at 3 days post fertilisation. Briefly, 6-well plates were filled with 10 mL of FSW per well, with three wells containing a small chip of crustose coralline algae (CCA; *Porolithon onkodes*), a known inducer of settlement for *Acropora kenti* (Whitman et al. 2020), and three wells without CCA as no-cue controls for settlement. Ten individual larvae were added to each well, and the plates were kept in the dark overnight at 28°C. Larval settlement was assessed under a Nikon SMZ740 photomicroscope by classifying larvae visually into categories: metamorphosed, attached but not metamorphosed, crawling, swimming or dead. This process was repeated daily until a competency rate of >90% was reached for all three replicate larval cultures, which was achieved at a larval age of 7 days.

For the 5-parent cohort, approximately 3000 competent larvae from each of the triplicate cultures were placed into each of six 48-L tanks containing FSW and pre-conditioned aragonite plugs ($n=2$ tanks per culture, $n=53$ plugs per tank) for settlement. For the 14-parent cohort, approximately 5000 larvae from each triplicate culture were placed into each of twelve 48-L tanks with FSW ($n=4$ tanks per culture, $n=165$ plugs per tank) for settlement. Plugs had been pre-conditioned in outdoor aquaria for 6 weeks prior to spawning to develop a mature biofilm, including CCA, to induce larval settlement. Plugs were transferred to a clean, unfouled tray prior to settlement to encourage larvae to settle on conditioned plug surfaces. Settlement tanks were provided with FSW at a flowrate of 0.8 L per hour (100% tank replacement every 24 h) and maintained under $1 \mu\text{mol}/\text{m}^2/\text{s}$ photosynthetically active radiation (PAR). Successful larval settlement was confirmed after 48 h by examining 20 haphazardly selected plugs per tank. All plugs were then placed randomly into new trays (and their plug location recorded) to eliminate spat that settled on trays rather than plugs, and transferred to a 1000-L indoor tank supplied with filtered seawater at 28°C. Fragments of adult broodstock were placed in the tank to supply algal symbionts (Symbiodiniaceae dinoflagellates) to the developing juveniles (Nitschke et al. 2015). For the first week, the tank was exposed to a maximum of $33 \mu\text{mol}/\text{m}^2/\text{s}$ PAR lighting, then ramped daily to a maximum of $300 \mu\text{mol}/\text{m}^2/\text{s}$ PAR by deployment (14-parent cohort only) on 24 November.

Prior to the field deployment of coral juveniles (14-parent cohort only), plugs were inserted into triangular ceramic deployment devices (Shanghai Gongtao Ceramics Co. Ltd.; designed by AIMS). Devices feature protrusions designed to provide coral recruits refuge from grazing pressure by herbivorous fishes (Whitman et al. 2024). Three plugs (one with juveniles from each triplicate 14-parent bulk culture, to control for batch effects) were placed in the upward-facing ('top') position, and three in a side-facing ('side') position (See Figure 1 'Reef Deployment'). Plugs with the highest number of individual juveniles were

selected visually, on the assumption that more starting juveniles would increase the chance of survival through field deployment (but see Smith et al. 2025). All plugs were photographed using a Nikon D810 camera prior to deployment. On 24 November 2021, devices were deployed to field sites at Yunbenun (Magnetic Island, GBRMPA permit G21/45348.1) with 60 devices in Arthur Bay ($-19.128894, 146.877496$) and 60 devices in Florence Bay ($-19.121912, 146.880308$). Devices were fastened to the reef substrate with nylon connectors onto threaded stainless-steel rods that were hammered into the benthos by SCUBA divers. Devices were censused visually for recruit survival every 3 months (see Smith et al. 2025 for survival dynamics) until retrieval approximately 2 years later, on 20 August 2023, when devices were transported by boat to SeaSim in 100-L bins of seawater.

2.2 | Tissue Sampling and Sequencing

Tissue samples were collected from corals at different stages of culture preparation and larval development to examine genetic diversity over time and between the parent cohorts (Table 1A). Samples of sperm from each parent colony were collected on each night of spawning (Supplementary Table 1) and used for genotyping parents. Developing embryos were collected from triplicate cultures at 12 and 84 h after fertilisation, during the period of larval development (Okubo et al. 2013). Larvae were collected immediately prior to settlement (7 days post spawning; termed herein as 'Pre'), post-settlement (13 days post spawning of 5-parent cohort; 12 days post spawning of 14-parent cohort; termed herein 'Post'), and 1 month post spawning (or approx. 2 weeks post-settlement). Following the approximately 2-year field deployment (14-parent cohort only), all surviving corals that were classified visually as *A. kenti* (and hence assumed to be the deployed corals) were collected for genetic analyses.

All tissue samples (sperm, embryos, larvae, and 2-year-old juveniles) were fixed in absolute ethanol and stored at -20°C until further processing. These tissue samples (12–15 mg; total $n=846$) were sent to Diversity Arrays Technology Pty Ltd. (DART, Canberra, Australia) for DNA extraction and genotyping of single nucleotide polymorphisms (SNPs). Genomic DNA was extracted following DART proprietary protocols (Kilian et al. 2012). DARTseq is a combination of genome complexity reduction methods based on restriction enzyme fragmentation, followed by DNA sequencing with an Illumina HiSeq 2500 next-generation sequencing platform (Kilian et al. 2012). SNPs were generated by DART with reference to an *A. kenti* genome (<http://aten.reefgenomics.org>; labelled as '*Acropora tenuis*') (For positional alignment of raw SNP mapping positions, see Supplementary Figure 1).

2.3 | Analysis of Genetic Diversity Markers, Parentage Assignment and Statistical Analysis

Sequencing data were returned pre-processed by a DART proprietary pipeline in a scored format (0/1/2 for homozygous reference allele, homozygous alternate allele, heterozygous, respectively), and SNP markers indicated as present or absent from the respective samples. The raw DARTseq SNP dataset contained 34,282 SNPs. For population level analyses, filtering of

SNPs was conducted on the full dataset concurrently (i.e., both 5- and 14-parent cohorts together) using the R package *dartR* (Table 1A) (Gruber et al. 2018). Filtering steps included removing secondary tags within each fragment, locus reproducibility > 95%, locus call rate > 90%, SNPs with minor allele frequency (MAF) < 5%, monomorphic loci were removed, and individuals with call rate < 80% of the filtered loci were removed. While loci under selection are generally removed for demographic studies, removing outlier loci could interfere with parentage assignment if a broodstock is under/over-represented in offspring, hence SNPs potentially under selection were retained. The filtered dataset retained 5278 SNPs and 548 individuals (Table 1B).

Principal component analysis (PCA) was performed using the package *ade4* (v. 1.7-22) (Dray and Dufour 2007) to visualise genotypic diversity among individuals and timepoints, with missing data imputed using the neighbour method. Upon visualisation, there was a clear cluster of outliers with genotypes outside the bounds of all parental samples, likely reflecting a group of wild-settled *Acropora kenti* individuals (Supplementary Figure 2). SNP filtering (using the same filtering parameters described above) was performed again excluding data from these individuals ($n = 27$), which were removed from all subsequent analyses (Table 1C). PCAs were repeated without the purported wild-settlers and differences in genotypic diversity between timepoints was visualised with 90% confidence ellipses. Significant differences in genetic dispersion among timepoints were tested using the function *PERMDISP2* in the package *vegan* (v.2.6-4) (Oksanen et al. 2015).

To assess variation within and among coral cohorts, we calculated measures of genetic diversity, including observed and expected heterozygosity unbiased by sample sizes (H_o and H_e , respectively), Wright's inbreeding coefficient (F_{IS}), and effective population size (N_e). Each metric was calculated for each timepoint for each triplicate culture within each parent mix. Heterozygosity values (H_o and H_e) are reported as mean values calculated by loci, using the package *hierfstat* (v. 11; Goudet 2005). Estimates of N_e with 95% jackknife confidence intervals (CIs) were calculated per timepoint using the linkage disequilibrium method with an allele frequency threshold of 0 to not exclude rare variants, with singletons included, in the R package *dartR.popen* (Gruber et al. 2018) as a wrapper for NeEstimator v2.1 (Do et al. 2014). To increase sample size and confidence in jackknife estimates, we pooled the three cultures for N_e analysis.

To identify whether observed heterozygosity (a proxy for genetic diversity at an allelic level and therefore indicative of adaptive potential) and F_{IS} (a measure of inbreeding) changed over time within parent cohorts, generalised linear mixed effects models (glmm) were fit using R package *glmmTMB* (v. 1.1.9) (McGillucuddy et al. 2025). Observed heterozygosity (H_o) and inbreeding (F_{IS}) were each modelled separately for each cohort as a function of timepoint, with triplicate culture tank as a random effect to account for shared tank-level variance. To identify whether H_o and F_{IS} varied between parent cohorts, an additional set of glmms were fitted, modelling H_o and F_{IS} as interactive functions of timepoint and parent cohort, with triplicate culture as a random effect. These analyses excluded the 2-year samples from the 14-parent cohort. The models for

heterozygosity (continuous positive data) used a gamma distribution and log-link, while inbreeding (continuous positive and negative data) used a gaussian distribution. Pairwise contrasts between heterozygosity values for timepoints and parent cohorts were made using estimated marginal means and Tukey's post hoc adjustment, calculated using the R package *emmeans* (v.1.10-0) (Lenth 2025). Model residuals were checked using the R package *DHARMa* (v. 0.4.6) (Hartig 2022).

Parentage was assigned for offspring corals among all timepoints for each of the 5-parent and 14-parent cohorts using the R package *sequoia* (v.2.9.0, Huisman, 2017). To maximise assignment of correct parent-offspring relationships, parentage assignment required more stringent filtering of SNPs than population summary metrics described above and were fine-tuned for each parent cohort using multiple *sequoia* runs. For the 14-parent cohort, filtering steps included removing secondary tags, loci reproducibility > 90%, loci call rate > 80%, MAF < 5%, individuals with call rate < 50%, and monomorphic loci were removed, retaining 540 loci and 367 individuals. For the 5-parent cohort, SNPs were filtered to remove secondary tags, by loci reproducibility > 95%, loci call rate < 95%, MAF < 5%, individual call rate < 95%, and to remove monomorphic loci, retaining 633 loci and 328 individuals (Table 1D). Different filtering parameters were used for each cohort to optimise parentage assignment.

Parental contribution (defined herein as the surviving component of each initial parent's input) was tallied for each parent for each timepoint, and relative contribution per parent was calculated as the percent of the total count of offspring per timepoint. To examine whether parental contributions deviated from a null expectation of equal contribution (20% for the 5-parent cohort, 7.14% for the 14-parent cohort), we fitted a series of Bayesian GLMMs separately for each parent using the package *brms* (Bürkner 2021). For each parent, the response variable was the proportion of offspring assigned to that parent at each timepoint, modelled using a beta distribution and logit link. Timepoint was included as a fixed effect, and culture tank as a random effect. All models used weakly informative priors, 10,000 MCMC sampling iterations each across three chains, with a warmup of 1000 and thinned to every fifth observation. Model diagnostics (trace plots, auto-correlation, r-hat, posterior predictive checks, effective sample sizes, residuals and dispersion) were checked using the R package *DHARMa* (Hartig 2022) and *rstan* (Stan Development Team 2025). For all models, diagnostics suggested model assumptions were met, chains were well mixed, and converged on a stable posterior. Bayesian probabilities were calculated to determine if parental contribution at each time point deviated from the expected equal value (i.e., 20% for 5-parent, or 7.14% for 14-parent).

To assess the evenness of family-cross representations over the study duration, the frequency of each parent pair across all timepoints was tallied using a contingency table of assigned offspring, and the resulting parent-pair frequency matrix was plotted as a directional chord diagram using package *circlize* (v 0.4.16; Gu et al. 2014). The parents and offspring were visualised as nodes and links, respectively, with the thickness of links corresponding to the number of offspring produced by the cross. To determine if endpoint survivors reflected random mating (and random survival), the contingency table of number of

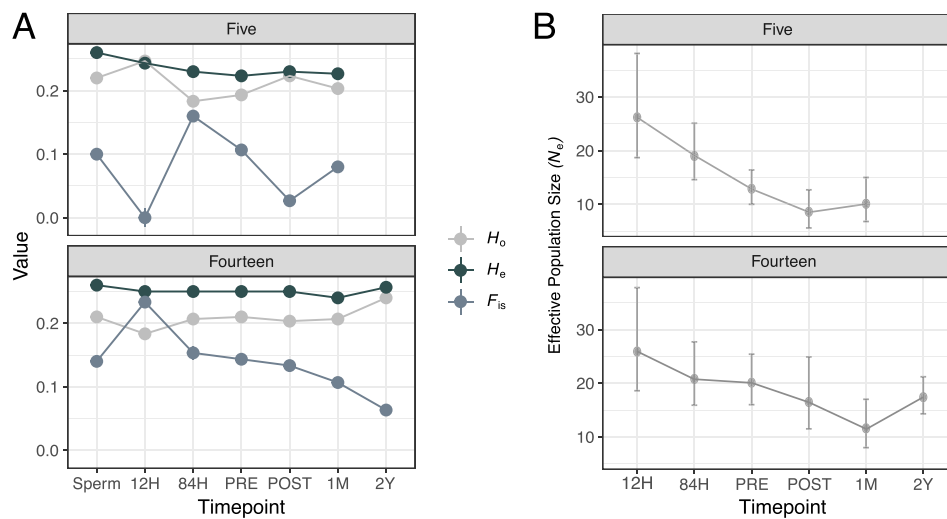


FIGURE 2 | Scatter plots showing mean observed heterozygosity ($H_o \pm SE$), mean expected heterozygosity ($H_e \pm SE$), and mean inbreeding coefficient ($F_{is} \pm SE$) of the seeded corals by each timepoint; blue points on the right of each timepoint show estimates of effective population size ($N_e \pm 95\%$ jackknife CI); within the (A) 5-parent mix (12h to 1 month post fertilisation) and (B) 14-parent mix (12h to 2 years). Sample sizes are indicated in Table 1. Sperm; representing parents, PRE; pre-settlement larvae (7 days), POST; post-settlement recruits (12–13 days). Parents' N_e were estimated as infinite for the 5-parent broodstock and the upper CI of the 14-parent broodstock (data not illustrated).

offspring produced per parent pairing was analysed using a chi-squared test. All statistical analyses were performed using R (R Core Team 2024), and figures were made using package *ggplot2* (Wickham 2016) unless otherwise specified.

3 | Results

3.1 | Temporal Patterns in Heterozygosity and Inbreeding Varied Between Parent Cohorts

A total of 237 coral samples consisting of embryos, planulae and recruits were retained for analyses of genetic diversity markers from the 5-parent fertilisation culture (5-parent cohort) across the five sampling time points (12h, 84h, 7 days, 13 days and 1 month; Table 1B). The observed heterozygosity (H_o) of the 5-parent cohort showed a significant increase from parent samples (i.e., sperm; model estimated mean $H_o = 0.21 \pm 0.01$ SE) to 12h embryos ($H_o = 0.25 \pm 0.01$; $t = -3.21$, $p = 0.02$; Figure 2, Supplementary Table 3). However, significant reductions in H_o relative to 12h samples were observed by the 84h timepoint ($H_o = 0.18 \pm 0.004$; $t = -8.61$, $p < 0.01$), and remained significantly reduced until the final 1-month timepoint ($H_o = 0.20 \pm 0.005$; $t = -5.67$, $p < 0.01$; Figure 2, Supplementary Table 3).

In the 5-parent cohort, the inbreeding coefficient (F_{is}) at the 12h timepoint (model estimated mean $F_{is} = 0 \pm 0.02$ SE) was significantly lower than that of parents ($F_{is} = 0.11 \pm 0.03$; $z = 3.29$, $p < 0.01$; Figure 2, Supplementary Table 4). There was a significant increase from 12h to the 84h timepoint ($F_{is} = 0.16 \pm 0.02$; $t = -6.77$, $p = 0.001$). After 84h, F_{is} declined over the 1-month aquarium rearing, reaching a similar value to the parents ($F_{is} = 0.076 \pm 0.02$; $t = -1.0$, $p = 0.91$; Figure 2, Supplementary Table 4).

A total of 310 coral samples consisting of sperm, embryos, planulae, recruits, and two-year-old juveniles were retained for

diversity analyses of genetic markers from the 14-parent batched fertilisation culture (14-parent cohort) across six sampling time points (12h, 84h, 7 days, 13 days, 1 month, and 2 years; Table 1B). There was no significant change in H_o between parent samples ($H_o = 0.21 \pm 0.01$ SE) and 12h embryos ($H_o = 0.18 \pm 0.01$; $z = 2.76$, $p = 0.08$; Figure 2, Supplementary Table 3). However, in contrast to the 5-parent cohort, there was a significant increase in observed heterozygosity over the time series, ending in significantly higher H_o relative to 12h samples at the end of both the one-month aquarium rearing phase ($H_o = 0.21 \pm 0.01$, $z = 3.36$, $p = 0.01$) and the 2-year field deployment ($H_o = 0.24 \pm 0.01$, $z = 6.91$, $p < 0.01$; Figure 2, Supplementary Table 3).

In the 14-parent cohort, F_{is} was 70% higher in the 12h embryo timepoint (model estimated mean $F_{is} = 0.24 \pm 0.01$ SE) compared to the parents ($F_{is} = 0.14 \pm 0.02$; $t = 3.73$, $p = 0.04$). The F_{is} also showed an overall decline (Figure 2, Supplementary Table 4) from the 12h sampling timepoint to the end of the one-month aquarium rearing period ($F_{is} = 0.10 \pm 0.01$; $t = 7.65$, $p < 0.01$). At the end of field deployment, F_{is} in the two-year juveniles ($F_{is} = 0.06 \pm 0.01$) was lower than the 1-month spat, but not statistically significantly lower ($t = 2.19$, $p = 0.38$; Figure 2, Supplementary Table 4).

3.2 | Larger Parent Pool Size Does Not Necessarily Increase Genetic Variation

When directly comparing H_o across the two cohorts, there was no difference in the starting heterozygosity of parent samples (i.e., sperm; $z = 0.0$, $p = 1.0$; Supplementary Table 3). At the 12h embryo timepoint, the 5-parent cohort had significantly greater heterozygosity (model estimated mean $H_o = 0.25 \pm 0.01 \pm SE$; $z = 8.8$, $p < 0.01$) than the 14-parent cohort ($H_o = 0.18 \pm 0.01$). However, by the end of the 1-month aquarium rearing phase, observed heterozygosity was the same between the two cohorts ($z = -0.45$, $p = 0.65$). Similarly, F_{is} did not vary between the two

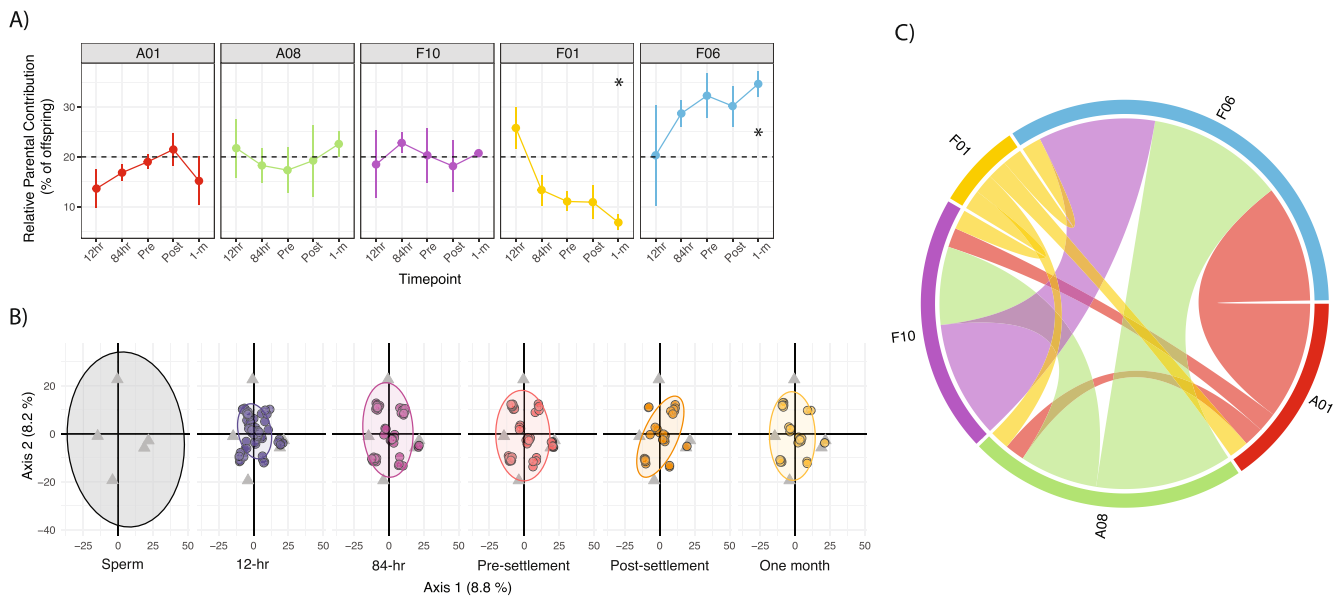


FIGURE 3 | (A) Relative parentage contribution of each parental genotype within each time point ($\pm$ SE), over the course of a one-month aquarium rearing, spanning larval development (12 and 84 h after fertilisation), pre-settlement larvae (PRE; 7 days), post-settlement recruits (POST; 12–13 days) and juveniles (1 m; 1 month) for the 5-parent cohort. Each colour and facet is representative of an individual parent assigned. Dashed black lines indicate the expected equal contribution of each parent given equal gamete input (i.e., 20%), with stars indicating strong evidence (Bayesian probability $\geq 95\%$) of deviations from equal contribution. (B) Principal Component Analysis (PCA), with 90% confidence ellipses, depicting the genetic dispersion of SNP genotypes spanning parents (represented by sperm), larval development (12 and 84 h after fertilisation), pre-settlement larvae (PRE; 7 days), post-settlement recruits (POST; 12–13 days) and juveniles (1 m; 1 month). Colours represent sampling timepoint and each point represents one individual. Triangular points shown in all facets represent parental (sperm) genotypes. The amount of variation explained by each axis is given in parentheses on axis labels. (C) Chord network diagram depicting all surviving parent crosses at the end of the 1-month aquarium rearing for the 5-parent cohort. Each colour node is representative of an individual parent assigned, each link represents resulting offspring, with link thickness correlated to the number of offspring. Sample sizes for all analyses are indicated in Table 1.

parent populations ($t = -0.83$, $p = 0.42$), was significantly lower in the 5-parent compared to 14-parent at 12 h embryo stage ($t = -11.62$, $p < 0.01$), and were the same by the 1-month timepoint ($t = -1.27$, $p = 0.22$; Supplementary Table 4).

Effective population size (N_e) for the 5-parent cohort showed a gradual decline from a maximum of 24.7 (95% CI: 18.3–34.0) at the 12 h larval stage to a minimum of 10.4 (95% CI: 7.5–14.5) at the sampling endpoint of 1-month old spat (Figure 2A). In contrast, the 14-parent cohort generally had higher estimates of effective population size, with no consistent decline over the study duration (Figure 2B). At the 12 h larval stage, N_e was 32.9 (95% CI: 25.4–44.1) and showed no significant change by the sampling endpoint of 2 years ($N_e = 26.56$; 95% CI: 20.8–33.9). The overlapping confidence intervals between early and late stages of this study suggest a relatively stable N_e over time.

As analysed in PCA, genotypic variation (assessed using PERMANOVA) of the 5-parent cohort showed no difference across timepoints ($F_{5,231} = 0.49$, $p = 0.94$), but there was a significant difference in dispersion among timepoints ($F_{5,231} = 6.59$, $p < 0.001$; Figure 3B). The difference in dispersion was driven by a generally reduced multivariate spread in larval stages compared to parents. In the 14-parent cohort, there was no variation across timepoints in observed multivariate dispersion ($F_{6,302} = 0.78$, $p = 0.59$) or genetic variation ($F_{6,302} = 1.52$, $p = 0.08$; Figure 4B). Two of the 14-parent colonies were highly similar, with overlapping multivariate genotypic profiles indicating possible clones (A09 and F03).

3.3 | Genetic Contributions Were Maintained Though Unequal Across Aquarium and Reef Deployment Phases

Of the 462 possible parent-offspring assignments (two assignments per individual offspring), 350 (75.8%) were successfully assigned within the 5-parent cohort (Supplementary Figure 3A). Offspring representing all five parent colonies were present in each sampling timepoint of the 1-month aquarium rearing phase (Figure 3A). Parental contribution did not deviate from expected equal contributions (i.e., 20% each) at the first time point, but two parents exhibited unequal contributions in subsequent timepoints despite normalised gamete contributions (Figure 3A, Supplementary Table 5). Specifically, the relative parental contribution of colony F06 displayed a positive trend over the rearing period: up to $35.9\% \pm 3\%$ of 1-month-old offspring were parented by F06, or 1.8 times the expected number of offspring given equal gamete input (Bayesian probability of parentage $> 20\% = 0.957$; Figure 3A; Supplementary Table 5). In contrast, the F01 derived offspring contribution had a negative trend over time, with no evidence for variation from equal parentage contribution at 12 h ($22.4\% \pm 4.4\%$), but strong evidence that the contribution was less than expected at 1-month, declining to $7\% \pm 1.5\%$ contribution (Bayesian probability of parentage $< 20\% = 0.979$; Figure 3A, Supplementary Table 5).

Out of the 622 possible parent-offspring assignments across the 14-parent cohort (two possible per offspring), 506 (81.4%) were successfully assigned (Supplementary Figure 3B). Offspring

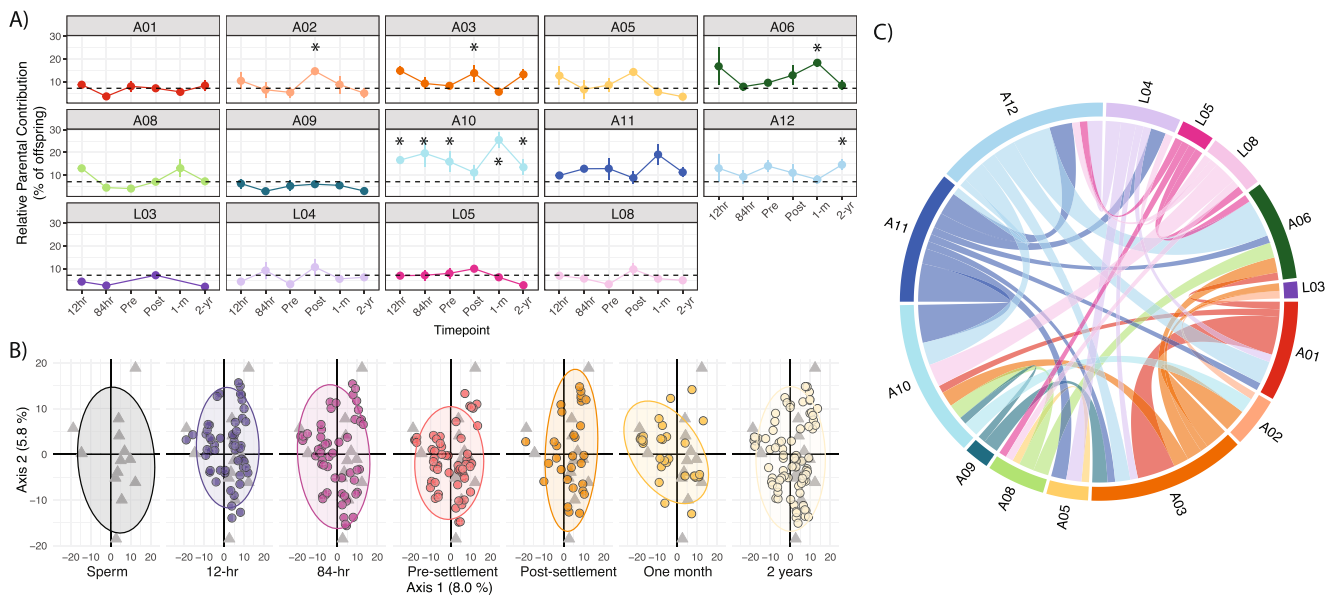


FIGURE 4 | (A) Relative parentage contribution of each parental genotype within each time point ($\pm$ SE), over the course of a one-month aquarium rearing, spanning larval development (12 and 84 h after fertilisation), pre-settlement larvae (PRE; 7 days), post-settlement recruits (POST; 12–13 days) and juveniles (1 m; 1 month), and after 2-year field growth (2 years) for the 14-parent cohort. Each colour and facet is representative of an individual parent assigned. Dashed black lines indicate the expected equal contribution of each parent given equal gamete input (i.e., 7.14%), with stars indicating strong evidence (Bayesian probability $\geq 95\%$) of deviations from equal contribution. (B) Principal Component Analysis (PCA), with 90% confidence ellipses, depicting the genetic dispersion of SNP genotypes spanning parents (represented by sperm), larval development (12 and 84 h after fertilisation), pre-settlement larvae (PRE; 7 days), post-settlement recruits (POST; 12–13 days) and juveniles (1 m; 1 month), and after 2-year field growth (2 years). Colours represent sampling timepoint and each point represents one individual. Triangular points shown in all facets represent parent (sperm) genotypes. The amount of variation explained by each axis is given in parentheses on axis labels. (C) Chord network diagram depicting all surviving parent crosses at the end of the 2-year field deployment (2 years) for the 14-parent cohort. Each colour node is representative of an individual parent assigned, each link represents resulting offspring, with link thickness correlated to the number of offspring. Sample sizes for all analyses are indicated in Table 1.

derived from each of the 14 parents were represented within the 2-year old timepoint, indicating that all parents' genetic contributions remained in the surviving population after 2 years (Figure 4A). Parental contributions were generally stable through time, with most parents contributing approximately expected equal contributions (i.e., 7.14% each) across time points. However, there were some deviations from expectations. For example, Bayesian probabilities showed that colony A10 had consistently higher relative contribution to the offspring cohort relative to other parents, comprising a maximum percentage of $31.2\% \pm 3.3\%$ of the offspring at 1-month (Figure 4A, Supplementary Table 6). At the end of the 2-year deployment, two parents (A10 and A12) had a Bayesian probability suggesting that their contribution was significantly higher than expected (i.e., Bayesian probability of parentage over $7.14\% \geq 0.95$; Figure 4A, Supplementary Table 6). While the parent colony F03 was not detected as a contributor to the 1-month spat, it was detected again in the 2-year timepoint, likely reflecting a stochastic sampling effect. Colony F03 had the lowest contribution at the 2-year timepoint but was still present ($2.4\% \pm 0.06\%$), indicating that it was not entirely winnowed from the population.

3.4 | Offspring Cohorts Were Derived From Diverse Family Crosses

In both the 5-parent and 14-parent cohorts, parentage analysis indicated that surviving offspring resulted from diverse parent

crosses, as opposed to being dominated by a few specific crosses. For the 5-parent cohort, each cross was equally represented at the one-month endpoint, suggesting mating and survival were random ($\chi^2 = 14.6$, $df = 9$, $p = 0.10$; Figure 3C). However, uneven family contributions were observed for the 14-parent cohort, with non-random survival documented at the end of the 2-year deployment ($\chi^2 = 171$, $df = 121$, $p < 0.01$; Figure 4C). Self-fertilisation occurred within the 14-parent mix by colony A10, with these offspring accounting for 4.25% of the total offspring across all timepoints.

4 | Discussion

Coral reefs are in crisis, increasingly prompting novel coral restoration trials (Boström-Einarsson et al. 2020; McLeod et al. 2022; van Oppen et al. 2017). Supportive breeding, a long-established conservation tool for replenishing declining populations (National Research Council 2004; Ripley 1973), is now being adapted to conserve the genetic diversity of coral reefs (Baums et al. 2019; Randall et al. 2020). The scale of restoration required to mitigate ongoing reef degradation will require scalable aquaculture breeding approaches that preserve genetic diversity across large numbers of out-planted corals (Baums 2008; van Oppen et al. 2017). Past attempts to scale coral aquaculture using sexual reproduction with more than two parents have revealed a dominance of specific crosses, with only full sibling colonies surviving a two-year

reef deployment (López-Nandam et al. 2022). Breeding with more parents is assumed to increase diversity of offspring (Banaszak et al. 2023), however, our results show that the effective population size of offspring did not differ between the 5- and 14-parent cohorts significantly, and genetic diversity of offspring did not correlate with parent number. Other important metrics for long-term population viability, observed heterozygosity and inbreeding coefficient estimates, showed variation between offspring produced from 5- versus 14- parents in early timepoints. However, by 1-month-old, there was no difference, suggesting that inbreeding and outbreeding depression were purged during early development. High heterozygosity can reduce genetic load by lowering the frequency of deleterious homozygotes. Importantly, the contribution of any single parent to offspring from either the 5- or 14-parent cohort was not completely winnowed during the breeding, settlement and grow-out periods, with no evidence of genetic bottlenecks across both aquarium and reef environments for 2 years in the case of the 14-parent cohort.

4.1 | Differential Parental Contribution to Offspring Cohorts Depicts Skewness of Reproductive Success

Previous investigation of fertilisation compatibility for batched cultures of Acroporids reported severe winnowing effects when more than two sperm genotypes are present in the broodstock population (Hagedorn et al. 2021; López-Nandam et al. 2022). For example, López-Nandam et al. (2022) reported that out of a 4-parent cohort, only one cross was successful, resulting in 22 full-sibling offspring surviving after 2 years. In direct contrast, our 5- and 14-parent crosses resulted in successful mating of all possible parental pairs, with all parents genetically contributing to the resultant offspring cohorts and the survival of offspring observed for 1 month and 2 years, respectively. Despite no evidence of a winnowing effect, the genetic contributions of individual parents varied from the equal contributions expected from standardised gamete input and theoretical random mating of all parents. While certain genotypes were consistently overrepresented, such as the contributions of colony A10 to the 14-parent cohort and F06 to the 5-parent cohort, their progeny did not completely dominate the offspring cohorts at any timepoint, highlighting the relative and stochastic nature of parental contributions during bulk fertilisation. It is possible that prezygotic and postzygotic barriers affected compatibility of some crosses, which may have underpinned the under-representation of some specific crosses. For example, varying gametic properties, such as sperm speed (Leviton 2000) or phenotypic disadvantages (Baums et al. 2013), can result in differential parental fertilisation success and may be a possible cause for the skewed yet persistent contributions from specific colonies. Furthermore, parental contribution by timepoint can be impacted by the non-random survival of progeny over time, as well as stochasticity due to sub-sampling for genotyping. If ensuring equal parental contributions is a goal for a given restoration program, it may be necessary to perform targeted fertilisation between specific crosses, rather than bulk crossing all gametes, followed by pooling of offspring families equally at later larval stages or post-settlement.

Importantly, our study demonstrated that parental diversity was more influential for genetic diversity estimators of the offspring cohort than the number of parent colonies per se (i.e., five vs. fourteen). Offspring produced from five parents with high genetic diversity and dispersion exhibited comparable levels of observed heterozygosity (H_o), effective population size (N_e), and inbreeding (F_{IS}), to those produced from fourteen parents. This suggests that a smaller number of well-selected, genetically diverse broodstock can be just as effective as a larger number of less diverse broodstock in maintaining offspring heterozygosity, and hence adaptive capacity. That said, a previous estimate of contemporary effective population size estimated an N_e of ~10,000 for *A. kenti* at the same study site (Cooke et al. 2020). Compared with that estimation, our study's calculated N_e was extremely low (<20 for the one-month timepoint of the 5-parent cohort, <40 for the 2-year timepoint of the 14-parent cohort). While low N_e in this study is likely a reflection of the low number of broodstock utilised compared to wild populations, it is critical to consider how seeding hundreds to thousands of juveniles derived from a small selection of parents may contribute to genetic bottlenecks beyond our 2-year sampling timeframe. In future work, it would be valuable to identify how many parents are required to capture sufficient diversity to retain an N_e comparable to the natural population. Nevertheless, N_e did not decline over time within either cohort's 1-month aquarium rearing phase, nor did the 14-parent cohort's N_e show evidence of decline over the reef deployment. These findings underscore the critical importance of broodstock selection for aquaculture-based sexual propagation of coral larvae. While previous studies have emphasised the importance of starting with a sufficient number of broodstock, our results show that genetic diversity within parental broodstock is an equally or more important factor for supporting restoration goals. Therefore, coral breeding programs should develop and implement genetic screening to maximise parental genetic diversity when designing large-scale, aquaculture-based restoration initiatives.

Interestingly, we observed self-fertilisation, albeit infrequent, by colony A10 within the 14-parent cohort as evidenced by parentage analysis. Low levels of self-fertilisation have previously been reported in individuals of several other Acroporid species, including *A. palmata* (Baums et al. 2005, 2013; Fogarty et al. 2012; Vasquez Kuntz et al. 2022), *A. cervicornis* (Fogarty et al. 2012; Koch et al. 2022), and *A. valida* (Willis et al. 1997), and may suggest that the allorecognition system within colony A10 is not entirely functional, allowing self-fertilisation (Puill-Stephan et al. 2009). These findings highlight that intrinsic biological factors such as gamete compatibility, competitive fertilisation dynamics and allorecognition mechanisms can shape parental contributions to offspring populations within coral aquaculture.

4.2 | The Three-Day Developmental Window Is an Initial Period of Selection in Aquarium-Reared Corals

At the 12-h timepoint, the 5-parent cohort exhibited an excess of heterozygotes and low F_{IS} , likely related to the genetic dissimilarity among the five parents. In contrast, the 14-parent offspring cohort derived from the broodstock including several genetically similar parents showed low heterozygosity and elevated F_{IS} at

12h. Compared to 12h samples, F_{IS} increased at the 84h point in the 5-parent cohort and decreased in the 14-parent cohort, suggesting a short-term increase and decrease in homozygosity, respectively, during early development. Within this timeframe, the 12 to 50h developmental window overlaps with gastrulation and other key developmental milestones, where developmental failures may manifest due to genetic defects (Ball et al. 2002). Based on our results, we hypothesize that purging of genetic defects due to inbreeding and outbreeding deficiency may occur during this time window. Alternatively, the peak/trough in F_{IS} followed by reversion to a mean level of diversity could reflect sampling stochasticity or random chance. Given the declining trends in N_e that coincide in both cohorts, suggesting overall declines in allelic variability, further studies are required to understand if these early mortality events are linked to the selection under the artificial environment. After this 3.5-day window, F_{IS} and H_o values stabilised in both cohorts, implying that sampling for genetic diversity at least 4-day old larvae post-fertilisation may better reflect the eventual genetic composition of the surviving cohort.

4.3 | Implications for Coral Restoration

One of key concerns in restoration genetics is that seeded corals subjected to captive rearing must succeed in subsequently out-planted reef destinations (Baums et al. 2019). In general, corals well-suited for propagation in aquaria may not necessarily be competitive genotypes on reefs, as observed in other aquaculture-based populations released into the wild (e.g., Atlantic salmon; Milot et al. 2013). This is especially the case if supportive breeding is necessary for successive generations (e.g., following catastrophic population decline), as the more captive generations they undergo, the more domestic selection it can face (Säisä et al. 2003). However, this study did not find evidence of differential success of offspring genotypes in aquaria compared to the reef, given the persistence of all 14 parental genotypes that were deployed to the reef. The bulk fertilisation culture using 14 parents reared under captive conditions survived for up to 2 years in the field, and did not experience a major loss of genetic diversity compared to the aquarium phase. While genetic bottlenecks in the studied population were not observed, diversity was largely assessed through summary estimators, lacking insights into the functional basis of genetic selection better explained by loci-level genetic information. As such, establishing further understandings of domestication selection on genetic outcomes among offspring is an area requiring further exploration. Furthermore, the reef deployment phase of this study coincided with a bleaching event affecting reefs around the study site (Great Barrier Reef Marine Park Authority, Australian Institute of Marine Science, and CSIRO 2022). Despite this disturbance, survival rates indicate that no parental genotypes were disproportionately vulnerable to bleaching. While some corals still present in our population at 2 years were somewhat under-represented, their continued presence highlights the importance of rare genotypes in maintaining overall population genetic diversity.

Overall, further studies are required to ascertain if aquaculture-based coral propagation can be successfully applied on a scale to form the foundation of successful restoration programs. The

impact of out-planting captive bred corals on the adaptive capacity of the reef population is unclear, without the knowledge of its standing genetic diversity and effective population sizes. For example, *A. kenti* corals at Yunbenun, the broodstock origin and field deployment site in this study, have likely experienced strong genetic bottlenecks and have a lower effective population size compared with populations of another Acroporid coral along the northern GBR ($N_e < 1000$, compared with $N_e \sim 10,000$ on northern GBR) (Cooke et al. 2020; Matias et al. 2023). Evolutionary effects of deploying coral cohorts with even smaller N_e (< 30) on the wild populations will likely depend on the scale of aquaculture and deployment. In other areas of aquaculture that rely on supportive breeding efforts to retain wild populations, such as fisheries management, maintaining a consistently high effective population size, especially in the founder generation, is recommended to preserve genetic diversity of the population as a whole (Leus et al. 2011). Aquaculture-based programs that are based in areas that have experienced catastrophic loss of corals may need to more carefully consider effective population size of the rescuing parental corals due to enhanced founder effects and higher risk of inbreeding (Baums 2008; Ryman and Laikre 1991; Shearer et al. 2009).

Our experimental design did not set out to assess allelic diversity across the coral broodstock and its implications for natural coral populations when out-planting offspring. However, our study did highlight that the relatedness of the parent colonies significantly impacted the population structure of offspring corals produced, which underscores the importance of genotype informed broodstock selection. Genetic variation is essential for adaptive resilience to rapidly changing environmental conditions, especially warming oceans (Barrett and Schluter 2008). While we can utilise aquaria-based rearing to select for heat tolerant corals (van Oppen et al. 2015), the selection of genetically diverse broodstock is imperative. The ability to effectively support genetic diversity through sexually propagated coral aquaculture and restoration will depend on establishing a further understanding of the mechanisms that shape offspring genetic diversity.

Author Contributions

H.A.S. and D.G.B. conceived and designed the study; H.A.S. and D.G.B. conducted experiments; H.A.S. and G.D.-D. collected data; G.D.-D. and H.A.S. analysed data; G.D.-D. and H.A.S. drafted and edited the manuscript; H.A.S., Y.S., D.G.B., and C.A.P. provided intellectual input and edited the manuscript.

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Ethics Statement

Ethics approval is not required for scleractinian coral research in Australia. This project was conducted with free, prior, and informed consent of the Wulgurukaba people, the Traditional Owners of Yunbenun, the Sea Country where field deployment took place.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data used in this study are available in the Research Data JCU repository at <https://doi.org/10.25903/2yve-a322>. The R script used in this study is available at <https://github.com/hillary-smith/CoralAquacultureGenetics/>. The data that support the findings of this study are openly available in Research Data JCU at <https://researchdata.jcu.edu.au/>.

References

- Babcock, R. 1985. "Growth and Mortality in Juvenile Corals (*Goniastrea*, *Platygyra* and *Acropora*): The First Year." *Proceedings of the 5th International Coral Reef Symposium* 4: 355–360.
- Babcock, R., and C. Mundy. 1996. "Coral Recruitment: Consequences of Settlement Choice for Early Growth and Survivorship in Two Scleractinians." *Journal of Experimental Marine Biology and Ecology* 206, no. 1: 179–201. [https://doi.org/10.1016/S0022-0981\(96\)02622-6](https://doi.org/10.1016/S0022-0981(96)02622-6).
- Ball, E. E., D. C. Hayward, J. S. Reece-Hoyes, et al. 2002. "Coral Development: From Classical Embryology to Molecular Control." *International Journal of Developmental Biology* 46, no. 4: 671–678. <https://doi.org/10.1387/jfdb.12141456>.
- Banaszak, A. T., K. L. Marhaver, M. W. Miller, et al. 2023. "Applying Coral Breeding to Reef Restoration: Best Practices, Knowledge Gaps, and Priority Actions in a Rapidly-Evolving Field." *Restoration Ecology* 31, no. 7: e13913. <https://doi.org/10.1111/rec.13913>.
- Barrett, R. D. H., and D. Schluter. 2008. "Adaptation From Standing Genetic Variation." *Trends in Ecology & Evolution* 23, no. 1: 38–44. <https://doi.org/10.1016/j.tree.2007.09.008>.
- Baums, I. B. 2008. "A Restoration Genetics Guide for Coral Reef Conservation." *Molecular Ecology* 17, no. 12: 2796–2811. <https://doi.org/10.1111/j.1365-294X.2008.03787.x>.
- Baums, I. B., A. C. Baker, S. W. Davies, et al. 2019. "Considerations for Maximizing the Adaptive Potential of Restored Coral Populations in the Western Atlantic." *Ecological Applications* 29, no. 8: e01978. <https://doi.org/10.1002/eap.1978>.
- Baums, I. B., V. F. Chamberland, N. S. Locatelli, and T. Conn. 2022. "Maximizing Genetic Diversity in Coral Restoration Projects." In *Coral Reef Conservation and Restoration in the Omics Age*, edited by M. J. H. van Oppen and M. Aranda Lastra, 35–53. Springer International Publishing. https://doi.org/10.1007/978-3-031-07055-6_3.
- Baums, I. B., M. K. Devlin-Durante, N. R. Polato, et al. 2013. "Genotypic Variation Influences Reproductive Success and Thermal Stress Tolerance in the Reef Building Coral, *Acropora palmata*." *Coral Reefs* 32, no. 3: 703–717. <https://doi.org/10.1007/s00338-013-1012-6>.
- Baums, I., C. Hughes, and M. Hellberg. 2005. "Mendelian Microsatellite Loci for the Caribbean Coral *Acropora palmata*." *Marine Ecology Progress Series* 288: 115. <https://doi.org/10.3354/meps288115>.
- Boström-Einarsson, L., R. C. Babcock, E. Bayraktarov, et al. 2020. "Coral Restoration—A Systematic Review of Current Methods, Successes, Failures and Future Directions." *PLoS One* 15, no. 1: e0226631. <https://doi.org/10.1371/journal.pone.0226631>.
- Bridge, T. C. L., P. F. Cowman, A. M. Quattrini, et al. 2023. "A Tenuis Relationship: Traditional Taxonomy Obscures Systematics and Biogeography of the '*Acropora tenuis*' (Scleractinia: Acroporidae) Species Complex." *Zoological Journal of the Linnean Society* 202: zlad062. <https://doi.org/10.1093/zoolinnean/zlad062>.
- Bürkner, P.-C. 2021. "Bayesian Item Response Modeling in R With Brms and Stan." *Journal of Statistical Software* 100, no. 5: 1–54. <https://doi.org/10.18637/jss.v100.i05>.
- Chamberland, V. F., D. Petersen, J. R. Guest, U. Petersen, M. Brittsan, and M. J. A. Vermeij. 2017. "New Seeding Approach Reduces Costs and Time to Outplant Sexually Propagated Corals for Reef Restoration." *Scientific Reports* 7, no. 1: 1. <https://doi.org/10.1038/s41598-017-17555-z>.
- Christie, M. R., M. L. Marine, R. A. French, and M. S. Blouin. 2012. "Genetic Adaptation to Captivity Can Occur in a Single Generation." *Proceedings of the National Academy of Sciences* 109, no. 1: 238–242. <https://doi.org/10.1073/pnas.1111073109>.
- Cooke, I., H. Ying, S. Forêt, et al. 2020. "Genomic Signatures in the Coral Holobiont Reveal Host Adaptations Driven by Holocene Climate Change and Reef Specific Symbionts." *Science Advances* 6, no. 48: eabc6318. <https://doi.org/10.1126/sciadv.abc6318>.
- Cruz, D. W., and P. L. Harrison. 2017. "Enhanced Larval Supply and Recruitment Can Replenish Reef Corals on Degraded Reefs." *Scientific Reports* 7, no. 1: 1. <https://doi.org/10.1038/s41598-017-14546-y>.
- Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. "NeEstimator v2: Re-Implementation of Software for the Estimation of Contemporary Effective Population Size (Ne) From Genetic Data." *Molecular Ecology Resources* 14, no. 1: 209–214. <https://doi.org/10.1111/1755-0998.12157>.
- Dray, S., and A.-B. Dufour. 2007. "The ade4 Package: Implementing the Duality Diagram for Ecologists." *Journal of Statistical Software* 22: 1–20. <https://doi.org/10.18637/jss.v022.i04>.
- Edwards, A. J., and E. Gomez. 2007. "Reef Restoration Concepts & Guidelines: Making Sensible Management Choices in the Face of Uncertainty. Coral Reef Targeted Research & Capacity Building for Management Programme, iv."
- Fogarty, N. D., S. V. Vollmer, and D. R. Levitan. 2012. "Weak Prezygotic Isolating Mechanisms in Threatened Caribbean *Acropora* Corals." *PLoS One* 7: 30486. <https://doi.org/10.1371/journal.pone.0030486>.
- Goudet, J. 2005. "HIERFSTAT, a Package for R to Compute and Test Hierarchical F-Statistics." *Molecular Ecology Notes* 5, no. 1: 184–186. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>.
- Graham, E. M., A. H. Baird, and S. R. Connolly. 2008. "Survival Dynamics of Scleractinian Coral Larvae and Implications for Dispersal." *Coral Reefs* 27, no. 3: 529–539. <https://doi.org/10.1007/s00338-008-0361-z>.
- Grant, W. S., J. Jasper, D. Bekkevold, and M. Adkison. 2017. "Responsible Genetic Approach to Stock Restoration, Sea Ranching and Stock Enhancement of Marine Fishes and Invertebrates." *Reviews in Fish Biology and Fisheries* 27, no. 3: 3. <https://doi.org/10.1007/s11160-017-9489-7>.
- Great Barrier Reef Marine Park Authority, Australian Institute of Marine Science, & CSIRO. 2022. *Reef Snapshot: Summer 2021–22*. Great Barrier Reef Marine Park Authority.

- Gruber, B., P. J. Unmack, O. F. Berry, and A. Georges. 2018. "DartR: An R Package to Facilitate Analysis of SNP Data Generated From Reduced Representation Genome Sequencing." *Molecular Ecology Resources* 18, no. 3: 691–699. <https://doi.org/10.1111/1755-0998.12745>.
- Gu, Z., L. Gu, R. Eils, M. Schlesner, and B. Brors. 2014. "Circize Implements and Enhances Circular Visualization in R." *Bioinformatics* 30, no. 19: 2811–2812. <https://doi.org/10.1093/bioinformatics/btu393>.
- Hagedorn, M., C. A. Page, K. L. O'Neil, et al. 2021. "Assisted Gene Flow Using Cryopreserved Sperm in Critically Endangered Coral." *Proceedings of the National Academy of Sciences of the United States of America* 118, no. 38: e2110559118. <https://doi.org/10.1073/pnas.2110559118>.
- Hartig, F. 2022. "DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models."
- Heyward, A. J., L. D. Smith, M. Rees, and S. N. Field. 2002. "Enhancement of Coral Recruitment by In Situ Mass Culture of Coral Larvae." *Marine Ecology Progress Series* 230: 113–118.
- Huisman, J. 2017. "Pedigree Reconstruction From SNP Data: Parentage Assignment, Sibship Clustering and Beyond." *Molecular Ecology Resources* 17, no. 5: 1009–1024. <https://doi.org/10.1111/1755-0998.12665>. R Package Version 3.2.0.
- Kardos, M., E. E. Armstrong, S. W. Fitzpatrick, et al. 2021. "The Crucial Role of Genome-Wide Genetic Variation in Conservation." *Proceedings of the National Academy of Sciences* 118, no. 48: e2104642118. <https://doi.org/10.1073/pnas.2104642118>.
- Kilian, A., P. Wenzl, E. Huttner, et al. 2012. "Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms." In *Data Production and Analysis in Population Genomics: Methods and Protocols*, edited by F. Pompanon and A. Bonin, 67–89. Humana Press. https://doi.org/10.1007/978-1-61779-870-2_5.
- Koch, H. R., B. Matthews, C. Leto, C. Engelsma, and E. Bartels. 2022. "Assisted Sexual Reproduction of *Acropora cervicornis* for Active Restoration on Florida's Coral Reef." *Frontiers in Marine Science* 9: 959520. <https://doi.org/10.3389/fmars.2022.959520>.
- Lenth, R. 2025. "emmeans: Estimated Marginal Means, Aka Least-Squares Means (1.11.1-00001)." <https://rvlenth.github.io/emmeans/>.
- Leus, K., K. Traylor-Holzer, and R. C. Lacy. 2011. "Genetic and Demographic Population Management in Zoos and Aquariums: Recent Developments, Future Challenges and Opportunities for Scientific Research." *International Zoo Yearbook* 45, no. 1: 213–225. <https://doi.org/10.1111/j.1748-1090.2011.00138.x>.
- Levitan, D. R. 2000. "Sperm Velocity and Longevity Trade Off Each Other and Influence Fertilization in the Sea Urchin *Lytechinus variegatus*." *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267, no. 1443: 531–534. <https://doi.org/10.1098/rspb.2000.1032>.
- López-Nandam, E. H., C. Y. Payne, J. C. Delbeek, et al. 2022. "Kinship and Genetic Variation in Aquarium-Spawned *Acropora hyacinthus* Corals." *Frontiers in Marine Science* 9: 961106. <https://doi.org/10.3389/fmars.2022.961106>.
- Loya, Y. 1976. "Settlement, Mortality and Recruitment in a Red Sea Scleractinian Coral Population." In *Coelenterate Ecology and Behavior*, edited by G. O. Mackie, 89–100. Plenum Press. https://doi.org/10.1007/978-1-4757-9724-4_10.
- Matias, A. M. A., I. Popovic, J. A. Thia, et al. 2023. "Cryptic Diversity and Spatial Genetic Variation in the Coral *Acropora tenuis* and Its Endosymbionts Across the Great Barrier Reef." *Evolutionary Applications* 16, no. 2: 293–310. <https://doi.org/10.1111/eva.13435>.
- McGillcuddy, M., G. Popovic, B. M. Bolker, and D. I. Warton. 2025. "Parsimoniously Fitting Large Multivariate Random Effects in glmTMB." *Journal of Statistical Software* 112: 1–19. <https://doi.org/10.18637/jss.v112.i01>.
- McLeod, I. M., M. Y. Hein, R. Babcock, et al. 2022. "Coral Restoration and Adaptation in Australia: The First Five Years." *PLoS One* 17, no. 11: e0273325. <https://doi.org/10.1371/journal.pone.0273325>.
- Miller, M. W., I. B. Baums, R. E. Pausch, et al. 2018. "Clonal Structure and Variable Fertilization Success in Florida Keys Broadcast-Spawning Corals." *Coral Reefs* 37, no. 1: 239–249. <https://doi.org/10.1007/s00338-017-1651-0>.
- Miller, M., L. Krw, A. Bickel, et al. 2021. "Settlement Yields in Large-Scale In Situ Culture of Caribbean Coral Larvae for Restoration." *Restoration Ecology* 30: 13512. <https://doi.org/10.1111/rec.13512>.
- Milot, E., C. Perrier, L. Papillon, J. J. Dodson, and L. Bernatchez. 2013. "Reduced Fitness of Atlantic Salmon Released in the Wild After One Generation of Captive Breeding." *Evolutionary Applications* 6, no. 3: 472–485. <https://doi.org/10.1111/eva.12028>.
- National Research Council. 2004. *Atlantic Salmon in Maine*. National Academies Press. <https://doi.org/10.17226/10892>.
- Nitschke, M. R., S. K. Davy, and S. Ward. 2015. "Horizontal Transmission of Symbiodinium Cells Between Adult and Juvenile Corals Is Aided by Benthic Sediment." *Coral Reefs* 35, no. 1: 1. <https://doi.org/10.1007/s00338-015-1349-0>.
- Oksanen, J., F. G. Blanchet, R. Kindt, et al. 2015. "Vegan: Community Ecology Package. R Package Version 2.2-1, 2, 1–2."
- Okubo, N., T. Mezaki, Y. Nozawa, et al. 2013. "Comparative Embryology of Eleven Species of Stony Corals (Scleractinia)." *PLoS One* 8, no. 12: e84115. <https://doi.org/10.1371/journal.pone.0084115>.
- Palumbi, S. R., D. J. Barshis, N. Traylor-Knowles, and R. A. Bay. 2014. "Mechanisms of Reef Coral Resistance to Future Climate Change." *Science* 344, no. 6186: 895–898. <https://doi.org/10.1126/science.1251336>.
- Penin, L., F. Michonneau, A. Baird, et al. 2010. "Early Post-Settlement Mortality and the Structure of Coral Assemblages." *Marine Ecology Progress Series* 408: 55–64. <https://doi.org/10.3354/meps08554>.
- Pollock, F. J., S. M. Katz, J. A. J. M. van de Water, et al. 2017. "Coral Larvae for Restoration and Research: A Large-Scale Method for Rearing *Acropora millepora* Larvae, Inducing Settlement, and Establishing Symbiosis." *PeerJ* 5: e3732. <https://doi.org/10.7717/peerj.3732>.
- Puill-Stephan, E., B. L. Willis, L. van Herwerden, and M. J. H. van Oppen. 2009. "Chimerism in Wild Adult Populations of the Broadcast Spawning Coral *Acropora millepora* on the Great Barrier Reef." *PLoS One* 4, no. 11: e7751. <https://doi.org/10.1371/journal.pone.0007751>.
- R Core Team. 2024. "R: A Language and Environment for Statistical Computing."
- Randall, C. J., C. Giuliano, A. J. Heyward, and A. P. Negri. 2021. "Enhancing Coral Survival on Deployment Devices With Microrefugia." *Frontiers in Marine Science* 8: 662263. <https://doi.org/10.3389/fmars.2021.662263>.
- Randall, C. J., A. Negri, K. Quigley, et al. 2020. "Sexual Production of Corals for Reef Restoration in the Anthropocene." *Marine Ecology Progress Series* 635: 203–232. <https://doi.org/10.3354/meps13206>.
- Ripley, S. D. 1973. "Saving the Wood Duck *Ah Sponsa* Through Captive Breeding." *International Zoo Yearbook* 13, no. 1: 55–58. <https://doi.org/10.1111/j.1748-1090.1973.tb02102.x>.
- Ritson-Williams, R., S. N. Arnold, and V. J. Paul. 2016. "Patterns of Larval Settlement Preferences and Post-Settlement Survival for Seven Caribbean Corals." *Marine Ecology Progress Series* 548: 127–138. <https://doi.org/10.3354/meps11688>.
- Ryman, N., and L. Laikre. 1991. "Effects of Supportive Breeding on the Genetically Effective Population Size." *Conservation Biology* 5, no. 3: 325–329.
- Säisä, M., M.-L. Koljonen, and J. Tähtinen. 2003. "Genetic Changes in Atlantic Salmon Stocks Since Historical Times and the Effective Population Size of a Long-Term Captive Breeding Programme."

Conservation Genetics 4, no. 5: 5. <https://doi.org/10.1023/A:1025680002296>.

Severati, A., F. M. Nordborg, A. Heyward, et al. 2024. “The AutoSpawner System—Automated Ex Situ Spawning and Fertilisation of Corals for Reef Restoration.” *Journal of Environmental Management* 366: 121886. <https://doi.org/10.1016/j.jenvman.2024.121886>.

Shearer, T. L., I. Porto, and A. L. Zubillaga. 2009. “Restoration of Coral Populations in Light of Genetic Diversity Estimates.” *Coral Reefs* 28, no. 3: 727–733. <https://doi.org/10.1007/s00338-009-0520-x>.

Smith, H. A., G. Dallmeyer-Drennen, D. G. Bourne, S. Egan, and C. A. Page. 2025. “Sea-Weeding Enhances Early Coral Survival on Seeding Devices, but Benefits of Seeding Diminish After One Year.” *Journal of Environmental Management* 383: 125322. <https://doi.org/10.1016/j.jenvman.2025.125322>.

Smith, H. A., A. Moya, N. E. Cantin, M. J. H. van Oppen, and G. Torda. 2019. “Observations of Simultaneous Sperm Release and Larval Planulation Suggest Reproductive Assurance in the Coral *Pocillopora acuta*.” *Frontiers in Marine Science* 6: 362. <https://doi.org/10.3389/fmars.2019.00362>.

Stan Development Team. 2025. “RStan: The R Interface to Stan. R Package Version 2.32.7.” <https://mc-stan.org/>.

van Oppen, M. J. H., R. D. Gates, L. L. Blackall, et al. 2017. “Shifting Paradigms in Restoration of the World’s Coral Reefs.” *Global Change Biology* 23, no. 9: 3437–3448. <https://doi.org/10.1111/gcb.13647>.

van Oppen, M. J. H., J. K. Oliver, H. M. Putnam, and R. D. Gates. 2015. “Building Coral Reef Resilience Through Assisted Evolution.” *Proceedings of the National Academy of Sciences of the United States of America* 112, no. 8: 2307–2313. <https://doi.org/10.1073/pnas.1422301112>.

Vasquez Kuntz, K. L., S. A. Kitchen, T. L. Conn, et al. 2022. “Inheritance of Somatic Mutations by Animal Offspring.” *Science Advances* 8, no. 35: eabn0707. <https://doi.org/10.1126/sciadv.abn0707>.

Whitman, T. N., M. O. Hoogenboom, A. P. Negri, and C. J. Randall. 2024. “Coral-Seeding Devices With Fish-Exclusion Features Reduce Mortality on the Great Barrier Reef.” *Scientific Reports* 14, no. 1: 13332. <https://doi.org/10.1038/s41598-024-64294-z>.

Whitman, T. N., A. P. Negri, D. G. Bourne, and C. J. Randall. 2020. “Settlement of Larvae From Four Families of Corals in Response to a Crustose Coralline Alga and Its Biochemical Morphogens.” *Scientific Reports* 10, no. 1: 1. <https://doi.org/10.1038/s41598-020-73103-2>.

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Willis, B. L., R. C. Babcock, P. L. Harrison, and C. C. Wallace. 1997. “Experimental Hybridization and Breeding Incompatibilities Within the Mating Systems of Mass Spawning Reef Corals.” *Coral Reefs* 16, no. 1: 1. <https://doi.org/10.1007/s003380050242>.

Wilson, J., and P. Harrison. 2005. “Post-Settlement Mortality and Growth of Newly Settled Reef Corals in a Subtropical Environment.” *Coral Reefs* 24, no. 3: 418–421. <https://doi.org/10.1007/s00338-005-0033-1>.

Zuchowicz, N., J. Daly, J. Bouwmeester, et al. 2021. “Assessing Coral Sperm Motility.” *Scientific Reports* 11: 61. <https://doi.org/10.1038/s41598-020-79732-x>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supplementary Figure 1.** Positional alignment of raw SNPs. **Supplementary Figure 2.** PCA depicting genetic dispersion of SNP genotypes. **Supplementary Figure 3.** Parental assignment rates to offspring within each time point. **Supplementary Table 1.** List of broodstock and nights of spawning. **Supplementary Table 2.** Egg counts per parent colony. **Supplementary Table 3.** Observed heterozygosity model outputs and pairwise comparisons. **Supplementary Table 4.**

F_{IS} model outputs and pairwise comparisons. **Supplementary Table 5.** Bayesian probabilities of equal parental contribution (5-parent cohort). **Supplementary Table 6.** Bayesian probabilities of equal parental contribution (5-parent cohort).