REEF RESTORATION & ADAPTATION PROGRAM

Genetic Basis of Key Traits (ECT-01)

Final Report June 2025

Riginos, C., Howells, E.J., Elder, H., Howlett, L. and Molinaro, G.

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Enquiries should be addressed to:

Cynthia Riginos, c.riginos@aims.gov.au

Lorna Howlett, lh.howlett@aims.gov.au

Cover Page: Coral reef, Credit: Gary Cranitch, Queensland Museum

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The RRAP partners acknowledge Aboriginal and Torres Strait Islander Peoples as the first marine scientists and carers of Country. We acknowledge the Traditional Owners of the places where RRAP works, both on land and in sea Country. We pay our respects to elders; past, present, and future; and their continuing culture, knowledge, beliefs, and spiritual connections to land and sea Country.

We specifically acknowledge and thank the following Traditional Owners of sea Country that this report relates to:

Location	**Traditional Owner Group**
Capricorn Bunker Group	Port Curtis Coral Coast
Swains Reefs	Port Curtis Coral Coast
Chicken and Davies Reefs	Bindal
Magnetic Island and Myrmidon Reef	Wulgurukaba and Gurambilbarra
Palm Island Group and Kelso Reef	Manbarra
Moore, Fitzroy, and Sudbury Reefs	Gunggandji
Mackay and St Crispin Reefs	Eastern Kuku Yalanji
Lizard Island Group, Martin Reef, No Name Reef and Hicks Reef	Hopevale Congress and Walmbaar Corporation
Tydeman Reef	Cape Melville
Keppels Islands	Woppaburra

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1 Executive Summary

Coral reefs are under increasing stress from climate change, with marine heatwaves causing widespread bleaching and mortality. The potential for reef-building corals to adapt to rising temperatures depends on the presence of heritable variation in traits like thermal and bleaching tolerance. The RRAP Project Genetic Basis of Key Traits (ECT-01), under the Enhanced Corals and Treatments Sub-Program, investigates the genetic basis of key traits such as thermal and bleaching tolerance for three key coral species: *Acropora hyacinthus, Acropora spathulata,* and *Acropora kenti.* The long-term goal is to improve understanding of the intrinsic adaptive capacity of coral populations and inform targeted restoration efforts.

The project involved field collection and tagging of coral colonies across the Great Barrier Reef (GBR), using rapid acute heat stress assays to measure phenotypic variation in bleaching and thermal tolerance. Whole-genome sequencing and genome-wide association studies (GWAS) are used to identify links between host genome regions, symbiont communities, and heat tolerance phenotypes.

Key findings include substantial evidence of intraspecific variation in thermal tolerance within GBR reefs. For example, thermal thresholds of *A. hyacinthus* and *A. spathulata* individuals differed by up to 7.3°C and 6.0°C, respectively, across scales from meters to over 1,250 km. Thus, there is substantial phenotypic large variation in heat tolerance within species and within populations on reefs. Preliminary results suggest that thermal tolerance is controlled by many loci, indicating a polygenic trait. The project also identified potential trade-offs among colonies, where individuals that perform well under moderate heat stress may perform poorly under acute heat stress. The findings contribute directly to broodstock selection for increasing heat tolerance, with plans to select broodstock based on phenotypes for the 2025 spawning season and based on genotypes for the 2026 spawning season.

The research undertaken under the RRAP Project Genetic Basis of Key Traits (ECT-01) represents a critical advancement in understanding, predicting, and supporting coral adaptation in a rapidly changing climate. By connecting genetic, phenotypic, and ecological data at large spatial scales, the project provides robust, actionable science to inform the future of coral reef restoration on the GBR and similar ecosystems globally. Future research recommendations include developing quick and cheap phenotyping methods for coral heat tolerance, leveraging additional insights from quantitative genetic techniques, and improving communication of genetic insights to stakeholders.

2 Background and Justification for the Research

Coral reefs around the world, including the Great Barrier Reef,are experiencing increasing stress due to climate change, with marine heatwaves causing widespread bleaching and mortality. The potential for reef-building corals to adapt to rising temperatures depends on the presence of heritable variation in traits such as thermal and bleaching tolerance. If such genetic variation exists within coral populations, then natural selection can shift population-level traits over time, increasing resilience. However, for corals, the extent, distribution, and genetic underpinnings of this variation remain poorly understood. This limits both our ability to accurately forecast reef futures under climate change scenarios and our capacity to implement targeted interventions such as selective breeding.

Although marker-assisted selection is widely used in human health, agriculture, and animal breeding, it has not yet been broadly applied to coral reef restoration. This project addresses a critical gap by linking phenotypic variation in thermal tolerance to genetic markers in three key coral species. The ability to identify tolerant individuals within native populations—rather than relying on translocation—would enable ecologically responsible, targeted restoration with fewer social and ecological risks.

In the early years of the Reef Restoration and Adaptation Program (RRAP), researchers focused on field collection and tagging of coral colonies across the GBR, using rapid acute heat stress assays to measure phenotypic variation in bleaching and thermal tolerance. These efforts yielded substantial evidence of intraspecific variation in tolerance in *Acropora hyacinthus* and *Acropora spathulata* (Naugle et al. 2024; Denis et al. 2024), providing a strong foundation for investigating the genetic basis of these traits.

As the Program progressed, the project moved into analysing whole-genome sequencing and genome-wide association studies (GWAS), enabling researchers to identify links between host genome regions, symbiont communities, and heat tolerance phenotypes. To enhance ecological relevance, the team also took advantage of natural bleaching events to collect paired phenotypic and genotypic data. The data allowed for cross-validation of controlled stress assay results under real-world conditions.

The project further expanded to assess performance under multiple heat stress contexts—including natural bleaching, acute stress, and ramp-and-hold experiments—on the same coral colonies. This approach enables testing for potential trade-offs among colonies, where individuals that perform well under one stress scenario may perform poorly under another. Results from these assays, in combination with reef-wide scans of neutral and adaptive genetic variation (in collaboration with RRAP Project Ecological and Genetic Adaptation (ECO-03)), and breeding experiments (in partnership with RRAP Project Assisted evolution (ECT-02)), directly support efforts to evaluate selective breeding as a climate adaptation tool.

The rationale for the RRAP Genetic Basis of Key Traits (ECT-01) research project lies in its capacity to directly inform practical management decisions. By identifying consistent genetic markers associated with heat and bleaching tolerance, the project provides the scientific foundation for developing diagnostic tools, such as polygenic scores, for selecting tolerant colonies. These tools can guide targeted breeding and propagation efforts that aim to increase population resilience in situ, without the ecological and regulatory risks of inter-reef coral movement.

This work package directly addresses the questions:

- **What is the extent and structure of phenotypic variation in heat and bleaching tolerance across environmental and latitudinal gradients on the GBR?** Understanding this variation is fundamental to mapping adaptive potential within and among coral populations.
- **Which genetic markers in coral hosts and symbionts are associated with these tolerance traits?** This allows identification of candidate genes and regions that underlie adaptive responses to heat stress.

- **Can predictive tools—such as phenotype-genotype assays or polygenic scores—be developed to identify high-performing individuals for selective breeding?** These tools are central to enabling scalable and targeted restoration strategies.
- **Do genetic markers and tolerance phenotypes remain consistent across different stress conditions (e.g. natural bleaching, acute assays, ramp-and-hold)?** Evaluating marker stability ensures applicability across varied environmental scenarios.
- **How do these tolerance traits relate to overall coral fitness, and are there trade-offs across stress conditions?** This informs risk assessment for restoration efforts, avoiding unintended consequences of trait selection.

This research represents a critical advancement in our ability to understand, predict, and support coral adaptation in a rapidly changing climate. By connecting genetic, phenotypic, and ecological data at large spatial scales, the project provides robust, actionable science to inform the future of coral reef restoration on the GBR and in similar ecosystems globally.

3 Research Objectives and Key Findings

A current list of project outputs are listed on the RRAP website: gbrrestoration.org. Key research objectives and findings are detailed below.

Table 1: Key findings of the Project aligned to the overarching and specific research questions for each sub-project.

Objective		Key Findings and/or Outcomes
1.	Measure phenotypic variation in heat and bleaching tolerance for a core set of coral species across environmental and latitudinal gradients on the Reef using rapid performance experiments and natural bleaching events and assays that span host and symbiont physiology.	This work has been achieved and reported in the two peer reviewed publications focused on *Acropora hyacinthus* and A*cropora spathulata* (Naugle et al. 2024; Denis et al. 2024). The major findings are that there is considerable variability within GBR reefs for heat and bleaching tolerance. For example, for *A. hyacinthus*, thermal thresholds of 569 individuals differed by up to 7.3 °C across scales from meters to >1250 km. For *A. spathulata*, thermal thresholds for photochemical efficiency and chlorophyll retention varied considerably among individual colonies both among reefs (approximately 6°C) and within reefs (approximately 3°C) as estimated across 709 colonies. This variation within reefs suggests that there is considerable variation for natural adaptation and that thermally tolerant individuals within reefs can potentially serve as broodstock for conservation aquaculture. The important next steps will be to measure how much of this whole-colony variation is due to host genetics, as the genetic portion is what supports both natural and assisted evolution to increase heat and bleaching tolerance.
2.	Perform genome-wide sequencing and phenotype association analyses to identify coral host and symbiont genetic markers linked to heat and bleaching tolerance, potential trade-offs with growth and reproduction and standing adaptive genetic variation across the Reef for a core set of coral species.	Genomic and phenotypic data were collected for three species (*Acropora hyacinthus*, *Acropora spathulata*, and *Acropora kenti)* across latitudinal and across-shelf gradients on the GBR. Genome wide association analyses are in progress with analyses most advanced for *Acropora hyacinthus.* Here, preliminary results suggest that thermal tolerance, as measured by photochemical efficiency (Fv/Fm) and chlorophyll content (NDVI) under acute heat stress, are controlled by many loci, and are therefore polygenic. Analyses for the remaining species' genome-wide sequencing and phenotype association analyses are currently ongoing and making good progress. The findings from this work will contribute directly towards broodstock selection to increase heat tolerance, as the individuals with the genetic attributes to be the best parents for creating heat tolerant offspring will be identifiable. Already, work in the Pilot Deployments Program (PDP) is planning to implement broodstock selection based on genetic attributes for 2026 spawning. This work will also contribute to three future peer-reviewed publications.
3.	Develop phenotype assays, deoxyribonucleic acid (DNA) markers or combined polygenic predictor scores to rapidly identify candidate coral	We have developed and evaluated methods to rapidly test heat tolerance in coral colonies both in the wild and ex situ using rapid heat stress assays. This allowed us to identify large

Objective	Key Findings and/or Outcomes
colonies for selective breeding and asexual propagation within coral populations.	amounts of standing variation in heat tolerance between species, within species, and between reefs (Denis et al. 2024; Naugle et al. 2024). While observed variation in heat tolerance could be attributed in part to local adaptation, acclimatisation, and recent thermal history, variation within reefs was largely not explained by environmental predictors, suggesting a role for adaptive genetic variation and thus the potential for selective breeding (Denis et al. 2024; Naugle et al. 2024). Accordingly, breeding of top performing colonies based on heat tolerance phenotypes led to increased heat tolerance and survival of offspring, although results varied by collection site (Lamb et al. in prep). Preliminary results further showed increased heat tolerance of colonies inoculated with heat-evolved symbionts. Physiological work under this project has primarily focused on measures under acute heat stress. However, broader understandings of heat tolerance – especially as relevant to what corals experience in nature – is needed. To address this, we have compared acute heat stress assays with moderate heat stress or a simulated bleaching event for the target species. We have found that traits commonly measured in acute assays such as chlorophyll content (NDVI) and photosynthetic efficiency (Fv/Fm), do not necessarily predict the coral genets that survive longest in a simulated bleaching event (Elder et al. in prep). The variation in heat tolerance rankings that is seen between tests within species, shows that the duration of the test and the different metrics change who the predicted survivors are, and this has implications for selective breeding (Elder et al. in prep). Choosing the right test and the right phenotype will be essential to the success of future restoration programs if the program relies on selecting broodstock using the results of these phenotyping tests. To further assess the ability of acute heat stress assays to identify survivors of bleaching, we leveraged resources in collaboration with AIMS to phenotype bleaching status, survival and percentage mortality for previously tagged ECT-01 corals. Here, chlorophyll content (NDVI) from acute heat stress assays performed in 2021 and 2022 was moderately predictive of the severity of bleaching observed in *A. hyacinthus* and *A. spathulata* in 2024. We also tagged additional colonies to further assess the heat tolerance within the same reefs to add to the number of individuals that can be leveraged for broodstock and tests of selective breeding measures in the future contributing to PDP. While the genetic analysis for the bleaching event of 2024 is ongoing, the data set of phenotypes in acute and moderate heat stress assays, including history of bleaching across five years at 30 reefs, and genetic samples for every coral tagged is an invaluable data set that will allow us to test the associations between genotype

Objective		Key Findings and/or Outcomes
		and heat tolerance phenotype and develop predictors of heat tolerance and survival for reefs across the GBR.

Adjustments to key research objectives

Table 2: Variation in the Project over time.

Initial Research Question	Explain when, how and why the research question changed
Bleaching response	An opportunity arose for the project team to conduct extra fieldwork in an unprecedented “natural” bleaching event, which has built the basis for new insights into phenotypic and genetic responses to acute heat stress events in nature that will be forthcoming over the next two years. The team tagged >2,100 coral colonies across seven species in the northern, central and southern GBR for phenotyping both during and after the GBR-wide bleaching event of 2024. Genetic samples were taken from 2,107 colonies and association analyses are planned for the resulting genomic data and corresponding measures of bleaching response.
Genetic diversity – baseline estimates of genetic diversity	This objective was added because the same genetic data used for heat tolerance studies can also inform baseline genetic diversity estimates. The geographic extent of sampling undertaken under this project (and supplemented by RRAP Project Ecological and Genetic Adaptation (ECO-03)) is rare and the ability to compare across multiple species provides unique insights into the processes that shape spatial differences in genetic diversity.
Quantitative genetics – beyond GWAS	As originally conceived, this project focused on deriving information about heritability and trait prediction from methods that fall under the umbrella term of “genome-wide association studies” (GWAS). GWAS, however, is not the only strategy for gaining insights on heritability and other quantitative genetic attributes. Indeed, often insights can be gained by piggybacking on other work, for example, by using contrasts between broodstock (parents) and deployed juveniles (offspring) as created by conservation aquaculture interventions. Additionally, the discovery of close kin among brooded corals opens up the possibility of using approaches based on kin-relationships, commonly referred to as “wild quantitative genetics”. To explore our future options, further support was sought from RRAP and AIMS. A workshop was convened in July 2024 that mixed experts in quantitative genetics and coral biology and emerged with robust recommendations that have greatly influenced plans for RRAP into the future and PDP, for example, showcasing how clonal fragmentation can give quick answers in some situations and developing experimental designs suitable for conservation aquaculture. A comprehensive white paper is nearing completion and will be published.

4 Future Research Recommendations

Research in progress

The overarching aim of **RRAP Project Genetic basis of key traits (ECT-01)** is to describe geographic variation for heat tolerance among GBR corals, where *Acropora hyacinthus*, *Acropora spathulata*, and *Acropora kenti* have been the focal taxa. As the project has matured, the focus has shifted from describing phenotypic variation and its environmental drivers (e.g. Naugle et al. 2024; Denis et al. 2024) to describing the genetic underpinnings of heat tolerance using genome wide association study (GWAS) frameworks. This work is continuing across the three species and is now also focusing on *Acropora kenti* and student-led investigations for *Acropora hyacinthus* and *Acropora spathulata.* Results from these GWAS analyses will be used to guide broodstock selection, as they will indicate which potential parents have the best genotypes for heat tolerance.

Additionally, through activities under the umbrella of “coral bleaching response”, efforts to tag colonies, phenotype, and genotype have been expanded for the same focal *Acropora* species (and *Acropora millepora*). 2,107 colonies have been tagged, phenotyped (with image analyses ongoing), and they will be genotyped in the 2025/26 financial year. These samples will provide independent validation of original GWAS work and are geographically well positioned to provide broodstock for reefs where Pilot Deployments Program activities will take place.

Recommendations

- ***Develop quick and cheap phenotyping for coral heat tolerance -*** There are two issues with phenotyping: i) relevancy to heat tolerance adaptation, ii) cost/time to undertake. Under the first phase of RRAP, phenotyping was primarily conducted as rapid heat stress response, with measurements of photosynthetic efficiency and chlorophyll content. The relevancy of these traits for survival in nature is increasingly being questioned (for example, field surveys of bleaching indicate a moderate association for chlorophyll content, but experiments comparing acute and longer-term heat stress responses did not show a correlation). Consequently, the project team augmented acute heat stress assays with records of survival following heat waves and image analyses of corals during heatwaves. However, these survival analyses require additional field work and tagged colonies can be difficult to find. Image analysis is also very laborious. We are moving to georeferencing rather than physical tagging (in collaboration with the RRAP Ecological Intelligence for Reef Restoration (EcoRRAP) Sub-program monitoring) to aid with survival records, but there is also great need to develop relevant measurements (biomarkers) that can be undertaken in the field efficiently to indicate relative performance (not simply, survival versus death).
- ***Not just GWAS: Leveraging additional insights on heat tolerance genetics from PDP and other quantitative genetic techniques - GWAS*** is the appropriate technique to use when samples do not include closely related individuals, as is the case for broadcast spawning *Acropora*. However, other quantitative genetic approaches based on the “animal model” leverage relatedness to estimate heritability and an individual’s genetic (breeding) value for traits like heat tolerance. Using this framework, we can also test for trait covariances that could constrain or synergistically accelerate adaptation in traits like growth and heat tolerance. Under PDP, conservation aquaculture deployments of families of siblings and half siblings will be created to advance genetic insights and resolve possible trade-offs among heat tolerance traits. For brooding species, we find close kin in nature and so the animal model approach could also be used to make these same estimates in natural populations, extending our inferences beyond just *Acropora*. Finally, under the first phase of RRAP the utility of quicker/simpler but less precise evaluations of heritability based on using clonal fragments, which can verify whether there is some genetic component for a trait of interest and also whether that trait differs by environment (genotype by environment interaction). Planning under future phases of RRAP incorporates work based on clonal fragments to greatly increase the number of species that we can examine. This will not only continue the project work of associating genotypes

with traits important for survival on the reef and during marine heat waves but will give us a more holistic and nuanced assessment of adaptation on the reef.

- ***Evolutionary Modelling to support efficacy and risk -*** Modelling is a key tool for developing predictions regarding intervention outcomes, forecasting system trajectories and resilience, and exploring which parameters are uncertain, unknown, or those which could greatly affect intervention outcomes. Most modelling under the first phase of RRAP did not explicitly include genetic-based evolutionary parameters and this oversight means that collectively our understanding of adaptation dynamics (baseline and with assisted evolution) for GBR corals is poor. To understand baseline natural adaptation to climate change and to explore the effects of any assisted evolution method requires genetically explicit models, where individuals have many independently assorting loci or 'genes' that contribute to the trait of interest. This allows for emergent variation in phenotypes and also captures the stochastic processes of genetic drift and natural selection. Eco-evolutionary models that simulate multiple proposed assisted evolution interventions on a single reef are in development but there is much scope to expand such modelling to incorporate other extensions, such as: (1) evaluating the scale of intervention (i.e. number of corals, number of years of intervention) required for evolutionary rescue of populations under increasingly pessimistic shared socioeconomic pathways (SSP) climate projections, (2) the effect of mass bleaching event(s) immediately preceding, during, or following intervention years, (3) evaluating reef adaptation when multiple simultaneous interventions are applied, and (4) the importance of connectivity and gene flow in maintaining genetic diversity and thus natural adaptation to climate change.

Shared recommendations across RRAP Projects Ecological and Genetic Adaptation (ECO-03) and Genetic Basis of Key Traits (ECT-01):

- **Validation of candidate adaptive loci through experiments, gene manipulation, and evolutionary modelling** - Although our work uncovers loci that are candidates for thermal adaptation (and other aspects of fitness), correlation is not the same as causation. Experimental manipulations (especially common garden or reciprocal transplantations) are the classic way to demonstrate effects on fitness. Ribonucleic acid (RNA) sequencing (RNAseq) data are already in hand to validate results from GWAS analysis from *Acropora hyacinthus* colonies with extreme thermal tolerance phenotypes \and may be combined with future in-situ validation of acute phenotypes. Gene manipulation can also be used to check effects of specific loci. The above experiments and analyses will aid to determine the genetic architecture of thermal adaptation for corals, which will inform evolutionary modelling and help determine the rate of natural adaptation as well as inform our assisted evolution interventions aimed at enhancing coral thermal adaptation to climate change.

- ***Opportunities to use 'omic and other technologies to strengthen inferences*** - Genotyping under the first phase of RRAP has focused on whole genome sequencing, some reduced representation sequencing, and symbiont characterisation with ITS2 (internal transcribed spacer) metabarcoding and k-mer analysis. Other omics approaches - for example, RNAseq gene expression can give insights on pathways, metabolites, and non-dinoflagellate microbial partners. An emerging result across ecological genetics is that large structural variants in genomes are important for adaptation - to uncover the role of structural variants in GBR corals would require improving genomic resources such as creating chromosomal level reference genomes and using long range sequencing to identify structural variants.

- **Future directions for genomics harnessing parent-offspring resources:** Recombination rate and mutation rate estimation to inform statistical analysis of captive breeding impacts on heterozygosity and identification of adaptive variation and ecological drivers (versus intrinsic processes). Parent-offspring phenotyping and genotyping would allow trait additive genetic covariances to be estimated and thus inform us of pleiotropy and/or phenotypic correlations.

- ***Better communication of genetic insights to Traditional Owners and stakeholders*** - Genetic based insights can be difficult to communicate to the general public. In the first phase of RRAP, our primary focus was on generating results, with less emphasis placed on outreach and communication. Strengthening this area will be an important focus moving forward."

- ***Unite RRAP Projects Ecological and Genetic Adaptation (ECO-03) and Genetic Basis of Key Traits (ECT-01) genomic work*** - linkages and collaborations between the two RRAP Sub-programs have been very productive, and we recommend uniting this work more cohesively in the future to benefit from shared genomic resources and procedures.

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