FIRST ASSESSMENT OF POPULATION GENETIC STRUCTURE ACROSS THE MARIANA ARCHIPELAGO

BY

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ABSTRACT

Quantifying population genetic patterns in reef-building corals is important for understanding the biology and fortitude of a species. However, such patterns, like connectivity and genotypic richness, can vary dramatically between species and environments, and are unknown for most coral species. Coral population genetics has never been studied on an archipelago-wide scale in the Mariana Islands, and no population genetic study has been performed on the important coral species *Porites rus*, a highly abundant and resilient reef builder. In this study, I quantify genomic patterns within and among populations of *P. rus* across the Mariana Archipelago. A low coverage whole genome resequencing approach was used to generate genome-wide sequencing data that was analyzed using genotype likelihood methods in ANGSD. Out of 163 sequenced colonies, I identified 105 unique genotypes ($N_G/N = 0.65$) with significant differences in clonality between islands and a negative correlation between clonality and *P. rus* density. I found high levels of genetic diversity (heterozygosity) across populations. There was a significant deficit of heterozygotes throughout the dataset, and all inbreeding coefficients (F_{IS}) were positive, but low. I found small amounts of population structure between populations following an isolation-by-distance pattern, which was corroborated by admixture analysis. Here, I quantify previously unknown population-level patterns in *P. rus* and show that Mariana Archipelago *P. rus* populations may be a species of concern due to high clonality at some sites and limited population connectivity throughout the archipelago.

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Dedicated to Chase Butler

1997-2022

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1.1 Introduction to Literature Review

Globally, coral reefs are declining due to anthropogenic impacts, such as climate change, pollution, and over-harvesting[1]. In some regions, large-scale phase shifts from coral dominance to macroalgae dominance have been observed and attributed partially to human impacts[2]. Ecological theory predicts that once a phase shift of this nature occurs, shifts back to coral dominance from algal dominance are often extremely unlikely or impossible [3]. A minimum of 33%-66% of coral reefs will be subject to long term degradation from climate change under relatively optimistic projected climate change scenarios[4], and over 90% of coral reefs worldwide are predicted to experience high frequency bleaching by 2100 if greenhouse gas emissions remain high, or even if they are dramatically reduced but the adaptive capacity of corals is low[5]. Global coral cover has already declined by more than 50% since the 1950s[6]. Due to the extreme current and projected threats affecting coral reef ecosystems, many conservation and management efforts have been implemented throughout the world.

Conservation of a species or ecosystem can be enhanced with basic knowledge of population-level characteristics. For example, understanding the magnitude of clonality within a group of populations can highlight conservation priorities. In habitat-forming seagrass, increased genotypic diversity has been shown to enhance disturbance resistance[7], biomass production[8], and organismal density[8]. In the coral *Acropora cervicornis*, white band disease transmission assays performed on 49 genotypes showed that only 3 genotypes (6%) were resistant[9], highlighting the importance of genotypic diversity in the context of stress resistance.

High clonality can make a population more vulnerable to certain stressors or disturbances, but clonality in corals can be highly variable. Corals can reproduce asexually and generate clones[10] via fragmentation[11] or through production of parthenogenic larvae[12]. Some species or populations of coral can be highly clonal and rely primarily on asexual reproduction[13],

while others have a relatively low ratio of clones[14] or none at all[15]. Within a single species, the number of clones can vary based on geography and colony density[16] or environmental conditions[17]. Due to the high variability in degrees of clonality within and among coral species, it is important to quantify the level of clonality within individual species and habitats. By quantifying the amount of genotypic diversity within populations and the differences in clonality between populations, population vulnerability can be forecasted, and populations with high numbers of unique genotypes can be prioritized for conservation.

Population-level genetic studies can also benefit a species by informing the design and management of marine protected areas (MPAs), which can positively impact the health and stability of coral reef ecosystems[18]. A global analysis of 310 MPAs found that MPAs can be a successful tool for maintaining coral cover[18]. Additionally, coral reef MPAs can reduce the magnitude of disturbance events and increase the rate of recovery post-disturbance[19]. Effective marine protected area design requires knowledge of the dynamics of larval import, export, and self-seeding within an area, patterns that can be understood through population genetics[20]. Different coral genera can exhibit dramatically different connectivity patterns within the same MPA network[21,22], so understanding the dynamics of a specific, ecologically important species can be highly informative for conservation and management.

Population genetic studies are important for informing conservation efforts of coral reefs, as their environment continues to dramatically degrade due to human activity. The aim of this study is to understand population-level patterns of an important coral species (*Porites rus)* within the Mariana Archipelago. This will provide knowledge about the basic biology of the species and allow its future in the Mariana Islands to be better forecasted.

1.2 Population Genetics

Many species of reef building coral can reproduce both sexually and asexually[10], and both modes of reproduction allow these sessile organisms to spread offspring beyond their immediate vicinity. In some species, the pelagic larval stage can allow for long distance dispersal, facilitating reproductive connection between populations across large spatial scales[23]. Dispersal patterns often vary greatly between species, and factors influencing dispersal include ocean currents, larval behavior, predator/prey interactions, and available settlement habitat[24].

In the Mariana Islands, the level of connectivity among islands was previously unknown, but studies of similar scales have been performed elsewhere, revealing archipelago-level patterns of population genetic structure. For example, in the Hawai‘ian Archipelago, geographic distance explained ~37% of genetic distance in *Porites lobata* populations[25]. In *Acropora hyacinthus*, population genetic structure exists on an inter-island scale in the Caroline Islands, with very limited gene flow among islands[26]. The majority of recruitment across twelve populations of *Montipora capitata* in the Hawai‘ian Archipelago and Johnston Atoll is driven by within-population sexual reproduction (rather than imported larvae from other locations), which indicates that management at the local level may be most effective for protecting these populations[27].

Corals can also exhibit population genetic structure on smaller scales as well, such as a small stretch of coast or a singular island. In *A. hyacinthus*, population structure exists within the islands of Palau and Yap as well as between them; sites on Palau were as differentiated from each other as they were from sites on Yap and Ngulu[26]. On Reunion Island, four populations of *Pocillopora damicornis* were significantly differentiated from each other, with colonies grouping into two distinct clusters[28]. In the Florida Reef Tract and Western Caribbean, *A. cervicornis*

exhibits extensive population structure, with significant isolation-by-distance[29]. Over a distance of ~85 kilometers in the Florida Reef Tract, *Montastraea cavernosa* exhibits population structure driven by geography, with northern and southern populations clustered together, and central sites acting as a genetic intermediary[30]. In the Tropical Eastern Pacific, *Pocillopora damicornis* exhibits fine-scale spatial genetic structure, with corals 10 m apart from each other being significantly more related to each other than to the rest of the population[31]. A similar pattern exists in *Seriatopora hystrix* at Scott Reef in Western Australia, where spatial structure exists within 300 m transects[32]. Population structure can exist on surprisingly small scales, such as on small islands.

1.3 Mariana Archipelago Population Connectivity

Although no previous archipelago-scale empirical study had evaluated larval connectivity within the Mariana Archipelago, potential connectivity has been modeled, and some larval exchange among islands is predicted. Oceanographic modeling predicts a clear break in connectivity between Guam and Rota due to the North Equatorial Current, which flows between the two islands[33]. The model predicted that larvae with a pelagic duration of 6-10 days would rarely be exchanged between Guam and Rota, and no larvae from Guam would be transported beyond Rota to islands further north. At this PLD, larvae from islands above the North Equatorial Current could be exchanged 150-200km from their origin point, enough distance to allow connectivity between islands[33]. At a pelagic larval duration of 12-20 days, the break point between Guam and Rota still exists, but allows for occasional larval exchange between Guam and islands to the north. However, in the region spanning from Rota to Farallon de Pajaros, sources could send larvae to other islands within the archipelago, either 150-200 km to the south,

or 300-400 km north of their position[33]. Modeling results predict some degree of larval exchange among Mariana Islands.

Connectivity patterns of corals across the Mariana Archipelago are unknown, but two studies have evaluated connectivity between the islands of Guam and Saipan. These studies have found small but significant population structure in *Acropora pulchra* between Guam and Saipan, and their results indicated some ongoing larval exchange[34,35].

These same two studies also analyzed the structure of *A. pulchra* within the island of Guam and revealed island-scale population structure. One study found significant population structure between the Cocos Island population and the rest of the populations sampled[34]. The more recent study quantified genetic structure among Guam populations and observed distinct genetic clusters between the northern and southern populations[35]. The population structure highlighted by these studies indicates that within Guam, connectivity between *A. pulchra* populations is present but somewhat limited.

1.4 *Porites rus*

Porites rus (Lit. Rev. Fig. 1) is a reef-building coral with a wide distribution across the Pacific and Indian Oceans (Lit. Rev. Fig. 2). It is an important species because it can be locally dominant and because it acts as a significant reef builder[36]. Ecologically, *P. rus* reefs generate over a dozen distinct habitat types for 100+ fish species[37].

Porites rus can reproduce sexually and asexually, and patterns related to *P. rus* reproduction such as connectivity and clonality were previously unknown. *P. rus* colonies are gonochoric, and reproduce by spawning[38], a form of reproduction known to potentially facilitate long distance dispersal. Before this study, the degree of reproductive connectivity among *P. rus* populations had not been quantified in the Mariana Archipelago, or anywhere in the world.

Regarding asexual reproduction, the amount of clonality within populations of *P. rus* was not fully understood, but seemed to vary by location or habitat type. In Moloka‘i, Hawai’i, *P. rus* was shown to be highly clonal. Fifteen samples were collected over a 70 m transect, and only two genotypes were detected[39]. Conversely, on Guam, a population of *P. rus* sampled at two depths (2 m and 10 m) did not exhibit any clonality[15].

Unlike many other coral species, *P. rus* is fairly resilient to stress. Tank experiments performed on *P. rus* revealed that the species’ calcification rate is insensitive to reduced pH and elevated temperature[40]. During Guam’s 2017 bleaching event, only 2% of surveyed *P. rus* living in reef slope habitat (where *P. rus* is most abundant) experienced bleaching, and no mortality was observed. Conversely, 63% of surveyed *P. rus* bleached in reef flat habitats (which are shallower and have less water turnover, resulting in higher temperatures), but only 4% experienced mortality[41].

Porites rus is an abundant but understudied species throughout its range, including within the Mariana Islands. Within the archipelago, *P. rus* has been documented on Guam, Rota, Tinian, Saipan, Sarigan, Guguan, Pagan, Asuncion, and Maug. The abundance and notable stress resilience of *P. rus* has been documented, but patterns of connectivity and clonality were either not fully understood, or entirely unknown as population genetic studies had not been performed on *P. rus* until now.

This thesis seeks to understand the intra-island (on Guam) and inter-island population genetic structure of *P. rus*. The results of this study generate knowledge of both the population genetics of the species, and more broadly, the dynamics of population structure and genotypic diversity among the islands of the Mariana Archipelago. These results not only increase basic

knowledge of coral population biology in the region, but also provide information relevant for predicting and managing the future of coral reefs in the Mariana Archipelago.

1.5 Chapter 1 References

1. Bellwood, D. R., Hughes, T. P., Folke, C. & Nyström, M. Confronting the coral reef crisis. Nature 429, 827–833 (2004).
2. Hughes, T. P. Catastrophes, Phase Shifts, and Large-Scale Degredation of a Caribbean Coral Reef. Science (80-.). 265, 1547–1551 (1994).
3. Mumby, P. J., Hastings, A. & Edwards, H. J. Thresholds and the resilience of Caribbean coral reefs. Nature 450, 98–101 (2007).
4. Frieler, K. et al. Limiting global warming to 2C is unlikely to save most coral reefs. Nat. Clim. Chang. 3, 165–170 (2013).
5. Kleypas, J. et al. Designing a blueprint for coral reef survival. Biol. Conserv. 257, 109107 (2021).
6. Eddy, T. D. et al. Global decline in capacity of coral reefs to provide ecosystem services. One Earth 4, 1278–1285 (2021).
7. Hughes, A. R. & Stachowicz, J. J. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc. Natl. Acad. Sci. U. S. A. 101, 8998–9002 (2004).
8. Reusch, T. B. H., Ehlers, A., Hämmerli, A. & Worm, B. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc. Natl. Acad. Sci. U. S. A. 102, 2826–2831 (2005).
9. Vollmer, S. V. & Kline, D. I. Natural Disease Resistance in Threatened Staghorn Corals. PLoS One 3, e3718 (2008).
10. Richmond, R. H. & Hunter, C. L. Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Mar. Ecol. Prog. Ser. 60, 185–203 (1990).

11. Highsmith, R. Reproduction by Fragmentation in Corals. Mar. Ecol. Prog. Ser. 7, 207–226 (1982).

12. Combosch, D. J. & Vollmer, S. V. Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. Ecol. Evol. 3, 3379–3387 (2013).

13. Williams, D. E., Miller, M. W. & Baums, I. B. Cryptic changes in the genetic structure of a highly clonal coral population and the relationship with ecological performance. Coral Reefs 33, 595–606 (2014).

14. van der Ven, R. M., Flot, J. F., Buitrago-López, C. & Kochzius, M. Population genetics of the brooding coral *Seriatopora hystrix* reveals patterns of strong genetic differentiation in the Western Indian Ocean. Heredity (Edinb). 126, 351–365 (2021).

15. Sartor, C. Transcriptomic Signatures of Acclimation in 'Susceptible' *Acropora surculosa* and 'Resistant' *Porites rus*. (University of Guam Marine Lab, 2021).

16. Baums, I. B., Miller, M. W. & Hellberg, M. E. Geographic Variation in Clonal Structure in a Reef-Building Caribbean Coral, *Acropora palmata*. Ecol. Monogr. 76, 503–519 (2006).

17. Torres, A. F., Forsman, Z. H. & Ravago-Gotanco, R. Shifts in coral clonality along a gradient of disturbance: insights on reproduction and dispersal of *Pocillopora acuta*. Mar. Biol. 2020 16711 167, 1–18 (2020).

18. Selig, E. R. & Bruno, J. F. A global analysis of the effectiveness of marine protected areas in preventing coral loss. PLoS One 5, 1–7 (2010).

19. Mellin, C., Aaron Macneil, M., Cheal, A. J., Emslie, M. J. & Julian Caley, M. Marine protected areas increase resilience among coral reef communities. Ecol. Lett. 19, 629–637 (2016).

20. Lagabrielle, E. et al. Connecting MPAs - eight challenges for science and management. Aquat. Conserv. Mar. Freshw. Ecosyst. 24, 94–110 (2014).

21. Miller, K. J. & Ayre, D. J. Population structure is not a simple function of reproductive mode and larval type: Insights from tropical corals. J. Anim. Ecol. 77, 713–724 (2008).

22. Miller, K. J. & Ayre, D. J. Protection of Genetic Diversity and Maintenance of Connectivity among Reef Corals within Marine Protected Areas. Conserv. Biol. 22, 1245–1254 (2008).

23. Huang, W. et al. Genetic diversity and large-scale connectivity of the scleractinian coral *Porites lutea* in the South China Sea. Coral Reefs 37, 1259–1271 (2018).

24. Cowen, R. K. & Sponaugle, S. Larval dispersal and marine population connectivity. Ann. Rev. Mar. Sci. 1, 443–466 (2009).

25. Polato, N. R., Concepcion, G. T., Toonen, R. J. & Baums, I. B. Isolation by distance across the Hawaiian Archipelago in the reef-building coral *Porites lobata*. Mol. Ecol. 19, 4661–4677 (2010).

26. Cros, A., Toonen, R. J., Davies, S. W. & Karl, S. A. Population genetic structure between Yap and Palau for the coral *Acropora hyacinthus*. PeerJ 2016, 1–17 (2016).

27. Concepcion, G. T., Baums, I. B. & Toonen, R. J. Regional population structure of *Montipora capitata* across the Hawaiian Archipelago. Bull. Mar. Sci. 90, 257–275 (2014).

28. Gélin, P. et al. Superclone expansion, long-distance clonal dispersal and local genetic structuring in the coral *Pocillopora damicornis* type β in Reunion Island, South Western Indian Ocean. PLoS One 12, 1–20 (2017).

29. Drury, C. et al. Genomic patterns in *Acropora cervicornis* show extensive population structure and variable genetic diversity. Ecol. Evol. 7, 6188–6200 (2017).

30. Dodge, D. L. et al. Population structure of the scleractinian coral *Montastraea cavernosa* in southeast Florida. Bull. Mar. Sci. 96, 767–782 (2020).

31. Combosch, D. J. & Vollmer, S. V. Population genetics of an ecosystem-defining reef coral *Pocillopora damicornis* in the Tropical Eastern Pacific. PLoS One 6, (2011).

32. Underwood, J. N., Smith, L. D., Van Oppen, M. J. H. & Gilmour, J. P. Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. Mol. Ecol. 16, 771–784 (2007).

33. Kendall, M. S. & Poti, M. Transport Pathways of Marine Larvae Around the Mariana Archipelago. NOAA Tech. Memo. NOS NCCOS i–134 (2015).

34. Boulay, J. N. Population Connectivity and Species Diversity of Pacific Coral Reefs. (2014).

35. Rios, D. Determination of the population genetic structure of *Acropora Pulchra* in Guam. (2020).

36. Veron, J. E. N., Stafford, M. G., Turak, E. & DeVantier, L. M. Corals of the World. http://www.coralsoftheworld.org/species_factsheets/species_factsheet_distribution/porites-rus/ (2016).

37. Brooks, A. J., Holbrook, S. J. & Schmitt, R. J. Patterns of microhabitat use by fishes in the Patch-Forming coral *Porites rus*. Raffles Bull. Zool. 2014, 245–254 (2007).

38. Penland, L., Kloulechad, J., Idip, D. & Van Woesik, R. Coral spawning in the western Pacific Ocean is related to solar insolation: Evidence of multiple spawning events in Palau. Coral Reefs 23, 133–140 (2004).

39. Jokiel, P. L., Rodgers, K. S. & Kar, S. A. Comparison of grafting versus a molecular genetic technique in identifying clone mates of the reef coral *Porites rus*. Mar. Ecol. Prog. Ser. 477, 87–92 (2013).

40. Lenz, E. A. & Edmunds, P. J. Branches and plates of the morphologically plastic coral *Porites rus* are insensitive to ocean acidification and warming. J. Exp. Mar. Bio. Ecol. 486, 188–194 (2017).

41. Raymundo, L. J. et al. Successive bleaching events cause mass coral mortality in Guam, Micronesia. Coral Reefs 38, 677–700 (2019).

1.6 Literature Review Figures

Literature Review Figure 1: *P. rus* displaying both branching and plate morphology.

Literature Review Figure 2: Global confirmed distribution of *P. rus*. Source: Corals of the World.

Chapter 2: Publication Formatted Manuscript

First Assessment of Population Genetics Across the Mariana Archipelago

This chapter is prepared for submission to Scientific Reports

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2.1 Abstract

Quantifying population genetic patterns in reef-building corals is important for understanding the biology and fortitude of a species. However, such patterns, like connectivity and genotypic richness, can vary dramatically between species and environments, and are unknown for most coral species. Coral population genetics has never been studied on an archipelago-wide scale in the Mariana Islands, and no population genetic study has been performed on the important coral species *Porites rus*, a highly abundant and resilient reef builder. In this study, I quantify genomic patterns within and among populations of *P. rus* across the Mariana Archipelago. A low coverage whole genome resequencing approach was used to generate genome-wide sequencing data that was analyzed using genotype likelihood methods in ANGSD. Out of 163 sequenced colonies, I identified 105 unique genotypes ($N_G/N = 0.65$) with significant differences in clonality between islands and a negative correlation between clonality and *P. rus* density. I found high levels of genetic diversity (heterozygosity) across populations. There was a significant deficit of heterozygotes throughout the dataset, and all inbreeding coefficients (F_{IS}) were positive, but low. I found small amounts of population structure between populations following an isolation-by-distance pattern, which was corroborated by admixture analysis. Here, I quantify previously unknown population-level patterns in *P. rus* and show that

Mariana Archipelago *P. rus* populations may be a species of concern due to high clonality at some sites and limited population connectivity throughout the archipelago.

2.2 Introduction

Population-level patterns such as genetic structure and clonality are important for understanding the basic biology of a coral species, and informing conservation and management efforts[20]. Yet, these patterns are complex and unknown in many species, and often vary dramatically across species and populations[21].

Understanding patterns of population connectivity in reef-building corals is a key issue in coral reef biology and conservation. Since corals are benthic organisms for most of their life cycle, populations are connected via the pelagic larval stage. Different coral genera can exhibit dramatically different connectivity patterns within the same area[21], and factors influencing connectivity include (but are not limited to) geography, temperature, ocean transport, and other climatic factors[42]. In some species, the pelagic larval stage can allow for long distance dispersal, facilitating reproductive connection between populations across smaller[43] and larger[23] spatial scales. Quantifying these often variable patterns of population connectivity is beneficial for informing marine protected area design[20] and for understanding a population's ability to respond to disturbance events and changing environments through factors such as ecological[44] and evolutionary rescue[45].

Quantifying genotypic richness in corals can reveal important information about the reproduction and local maintenance of a species. Corals can reproduce asexually and generate clones[10] via fragmentation[11] or through the production of parthenogenic larvae[12]. Some species or populations of coral can be highly clonal[13], while others have a relatively low ratio of clones[14]. Within a single species, the amount of clones can vary based on geography and colony

density[16,46] and/or environmental conditions[17]. Quantifying clonality is relevant to understanding a population's vulnerability to stressors: modeling has shown that genetically diverse coral populations may have higher adaptive capacity[47]. Tank experiments with the coral *Acropora cervicornis* demonstrated that only 6% of genotypes in the experiment were resistant to a prominent coral disease[9]. These studies demonstrate potential vulnerability in highly clonal populations, which inherently have low genetic and genotypic diversity. Because the amount of clonality in a species or population of coral can vary greatly, understanding genotypic diversity can improve our understanding of reproductive strategies and population-level vulnerabilities to certain stressors.

The Mariana Islands are an isolated archipelago located in Micronesia, a region in the tropical Northwest Pacific. There are 15 islands spread over ~800 kilometers, consisting of two United States territories: Guam, the largest and southernmost island, and the other 14 islands, the Commonwealth of the Northern Mariana Islands (CNMI). Although one study used population genetics to quantify population structure and connectivity in *A. pulchra* between Guam and Saipan[35], population structure has never been assessed between any of the other 13 islands, in any marine species. However, oceanographic modeling predicts a major break in connectivity between Guam and Rota in species with a short pelagic larval duration due to the North Equatorial Current, which flows between the two islands. For the other islands north of the North Equatorial Current, the model predicts that there is likely some larval exchange at most PLDs, which increases with greater pelagic larval duration[33]. The model predicts that at greater pelagic larval durations, connectivity between islands is much less limited. For example, at a PLD of 12-20 days, larvae can be sent 300-400 km north of their starting position, and at a PLD of 30-50 days, larvae can be sent northward from any island to any other island in the archipelago.

Quantifying population structure among these islands will facilitate the first genetic assessment of connectivity in the Mariana Archipelago.

Porites rus is an abundant, important, and resilient coral in the Mariana Archipelago, but many aspects of its biology, including population connectivity and clonality, are poorly understood. *Porites rus* can generate over a dozen distinct habitat types for 100+ fish species[37] and is often locally dominant, covering large areas, sometimes with so much abundance that it is almost monospecific. Unlike many other coral species, *P. rus* is particularly resilient to thermal stress in both tank experiments[40] and in the field during bleaching events[41,48]. The degree of population connectivity among *P. rus* populations has not been studied in the Mariana Archipelago, or anywhere in the world. It is known to be a broadcast spawning gonochoric species[38,49]. The amount of clonality within *P. rus* populations is not well understood and seems to vary across locations. In Moloka‘i, Hawai‘i, *P. rus* was shown to be highly clonal. Fifteen samples were collected over a 70 m transect, and only two genotypes were detected[39]. Conversely, on Guam, a population of *P. rus* sampled at two different depths (2 m and 10 m) did not exhibit any clonality[15]. Before this study, many population-level patterns in *Porites rus* were understudied or entirely unknown.

In this study, I present the first archipelago-scale population genetics study of a coral in the Mariana Islands, using the highly abundant and resilient *P. rus*. I used low-coverage whole genome resequencing to generate genome-wide datasets, containing 163 individual colonies from 11 populations spanning 7 islands. These datasets were used to assess clonality, genetic diversity, population structure and pairwise relatedness among the Mariana Islands and around the island of Guam. This research increases basic knowledge of the biology of an important reef-building coral and allows the future of *P. rus* to be better forecasted.

2.3 Methods

2.3.1 Sample Collection

Fragments of 330 *Porites rus* colonies were collected from January-May 2022 from eleven populations on seven islands throughout the Mariana Archipelago. This chain of islands spans ~800 km in North-South orientation, from ~13.7° to ~20.0° N. Sampling sites included four sites on Guam, two sites on Maug, and one site per island on Rota, Tinian, Saipan, Sarigan, and Pagan. On Pagan and at one Maug site (Maug A), samples were collected from several nearby sub-sites and grouped together for archipelago-scale analysis. Samples were collected via SCUBA diving and snorkeling, using clippers.

On Guam, samples were collected at 10 m intervals along a transect. At one Guam site, Haps Reef, only the first 10 samples could be collected in this fashion due to patchy *P. rus* distribution after the initial 110 meters, forcing haphazard sample collection after that. At another site, Pago Bay, it was not possible to collect samples at these intervals due to low colony density, so samples were collected opportunistically. On the other six islands, samples were collected with minimum distance intervals of 5 m, estimated visually by sample collectors. Samples were collected at a depth of 3-7 m whenever possible.

Samples were stored in Whirl-Pak bags in the field and subsequently preserved in DESS buffer[50] at -20°C.

2.3.2 DNA extraction, Quality Control, and Sequencing

Genomic DNA was extracted using the Epoch MiniPrep GenCatch extraction kit following the manufacturer's instructions. DNA concentrations were measured using a Qubit fluorometer using the high sensitivity (HS) assay, and gel electrophoresis was used to visualize fragment length and confirm high molecular weight prior to library preparation. Samples were

treated with 5µl of 100mg/mL RNAse during extraction, and RNA concentrations were quantified via Qubit fluorometer using the HS assay to confirm low RNA content and avoid RNA contamination. Extracted DNA from 163 colonies and 6 technical replicates was then shipped to the UC Davis Sequencing Center for low coverage whole genome library preparation and sequencing. Illumina libraries were prepared using custom WGS 384 reaction plexWell Library Preparation Kits and sequenced on the NovaSeq 6000 platform.

2.3.3 Data Curation

Reads were demultiplexed by UC Davis using bcl2fastq version 2.20, and then sent to me. Reads were then trimmed using fastp[51] and aligned to the *P. rus* genome[52] using bwa-mem2[53]. Duplicate reads were removed using Picard MarkDuplicates[54]. Overlapping reads within read pairs were clipped using BamUtil clipOverlap, and the higher quality read in the overlapping segment was retained[55].

2.3.4 Identification and Assessment of Clonality

To assess the amount of genotypic diversity in *P. rus* populations, clonal genotypes were identified using an identity-by-state matrix including all samples and technical replicates, generated with the program ANGSD[56]. A dendrogram of IBS distances was plotted using a hierarchical clustering analysis in R, and distances were used to determine whether samples were clones or unique genotypes. Technical replicates were included because clonal genotypes do not appear completely identical due to sequencing errors and differences in genome coverage between sequencing libraries, blurring the difference between clones and unique genotypes. So, technical replicates were used to determine the cutoff that distinguishes minor differences between clones due to sequencing artifacts from actual differences between unique genotypes.

Groups of samples that have similar degrees of "identity" as technical replicate pairs were determined to be clones; samples that are substantially less similar are considered unique, sexually-derived genotypes.

To assess the relationship between clonality and *P. rus* density, I qualitatively categorized each site as low, high, or almost monospecific *P. rus* density. This rough categorization is based on estimated, not measured, coral cover and should thus be treated accordingly. I binned samples together based on the density category of their site, and then I used Fisher's exact tests to test for differences in clonality between density bins. For the two sites where samples were collected from multiple nearby sub-sites and grouped together for other analyses (Pagan and Maug A), only the samples from the sub-site with the highest sample size were used for the clonality by density analysis. Differences in clonality between islands were also tested via Fisher's exact tests.

Because clones skew population genetic analysis, all but one individual from each clonal genotype or technical replicate pair were removed. Additionally, a single unique individual with an exceptionally low number of mapped reads relative to other samples (<400,000, compared to all other samples, which had >1 million) was removed. ANGSD was then run again using the same parameters as before, but with the reduced sample list, to generate a clone-free dataset. Genotypes from Pagan and Sarigan were retained for comparisons based on individual colonies (admixture and relatedness) but removed due to low sample size for comparisons based on population-level comparisons (heterozygosity, F_{ST}, IBD, and PCoA) (Table S1). The comparisons based on individual colonies included 104 colonies from 11 populations, and the population-level comparisons included 97 colonies from 9 populations.

Due to the low coverage sequencing used for this project, genotype likelihoods were used for all analysis. Genotype likelihoods account better for sequencing errors and thus introduce less

bias in population genetic analyses than standard genotype calling methods[57]. Genotype likelihoods were generated with ANGSD[56], using a minimum mapping quality score of 20, minimum base quality score of 20, and only retaining sites present in at least 70% of all samples. For SNP-based analysis, a minimum SNP calling p-value of 2×10^{-6} was used. To account for linkage disequilibrium, SNPs were subjected to a distance thinning approach where only one in every 100 SNPs were retained for analysis.

2.3.5 Calculating Heterozygosity and F_{IS}

To evaluate heterozygosity, observed (H_O) and expected (H_E) heterozygosity were estimated from ANGSD genotype likelihoods. To estimate H_O, site allele frequencies were calculated with ANGSD. Site allele frequencies were then used to generate per-sample site-frequency spectrums for all sites with the ANGSD subprogram realSFS. These per-sample site-frequency spectrums function as an estimate of the number of homozygous and heterozygous sites. H_E was estimated from genotype likelihoods, using a custom R script[58,59] based on the expectation maximization algorithm in realSFS. Due to the intense computational demands of this method, 1 in 10 sites were subsampled and retained for the H_E calculation. F_{IS} ($1 - H_O/H_E$) was calculated based on the H_O and H_E estimates in R. An ANOVA was used to test for significant differences in H_O and H_E among populations, and Wilcoxon rank-sum tests were used to test for significant differences between H_O and H_E within each population and across the entire dataset.

2.3.6 Assessing Population Structure and Connectivity

To quantify population structure, F_{ST} values were calculated with the ANGSD subprogram realSFS[56]. First, per-population site allele frequencies were generated from all

monomorphic and polymorphic sites. For each pair of populations, a 2-dimensional folded site frequency spectrum was generated. Then, F_{ST} was calculated from each 2-dimensional site frequency spectrum using the F_{ST} function in realSFS.

To evaluate the effect of geographic distance on population structure, isolation-by-distance was assessed in R. First, distances between populations were calculated using the geosphere package[60] for inter-island comparisons, and Google Maps "Measure Distance" feature for intra-island populations. Using the R package vegan[61], Mantel tests were performed to assess significant correlation between geographic and genetic (F_{ST}) distances within Guam and on the archipelago scale. For the archipelago-scale analysis, a stratified Mantel test was performed to account for false positive IBD assessments which are sometimes associated with standard Mantel tests, due to geographic clustering[62]. For the stratified Mantel test, populations were grouped into three clusters based on ngsAdmix results, which aligned with geographical distribution of populations. A scatter plot was used to visualize potential IBD, and to further evaluate significant correlations by calculating R^2 values.

For a different visualization of population structure, an admixture analysis was performed using ngsAdmix, and admixture plots with K values of 2-7 were generated. The optimal K was determined with a custom script using the CLUMPAK method[63] and identified as K = 3.

To further assess population structure, a covariance matrix was generated in ANGSD. The covariance matrix was used to generate and plot a PCoA in R using the package vegan[61,64].

To quantify direct, sample-specific evidence of gene flow between islands, the program ngsRelate[65] was used to calculate R_{ab} values[66], a measurement of the proportion of shared alleles between two individuals. R_{ab} values between all sample pairs were evaluated, and sample pairs

with R_{ab} values > 0.125 were considered closely related, because if a pair of colonies has a R_{ab} value above 0.125, then the pair is separated by 3 generations maximum.

2.4 Results

In total, 169 sequencing libraries consisting of genomic DNA from 163 *Porites rus* colonies and 6 technical replicates were sequenced. These samples represent 11 populations from 7 islands (N = 13-16 per population). Sequencing generated over 1.4 billion mapped read pairs, covering an average of 58.4% of the genome with a mean coverage depth of 4.4x across all samples. 3,675,319 SNPs were identified, resulting in 36,754 SNPs retained for SNP-based analysis after thinning 1/100 to account for linkage. Sequencing libraries from all colonies and technical replicates passed initial quality control and were used to identify clones using hierarchical clustering analysis.

2.4.1 Clonality

Using hierarchical clustering analysis based on identity-by-state distances, I identified two distinct types of relationships among genotypes. One relationship type consisted of IBS distances below 0.19, which included all technical replicate pairs and several other groups of samples. Because samples within these groups had comparable genetic distances from each other as the technical replicate pairs did, they were determined to be clones. A second set of samples was much less similar, with IBS distances of 0.22-0.28, and were determined to be unique genotypes (Fig. 1). Among the 163 *P. rus* colonies sequenced, I identified 105 unique genotypes.

Clones were only detected within sampling sites.

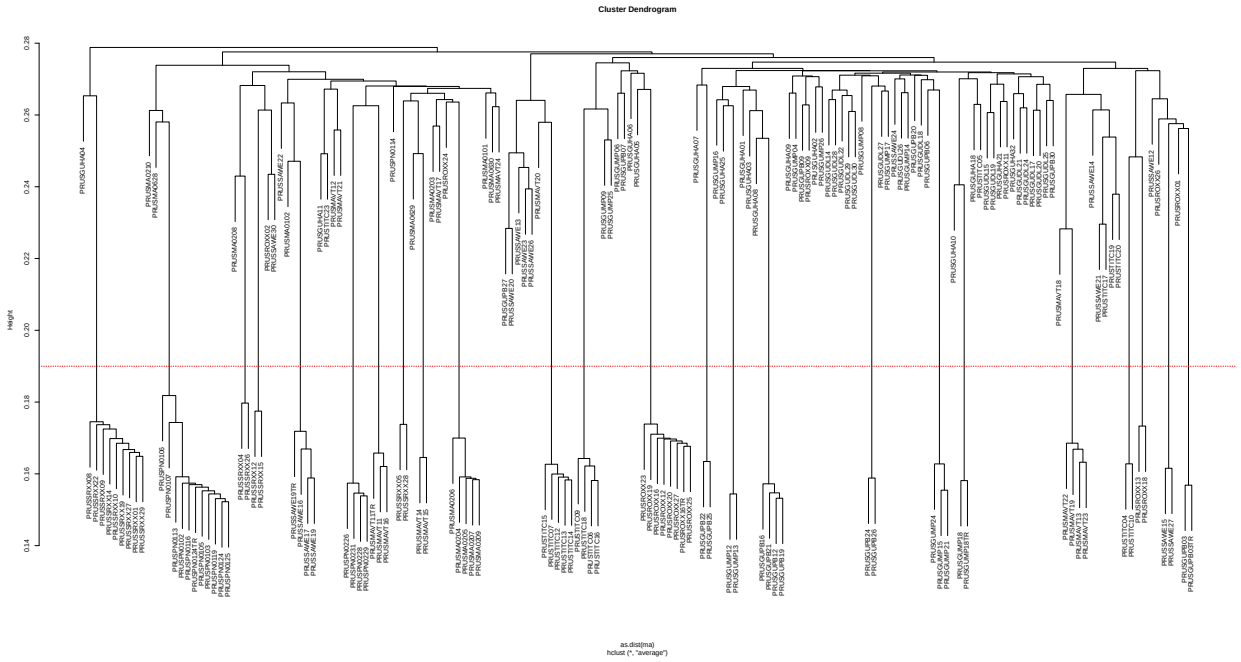


Figure 1: Clonality dendrogram showing genetic distance between samples. The red dashed line indicates the cutoff between clones/technical replicates (node below the line) and unique genotypes (node above the line). Because there is such a clear, large divide between the technical replicates/clonal groups and the unique genotypes, distinguishing clones from unique individuals was obvious and non-ambiguous.

Among islands, Guam had the highest proportion of unique genotypes ($N_G/N = 0.88$), and Pagan and Sarigan had the lowest ($N_G/N = 0.27$ and 0.20, respectively). Pairwise Fisher's exact tests showed that Guam, Saipan, and Maug had significantly less ($p < 0.05$) clonality than Pagan and Sarigan. Additionally, Guam had significantly less clonality than Maug, Rota, and Tinian (Table 2, Fig. 2).

Table 1: Clonality and Genetic Diversity Statistics of All Populations. H_E, H_O, and F_{IS} are unavailable for Pagan and Sarigan due to the low number of unique genotypes.

Site	**N**	**N_G**	**N_G/N**	**H_E**	**H_O**	**F_{IS}**
Guam - Dog Leg Reef	15	15	1.00	0.008	0.007	0.089
Guam - Haps Reef	15	15	1.00	0.008	0.008	0.065
Guam - Merizo	15	12	0.80	0.008	0.007	0.091
Guam - Pago Bay	15	10	0.67	0.008	0.007	0.101
Rota	15	8	0.53	0.008	0.007	0.065
Tinian	16	8	0.50	0.008	0.008	0.073
Saipan	15	12	0.80	0.008	0.007	0.100
Sarigan	15	4	0.27	N/A	N/A	N/A
Pagan	15	3	0.20	N/A	N/A	N/A
Maug A	13	9	0.69	0.008	0.008	0.002
Maug B	14	9	0.64	0.008	0.008	0.013
Total	163	105	0.65	0.008	0.007	0.067

N = number of sequenced samples. N_G = number of genotypes detected. N_G//N = number of genotypes divided by number of sequenced samples. H_E = expected heterozygosity. H_O = observed heterozygosity. F_{IS} = inbreeding coefficient.

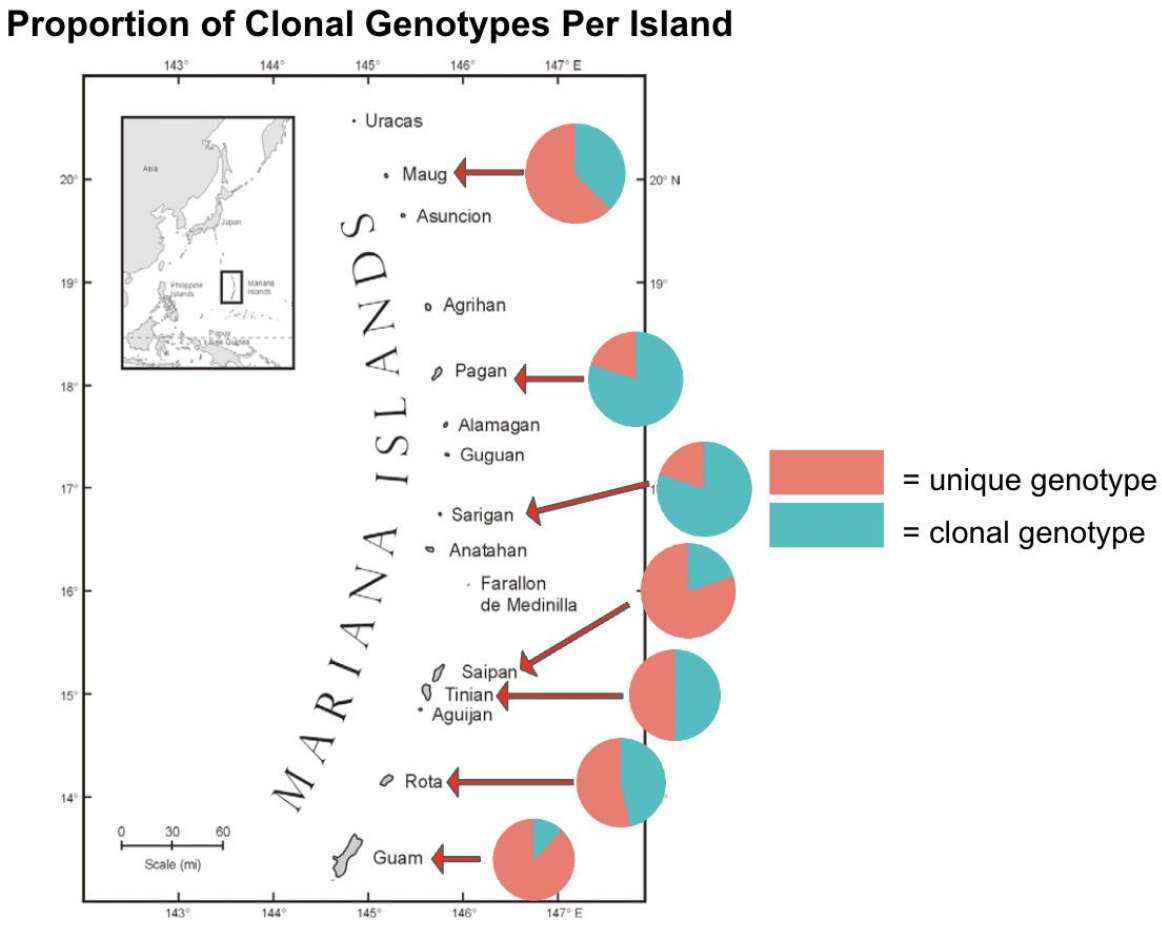


Figure 2: Proportion of unique:clonal genotypes on each island. Guam had significantly less clonality than all islands besides Saipan, Saipan had significantly less clonality than Pagan and Sarigan, and Maug had significantly less clonality than Pagan. See Table 2 for p-values from Fisher's exact tests.

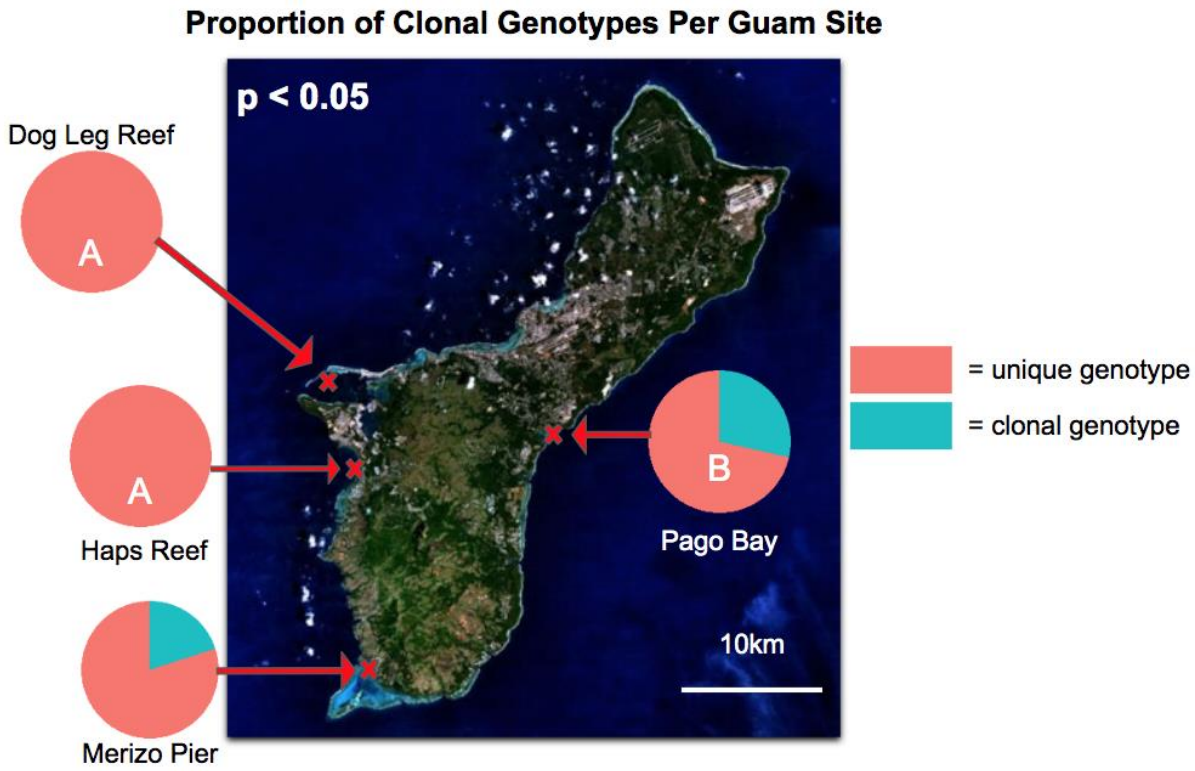


Figure 3: Proportion of unique:clonal genotypes at each site on Guam. Different numbers on the pie charts indicate significant differences in clonality between sites, according to a pairwise Fisher's exact test. Pago Bay, which has significantly more clones than Dog Leg Reef or Haps Reef, is the site with the lowest Porites rus density among Guam sites.

Table 2: P-values from pairwise Fisher's exact test, showing significant differences (green) in clonality among islands. Green values are significant (< 0.05).

	Guam	Rota	Tinian	Saipan	Sarigan	Pagan
Rota	0.008					
Tinian	0.003	1.000				
Saipan	0.683	0.245	0.135			
Sarigan	<0.001	0.264	0.273	0.009		
Pagan	<0.001	0.128	0.135	0.003	1.000	
Maug	0.012	0.511	0.343	0.485	0.023	0.009

On Guam, no clones were detected at two sites, Haps Reef and Dog Leg Reef, but moderate clonality was detected at Pago Bay ($N_G/N = 0.67$) and Merizo Pier ($N_G/N = 0.80$)

(Table 2, Fig. 3). Pairwise Fisher's exact tests ($p < 0.05$) revealed that these differences were significant for Haps Reef and Dog Leg Reef vs. Pago Bay. Interestingly, Pago Bay had the lowest *P. rus* density of any Guam site, so unexpectedly, the site with the lowest *P. rus* density and most distance between colonies had the highest clonality. In fact, this was the only site on Guam where *P. rus* was not a dominant species, and could not be sampled on a transect.

To further evaluate this pattern throughout the archipelago, each site was qualitatively categorized into one of three *P. rus* density estimate categories: low, high, and almost monospecific, and samples were binned together based on the density category of their site. A Fisher's exact test revealed that the "low" *P. rus* density bin had significantly more ($p < 0.05$) clonality than the "high" and "almost monospecific" bins (Fig. 4). In other words, low *P. rus* density is correlated with higher clonality throughout the archipelago, recapitulating the pattern observed on Guam.

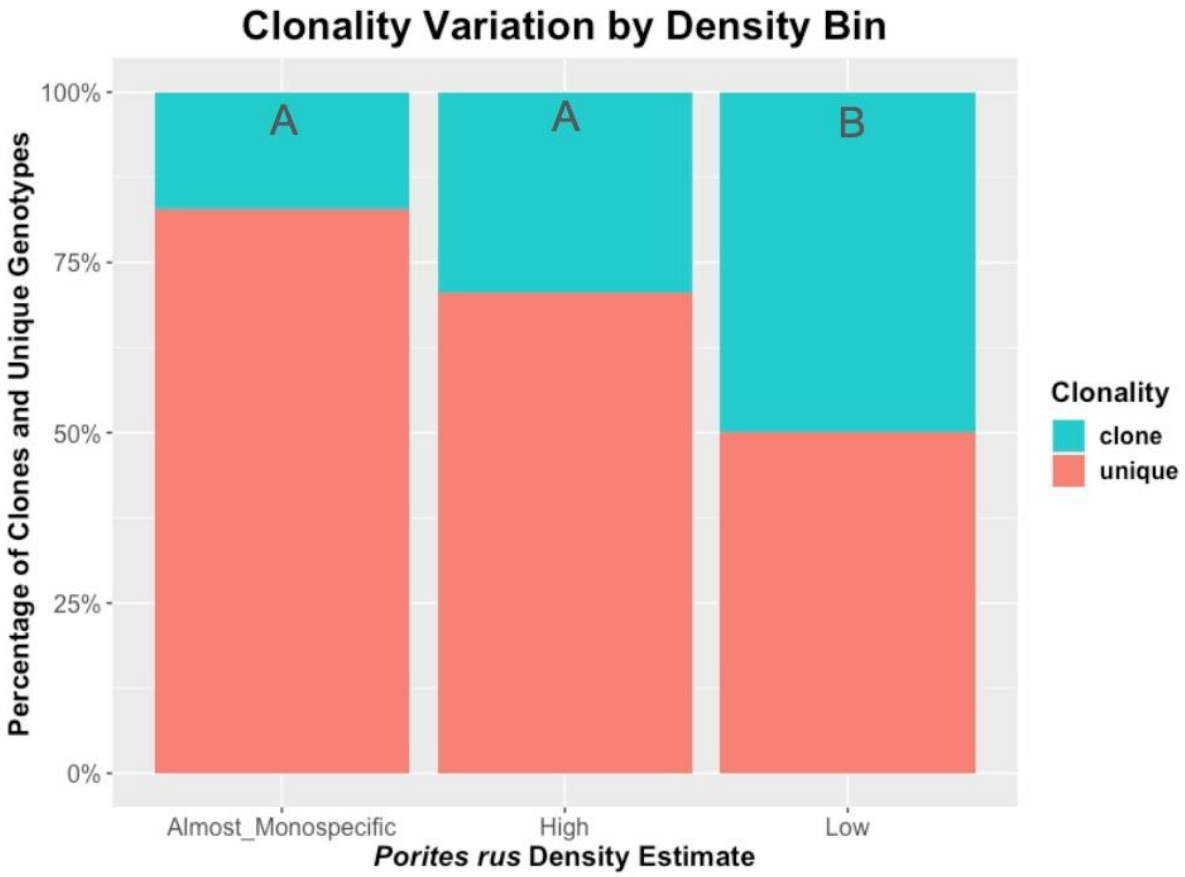


Figure 4: Clonality Variation by Density Bin. Different numbers indicate significant differences in clonality; the "low" *P. rus* density bin had significantly higher clonality than other density bins.

2.4.2 Heterozygosity and F_{IS}

To evaluate expected and observed heterozygosity, H_O and H_E values were calculated based on site frequency spectrum (SFS) estimates over all sites. H_O and H_E were similar across populations (0.0066 - 0.0084; Table 1), with no significant differences among populations based on ANOVA tests. F_{IS} values (Table 1) were an order of magnitude higher and similar across populations (mean = 0.066, median = 0.073, SD = 0.036), indicating small but consistent deficits of heterozygotes in all populations. H_O was significantly lower than H_E overall and within three populations, Saipan and Guam's Dog Leg Reef and Pago Bay (Fig. 5), after adjusting for multiple comparisons via Bonferroni correction.

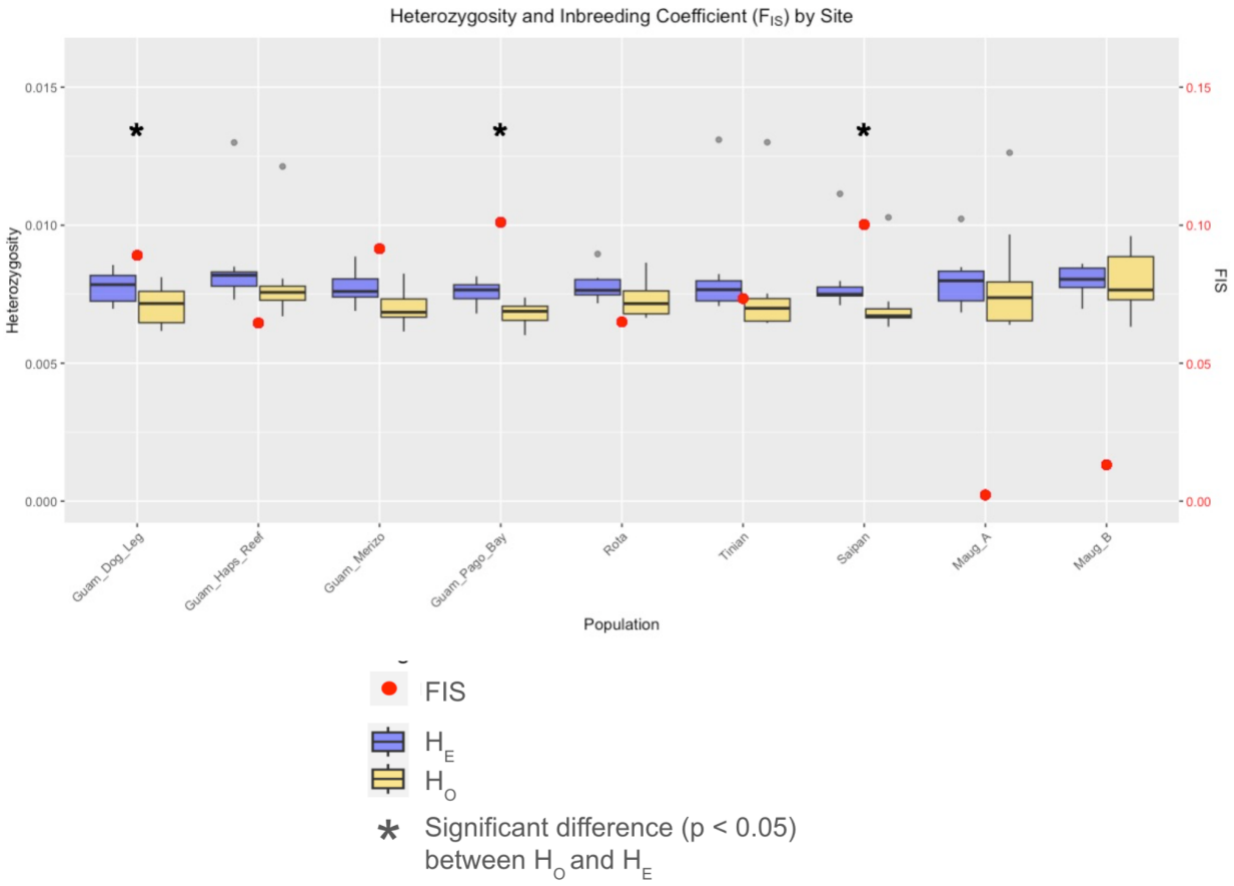


Figure 5: Expected Vs. Observed Heterozygosity By Site. Asterisks indicate significant differences between H_E and H_O within a site; Dog Leg Reef and Pago Bay on Guam and the Saipan site had significantly less H_O than H_E. Red dots represent inbreeding coefficients (F_{IS}), which were all low and positive.

2.4.3 *F_{ST}* and IBD Analysis

F_{ST} values were calculated from the same SFS estimates and ranged from 0.015 to 0.040 (Table 2). These values suggest small to moderate degrees of population differentiation, indicating limited connectivity around Guam and among islands in the Mariana Archipelago.

All intra-island pairwise comparisons (n = 6 on Guam, n = 1 on Maug) were below 0.022 and represent seven of the eight lowest *F_{ST}* values in the dataset; Guam HA-Rota was the only inter-island comparison with a comparable *F_{ST}* of 0.017. Aside from this one inter-island comparison, *F_{ST}* values among the four southern islands (Guam, Rota, Tinian, and Saipan)

ranged from 0.024 to 0.033. In contrast, F_{ST} values between Maug and the Southern Islands ranged from 0.028 to 0.043, including the nine highest F_{ST} values in the dataset. Generally, the farther two sites are from each other, the higher the F_{ST} values, alluding to an overall pattern of isolation-by-distance (IBD)[67].

Table 3: Weighted F_{ST} values. Darker green indicates higher F_{ST}.

	Guam - Dog Leg	Guam - Haps Reef	Guam - Merizo Pier	Guam - Pago Bay	Rota	Tinian	Saipan	Maug A
Guam - Haps Reef	0.017							
Guam - Merizo Pier	0.016	0.016						
Guam - Pago Bay	0.021	0.023	0.017					
Rota	0.026	0.016	0.027	0.030				
Tinian	0.027	0.027	0.023	0.027	0.027			
Saipan	0.031	0.031	0.025	0.026	0.033	0.024		
Maug A	0.039	0.030	0.038	0.040	0.028	0.042	0.043	
Maug B	0.036	0.029	0.034	0.036	0.028	0.030	0.040	0.017

IBD analyses revealed significant correlations between genetic and geographic distances, indicating a pattern of isolation-by-distance throughout the archipelago and around the island of Guam. On the archipelago scale (Fig. 6), there was a substantial ($R^2 = 0.590$) and highly significant ($p = 4.24 \times 10^{-8}$) correlation between genetic and geographic distance and a large ($r = 0.4881$) and significant ($p = 0.016$) Mantel statistic, from a stratified Mantel test. On Guam (Fig. 7), there was a tight ($R^2 = 0.890$) and significant ($p = 0.005$) correlation between genetic and geographic distance, and a large ($r = 0.827$) and significant ($p = 0.001$) Mantel statistic.

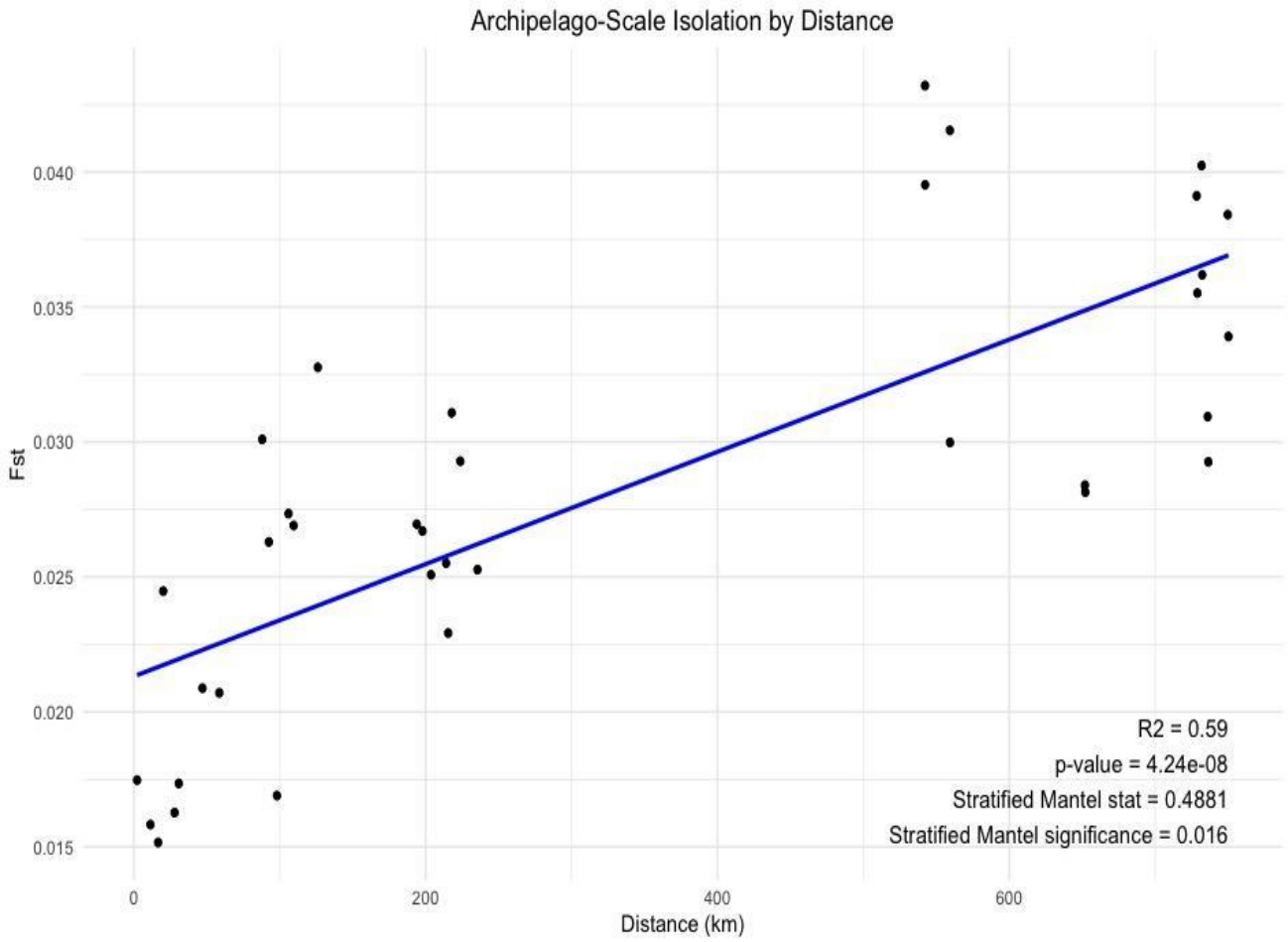


Figure 6: Genetic distance (Weighted $F_{ST,}$ y-axis) vs. geographic distance (km, X-axis) between all pairs of populations (excluding Pagan and Sarigan populations due to low sample size), showing a strong and significant pattern of isolation-by-distance on the archipelago scale.

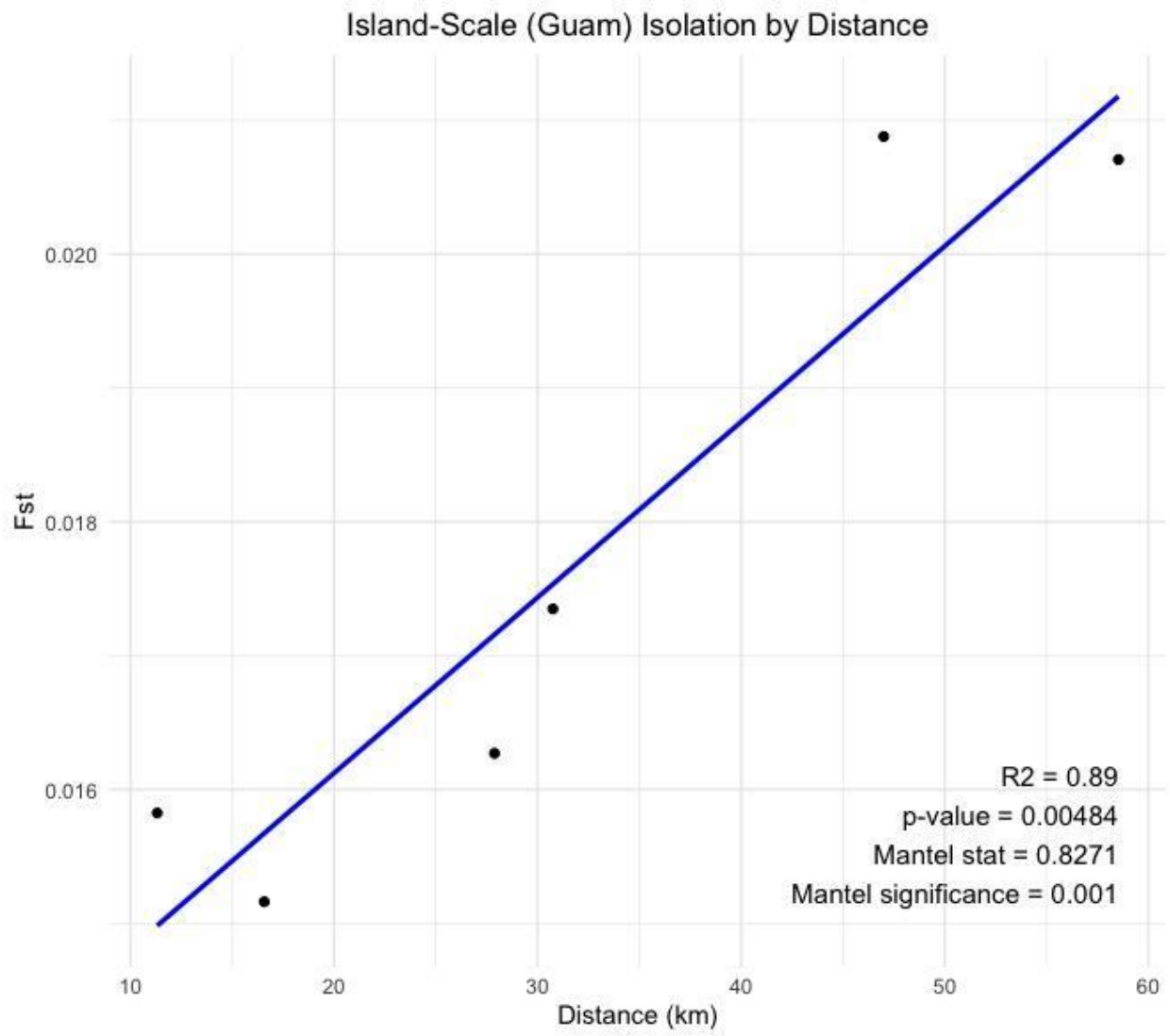


Figure 7: Genetic distance (Weighted $F_{ST,}$ y-axis) vs. geographic distance (km, X-axis) between all pairs of populations on Guam, showing a strong pattern of isolation-by-distance among populations.

2.4.4 Admixture, PCoA, and Relatedness

The admixture plot for the best K (n = 3) shows that the distribution of these 3 genetic clusters among islands is not equal but occurs across a geographic gradient, indicating some population structure in line with isolation-by-distance. Guam and Rota are dominated by the "blue lineage", and Maug is dominated by the "red lineage" (Fig. 8). These are the two

geographic ends of the sampling range and represent the two most homogenous groups of populations with regards to admixture. Saipan and Tinian, in the middle of the sampling range, are dominated by the “yellow lineage”. Although each island is dominated by a single ancestry group, each population also had admixture from the other two groups, indicating some population connectivity. Generally, admixture analysis aligned with isolation-by-distance patterns throughout the Mariana Archipelago.

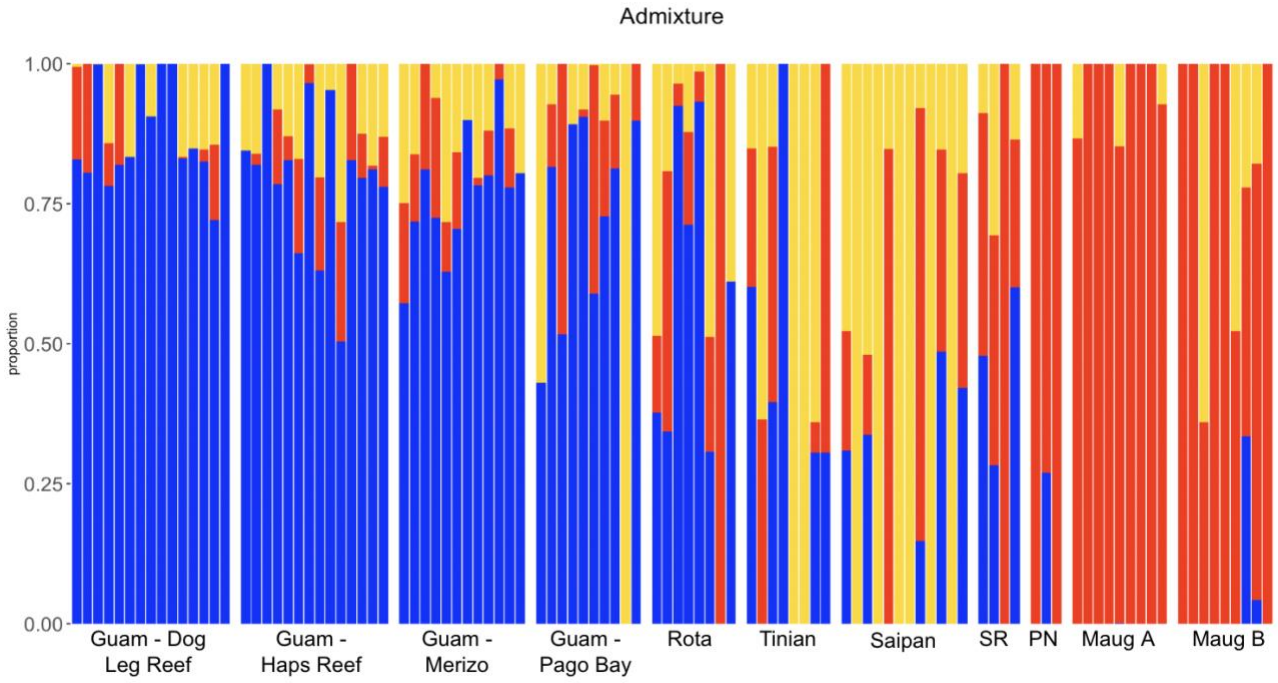


Figure 8: Admixture plot (k = 3) of all non-clonal individuals. SR = Sarigan, PN = Pagan. In line with F_{ST} and IBD analysis, ancestry groups are well mixed, but the admixture plot displays a gradient, where the dominant lineage changes from "blue" (centered around Guam) to "yellow" (on Tinian and Saipan) to "red" (on Maug), from south to north.

Relatedness was assessed by calculating pairwise R_{ab} values for all sample pairs using ngsRelate[65]. 16 out of 1553 (~1.0%) intra-island comparisons were relatives, while only 21 out of 3803 (~0.6%) inter-island comparisons were relatives. Notably, there was a pair of relatives between Maug and Saipan, which are ~540 km apart, and a pair of relatives between Tinian and

Maug, which are ~560 km kilometers apart. Overall, R_{ab} values revealed related individuals living on different islands, tentatively confirming some population connectivity (Table 3).

Table 4: Number and percentage of relative pairs between islands. Percentages above the diagonal represent the percentage of comparisons between two islands that were relatives, and values below the diagonal are the number of relatives between two islands.

	Guam	**Rota**	**Tinian**	**Saipan**	**Sarigan**	**Pagan**	**Maug**
Guam		**0.0%**	**0.7%**	**0.7%**	0.0%	0.0%	0.0%
Rota	**0**		**3.1%**	**2.1%**	0.0%	0.0%	0.0%
Tinian	**3**	**2**		**3.1%**	0.0%	0.0%	0.7%
Saipan	**4**	**2**	**3**		0.0%	0.0%	0.5%
Sarigan	0	0	0	0		0.0%	**2.8%**
Pagan	0	0	0	0	0		**9.3%**
Maug	0	0	1	1	2	3	

No PCoA axis explained more than 2% of the variation in the dataset and showed no clear, consistent patterns. PCoA results were therefore not considered meaningful and are not included here.

2.5 Discussion

2.5.1 Results Summary

This study examined clonality and population structure in *P. rus* across the Mariana Archipelago. Clonality varied among sites on Guam and throughout the Mariana Islands, and sites with higher densities of *P. rus* had significantly less clonality. Genetic diversity was relatively high overall and not significantly different among sites. Inbreeding coefficients (F_{IS}) were positive for all populations but low, indicating consistent minor heterozygote deficits across populations. F_{ST} analysis revealed small levels of population structure among islands, following a significant IBD pattern. Admixture analysis further supported the IBD results, and relatedness analysis revealed closely related individuals on different islands, providing evidence of at least occasional inter-island connectivity.

2.5.2 Clonality

Porites rus populations across the Mariana Archipelago show significantly different degrees of clonality, ranging from a total absence of clones to almost entirely clonal populations. This is interesting because *P. rus* can create large, almost monospecific reefs, and the only two previous studies found very different levels of clonality: Sartor[15] found no clonality (N_G/N = 1.00) within a population of *P. rus* in Apra Harbor, Guam (near my Dog Leg Reef site) while Jokiel[39] found very high (N_G/N = 0.13) clonality in a population on Moloka‘i, Hawai‘i. My results span almost this entire range of clonality, with N_G/N values ranging from 0.20 to 1.00, and indicate that *P. rus* clonality can be highly variable in different populations in the same region, and even on the same island. Significant differences in clonality between sites on Guam and among neighboring islands indicate that clonality may be influenced by local conditions rather than large-scale biogeographic differences. Local factors like reef slope steepness[68] and

wave exposure[17] can affect clonality in corals, so it is possible that environmental heterogeneity between sampling sites is partially responsible for the differences in clonality. However, based on preliminary exploration (Fig. S1), wave exposure does not seem to be a major driver of clonality in this study.

In contrast, I observed a pattern where low *P. rus* density was associated with high clonality (Fig. 4). Significant correlations between density and clonality have been demonstrated in other corals before, such as in locally dominant Caribbean *Acropora* corals. In *A. palmata*, positive correlations between clonality and colony density[16] and colony abundance[13] indicate that asexual reproduction is driving high colony density. In contrast, in *A. cervicornis*, negative correlations between coral cover and clonal abundance in Florida and the Dominican Republic suggest that high genotypic diversity may be driving high coral cover[46], similar to my observation here.

One possible explanation for this pattern are selection effects. For example, higher genotypic diversity increases the probability that genotypes conducive to high coral density are present in the population[69]. This type of selection effect relies on some genotypes being particularly successful and increasing overall population density; it is dependent on *some* clonality combined with genotypic richness. This is unlikely to be driving the negative correlation between clonality and density here, because some high-density sites, such as Dog Leg Reef, had no clonality detected.

In contrast, selection could also lead to reduced genotypic diversity at low density sites. For example, environmental factors or disturbances may have removed most genotypes from some populations, and only genotypes that are particularly resilient to these conditions or disturbances are able to persist. Such a pattern was observed in Hawai'i, where heavily impacted

inshore sites were dominated by a particular subset of *Porites lobata* haplotypes, suggesting that the habit was driving selection for stress resistant genotypes[70]. It is unclear what environmental characteristics or disturbances may have acted on the genotypic diversity of *P. rus* in the Marianas, but it is possible that conditions have made certain sites uninhabitable for most *P. rus* genotypes, allowing only particularly well-adapted genotypes to survive and persist locally via clonal reproduction.

Another possible factor is complementarity, where some type of positive ecological interaction between diverse genotypes, such as facilitation or niche partitioning, benefits genotypically rich populations and enables them to increase their population density[71,72]. The distribution patterns of *P. rus* supports this hypothesis since *P. rus* is often extremely abundant within a highly localized area, and then absent or uncommon on other sections of the reefs, in the Mariana Islands (pers. observ.) but also in French Polynesia (Combosch, pers. comm.). Similarly, in Hawai‘i, Jokiel[39] noted that *P. rus* is uncommon, but if it is present, it tends to cover extensive areas of the reef. Perhaps when genotypic richness is high, facilitation between genotypes of *P. rus* increases abundance within the local area. Although intraspecific complementarity between genotypes has not yet been explicitly quantified in corals, it has been shown in other foundational marine species: for example, in the seagrass *Zostera marina*, genotypic diversity increases disturbance resistance[7], biomass production[8], and notably, population density[8]. Genotypically rich *P. rus* populations may have similar positive interactions that lead to increased abundance and population densities.

The negative correlation between clonality and density is in line with Sartor's results[15], which showed no clonality in a site with near monospecific *P. rus* density (personal observation). In contrast, the high clonality population in Moloka‘i also seems to have had a substantial *P. rus*

density, although no direct measurements or estimates are provided[39]. Differences in clonality between studies could also be due to methodological differences: I used whole genome sequencing and Sartor[15] used RNA-Seq, two high-resolution genomic approaches, while Jokiel[39] used a combination of microsatellites and grafting experiments to determine clonality. Alternatively, *P. rus* populations in the Hawai'ian Islands may generally have lower genotypic richness compared to the Mariana Islands, possibly due to the isolation of Hawai'i relative to the Marianas or differences in disturbance regimes[73,74] between the two archipelagos. And importantly, only one *P. rus* population was studied in Hawai'i, so it is not possible to properly assess the relationship of population density and clonality there.

2.5.3 Genetic diversity

In contrast to clonality, there were no significant differences in genetic diversity (H_O, H_E) among populations. In fact, *P. rus* genetic diversity was surprisingly high compared to other corals that have been assessed with genotype likelihoods and the same custom script: mean H_E values for *Montastraea cavernosa* were 0.002 - 0.003 across 2 depths (shallow and mesophotic) at 4 sites in Florida[75], and mean H_E values for *A. hyacinthus* were between 0.002 - 0.004 among 5 populations in Japan[76], compared to H_E = 0.008 observed here.

In the Mariana Islands, H_E has been measured in three *Acropora* species and three massive *Porites* species. Among massive *Porites*, all mean H_E values were under 0.005, including *P. cf. murrayensis* (H_E = 0.005), *P. cf. australiensis* (H_E = 0.004), and *P. cf. lutea* (H_E = 0.004)[77]. Among *Acropora* species, H_E values were even lower, all under 0.004. This includes *A. pulchra (*H_E = 0.001)[35], *A. surculosa* (H_E = 0.004), and *A verweyi* (H_E = 0.002)[78]. *Porites rus* populations in the Mariana Islands thus have high levels of genetic diversity and presumably a much larger effective population size than most other species.

Inbreeding coefficients (F_{IS}) were all positive and low, indicating small heterozygote deficits across populations. In fact, observed heterozygosity was significantly lower than expected heterozygosity in the dataset as a whole and at 3 out of 9 sites. A broad pattern of heterozygote deficiency has been documented in many broadcast spawning marine invertebrates[79], including coral species such as *P. lobata*[25], *Seriatopora hystrix*[80], *Montipora capitata*[27], *Acropora digitifera*[81], and *Acropora tenuis*[81]. Possible explanations for heterozygote deficits include inbreeding[82], assortative mating[83], or unrecognized substructure within populations[84]. Just like in many other corals, my results show a broad pattern of heterozygote deficiency in *P. rus* throughout the Mariana Islands, but the exact mechanism driving this pattern is unknown.

2.5.4 Population Structure and Connectivity

In this study, I show that *P. rus* populations are structured on the island scale and throughout the Mariana Archipelago, following a significant pattern of isolation-by-distance (IBD). The IBD pattern on Guam indicates that dispersal among populations is limited over ~10-60+ km. Small scale intra-island population structure has been documented in corals before, such as in Palau, where *A. hyacinthus* displays significant population structure between sites as close as 5 km[26], on Reunion Island, where *Pocillopora damicornis* exhibits population structure among nearby sites ~20-40 km apart[28], and on Hawai'i, where *Porites lobata* exhibits significant population structure between sites less than 2 km apart[70]. On Guam, previous coral population genetics work focused on *Acropora* corals: in forereef *A. hyacinthus* and *A. verweyi* populations, no significant population structure was detected[78]. In contrast, small but significant population structure was documented among reef flat *A. pulchra* populations[35], also following an isolation-by-distance pattern. The greatest differentiation among *P. rus* forereef populations was detected

between the eastern site, Pago Bay, and two populations on the west coast, Haps Reef and Dog Leg Reef. Merizo Pier, roughly halfway between the western sites and Pago Bay, had lower and highly similar levels of differentiation compared to populations on either side, indicating that it is equally connected to both sides of the island. This suggests that on Guam, *P. rus* populations within 30 km of each other are more connected than populations >45 km apart. The identical F_{ST} values between Haps Reef-Merizo Pier and Dog Leg-Merizo Pier also indicate that Apra Harbor, a deep lagoon containing Dog Leg Reef (Table 3), does not act as a substantial barrier to dispersal between sites inside and outside of the lagoon. These results are consistent with a previous larval transport study in Guam[85], which used drifters to assess larval transport originating in Apra Harbor and found evidence of current patterns capable of transporting larvae both north and south out of the harbor. Overall, *P. rus* dispersal is limited within Guam, with increased population structure among sites at distances of 45+ km.

Coral population structure between Mariana Islands has so far only been assessed for *A. pulchra* between Guam and Saipan. Interestingly, mean F_{ST} values between Guam and Saipan were nearly identical for *P. rus* (F_{ST} = 0.028) and *A. pulchra* (F_{ST} = 0.026), indicating similar levels of population connectivity between the two islands in both species, with significant isolation-by-distance. The two species are phylogenetically distant[86,87], but both reproduce via broadcast spawning[49,88]. Similar IBD patterns have been documented in other coral species and are particularly common across oceanic islands. Across Micronesia (from Guam and Palau to the Marshall and Phoenix Islands), both *A. digitifera* and *A. hyacinthus* exhibit patterns of isolation-by-distance[89]. *A. hyacinthus* also displayed a significant pattern of isolation-by-distance across the Ryukyus Islands and the southern end of the main islands of Japan[90]. In the Hawai‘ian Archipelago, *Porites lobata* displays a pattern of IBD over large scales[25] but exhibits population

structure driven by environment (IBE) over smaller scales, within and between Maui and Oahu[70]. Similar to many other species of coral throughout the Pacific, population connectivity in *P. rus* seems to be limited over small and large geographic distances, as indicated by the significant IBD pattern.

2.5.5 Significance and Conclusion

Porites rus is widespread[36], locally highly abundant[91], and particularly important in the Mariana Islands because it contributes substantially to the reef structure at many sites. The results of my study indicate that *P. rus* in the Mariana Archipelago could be vulnerable to disturbance events and environmental changes due to high clonality in some populations, and limited connectivity throughout the archipelago.

Significantly different levels of clonality among populations indicate that some populations may be vulnerable to disturbance, but overall, moderate to high genotypic richness at most sites is beneficial for *P. rus*. Highly clonal populations have been shown to have less resistance to disturbances[7] and may be particularly vulnerable to changing environments due to their more limited adaptive capacities[47,92,93], which is especially concerning for corals, as climate change, pollution and other anthropogenic factors continue to massively alter coral reef ecosystems throughout the world[94]. Since some genotypes may exhibit disease resistance[9,95], populations with higher genotypic richness may be less vulnerable to disease. The highly clonal populations of Pagan and Sarigan may be more vulnerable to some stressors than minimally clonal populations like Dog Leg Reef and Haps Reef on Guam. Besides those two highly clonal populations, most other populations have many unique genotypes and are of low concern for clonality-related issues. Additionally, my results show that low clonality is associated with high

P. rus density, so sites that are dominated by *P. rus* (and thus highly dependent on *P. rus* for reef structure) are unlikely to suffer from issues caused by low genotypic richness.

Although I found positive inbreeding coefficients (F_{IS}) in all populations and a significant heterozygosity deficit overall and in three sites, inbreeding is likely not a major concern for *P. rus* in the Mariana Islands. Although inbreeding generally reduces fitness [82,96,97] these F_{IS} values were low compared to what is typically considered moderate or severe inbreeding ($F_{IS} > 0.2$)[98–100]. I also found high genetic diversity in *P. rus* compared to other coral species. Additionally, all populations exhibit some connectivity, which allows new alleles to be introduced to potentially inbred populations, counteracting negative effects associated with inbreeding[101] with as little as only one migrant per generation[101,102]. This idea is not based on theory alone; genetic rescue, or the artificial introduction of migrants into inbred populations, has been shown to reduce inbreeding depression[103,104]. Because of the relatively low F_{IS} values and evidence of population connectivity, inbreeding is not a major concern for *P. rus*.

Porites rus populations exhibit limited connectivity within the Mariana Archipelago, and because of this, these populations cannot be expected to benefit from positive forces associated with connectivity such as ecological rescue and evolutionary rescue. Ecological rescue is when extinction caused by local disturbance events is mitigated by larval import from other populations[44]. Evolutionary rescue, or adaptation to severe disturbance through natural selection[45], is particularly suitable for well-connected populations[105], because beneficial mutations (or a pre-existing local adaptation which becomes beneficial for the whole meta-population as the environment changes) could be spread throughout the archipelago. Although evolutionary and ecological rescue could theoretically occur between populations with limited connectivity (as long as they are not completely isolated), low connectivity would slow these

processes down to the point where they are too slow for the current pace of climate change and other anthropogenic factors that are causing large scale degradation of coral reef ecosystems[94].

In conclusion, I estimate that *P. rus* in the Mariana Islands is a species of concern due to limited connectivity between populations, and occasionally, high clonality. Limited connectivity indicates that populations may be unable to substantially benefit from ecological or evolutionary rescue, and high clonality indicates that populations may be vulnerable to disease and environmental change. Because of the patterns documented in this study and because of the importance of *P. rus* to coral reefs in the Mariana Islands, *P. rus* populations should be managed on a small-scale with expectations of limited connectivity, and overall, should be considered a species of conservation priority and concern.

2.6 References

1. Bellwood, D. R., Hughes, T. P., Folke, C. & Nyström, M. Confronting the coral reef crisis. *Nature* **429**, 827–833 (2004).
2. Hughes, T. P. Catastrophes, Phase Shifts, and Large-Scale Degredation of a Caribbean Coral Reef. *Science (80-.).* **265**, 1547–1551 (1994).
3. Mumby, P. J., Hastings, A. & Edwards, H. J. Thresholds and the resilience of Caribbean coral reefs. *Nature* **450**, 98–101 (2007).
4. Frieler, K. *et al.* Limiting global warming to 2C is unlikely to save most coral reefs. *Nat. Clim. Chang.* **3**, 165–170 (2013).
5. Kleypas, J. *et al.* Designing a blueprint for coral reef survival. *Biol. Conserv.* **257**, 109107 (2021).
6. Eddy, T. D. *et al.* Global decline in capacity of coral reefs to provide ecosystem services. *One Earth* **4**, 1278–1285 (2021).
7. Hughes, A. R. & Stachowicz, J. J. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 8998–9002 (2004).
8. Reusch, T. B. H., Ehlers, A., Hämmerli, A. & Worm, B. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 2826–2831 (2005).
9. Vollmer, S. V. & Kline, D. I. Natural Disease Resistance in Threatened Staghorn Corals. *PLoS One* **3**, e3718 (2008).
10. Richmond, R. H. & Hunter, C. L. Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Mar. Ecol. Prog. Ser.* **60**, 185–203 (1990).

11. Highsmith, R. Reproduction by Fragmentation in Corals. *Mar. Ecol. Prog. Ser.* **7**, 207–226 (1982).

12. Combosch, D. J. & Vollmer, S. V. Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. *Ecol. Evol.* **3**, 3379–3387 (2013).

13. Williams, D. E., Miller, M. W. & Baums, I. B. Cryptic changes in the genetic structure of a highly clonal coral population and the relationship with ecological performance. *Coral Reefs* **33**, 595–606 (2014).

14. van der Ven, R. M., Flot, J. F., Buitrago-López, C. & Kochzius, M. Population genetics of the brooding coral *Seriatopora hystrix* reveals patterns of strong genetic differentiation in the Western Indian Ocean. *Heredity (Edinb).* **126**, 351–365 (2021).

15. Sartor, C. Transcriptomic Signatures of Acclimation in 'Susceptible' *Acropora surculosa* and 'Resistant' *Porites rus*. (University of Guam Marine Lab, 2021).

16. Baums, I. B., Miller, M. W. & Hellberg, M. E. Geographic Variation in Clonal Structure in a Reef-Building Caribbean Coral, *Acropora palmata*. *Ecol. Monogr.* **76**, 503–519 (2006).

17. Torres, A. F., Forsman, Z. H. & Ravago-Gotanco, R. Shifts in coral clonality along a gradient of disturbance: insights on reproduction and dispersal of *Pocillopora acuta*. *Mar. Biol. 2020 16711* **167**, 1–18 (2020).

18. Selig, E. R. & Bruno, J. F. A global analysis of the effectiveness of marine protected areas in preventing coral loss. *PLoS One* **5**, 1–7 (2010).

19. Mellin, C., Aaron Macneil, M., Cheal, A. J., Emslie, M. J. & Julian Caley, M. Marine protected areas increase resilience among coral reef communities. *Ecol. Lett.* **19**, 629–637 (2016).

20. Lagabrielle, E. *et al.* Connecting MPAs - eight challenges for science and management. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **24**, 94–110 (2014).

21. Miller, K. J. & Ayre, D. J. Population structure is not a simple function of reproductive mode and larval type: Insights from tropical corals. *J. Anim. Ecol.* **77**, 713–724 (2008).

22. Miller, K. J. & Ayre, D. J. Protection of Genetic Diversity and Maintenance of Connectivity among Reef Corals within Marine Protected Areas. *Conserv. Biol.* **22**, 1245–1254 (2008).

23. Huang, W. *et al.* Genetic diversity and large-scale connectivity of the scleractinian coral *Porites lutea* in the South China Sea. *Coral Reefs* **37**, 1259–1271 (2018).

24. Cowen, R. K. & Sponaugle, S. Larval dispersal and marine population connectivity. *Ann. Rev. Mar. Sci.* **1**, 443–466 (2009).

25. Polato, N. R., Concepcion, G. T., Toonen, R. J. & Baums, I. B. Isolation by distance across the Hawaiian Archipelago in the reef-building coral *Porites lobata*. *Mol. Ecol.* **19**, 4661–4677 (2010).

26. Cros, A., Toonen, R. J., Davies, S. W. & Karl, S. A. Population genetic structure between Yap and Palau for the coral *Acropora hyacinthus*. *PeerJ* **2016**, 1–17 (2016).

27. Concepcion, G. T., Baums, I. B. & Toonen, R. J. Regional population structure of *Montipora capitata* across the Hawaiian Archipelago. *Bull. Mar. Sci.* **90**, 257–275 (2014).

28. Gélin, P. *et al.* Superclone expansion, long-distance clonal dispersal and local genetic structuring in the coral *Pocillopora damicornis* type β in Reunion Island, South Western Indian Ocean. *PLoS One* **12**, 1–20 (2017).

29. Drury, C. *et al.* Genomic patterns in *Acropora cervicornis* show extensive population structure and variable genetic diversity. *Ecol. Evol.* **7**, 6188–6200 (2017).

30. Dodge, D. L. *et al.* Population structure of the scleractinian coral *Montastraea cavernosa* in southeast Florida. *Bull. Mar. Sci.* **96**, 767–782 (2020).

31. Combosch, D. J. & Vollmer, S. V. Population genetics of an ecosystem-defining reef coral *Pocillopora damicornis* in the Tropical Eastern Pacific. *PLoS One* **6**, (2011).

32. Underwood, J. N., Smith, L. D., Van Oppen, M. J. H. & Gilmour, J. P. Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Mol. Ecol.* **16**, 771–784 (2007).

33. Kendall, M. S. & Poti, M. Transport Pathways of Marine Larvae Around the Mariana Archipelago. *NOAA Tech. Memo. NOS NCCOS* i–134 (2015).

34. Boulay, J. N. Population Connectivity and Species Diversity of Pacific Coral Reefs. (2014).

35. Rios, D. Determination of the population genetic structure of *Acropora Pulchra* in Guam. (2020).

36. Veron, J. E. N., Stafford, M. G., Turak, E. & DeVantier, L. M. Corals of the World. http://www.coralsoftheworld.org/species_factsheets/species_factsheet_distribution/porites-rus/ (2016).

37. Brooks, A. J., Holbrook, S. J. & Schmitt, R. J. Patterns of microhabitat use by fishes in the Patch-Forming coral *Porites rus*. *Raffles Bull. Zool.* **2014**, 245–254 (2007).

38. Penland, L., Kloulechad, J., Idip, D. & Van Woesik, R. Coral spawning in the western Pacific Ocean is related to solar insolation: Evidence of multiple spawning events in Palau. *Coral Reefs* **23**, 133–140 (2004).

39. Jokiel, P. L., Rodgers, K. S. & Kar, S. A. Comparison of grafting versus a molecular genetic technique in identifying clone mates of the reef coral *Porites rus*. *Mar. Ecol. Prog.*

Ser. **477**, 87–92 (2013).

40. Lenz, E. A. & Edmunds, P. J. Branches and plates of the morphologically plastic coral *Porites rus* are insensitive to ocean acidification and warming. *J. Exp. Mar. Bio. Ecol.* **486**, 188–194 (2017).

41. Raymundo, L. J. *et al.* Successive bleaching events cause mass coral mortality in Guam, Micronesia. *Coral Reefs* **38**, 677–700 (2019).

42. Selkoe, K. A. *et al.* A decade of seascape genetics: Contributions to basic and applied marine connectivity. *Mar. Ecol. Prog. Ser.* **554**, 1–19 (2016).

43. Shilling, E. N., Eckert, R. J., Sturm, A. B. & Voss, J. D. *Porites astreoides* coral populations demonstrate high clonality and connectivity in southeast Florida. *Coral Reefs* **42**, 1131–1145 (2023).

44. Brown, J. H. & Kodric-Brown, A. Turnover Rates in Insular Biogeography: Effect of Immigration on Extinction. *Ecology* **58**, 445–449 (1977).

45. Bell, G. Evolutionary Rescue. *Annu. Rev. Ecol. Evol. Syst.* **48**, 605–627 (2017).

46. Drury, C., Greer, J. B., Baums, I., Gintert, B. & Lirman, D. Clonal diversity impacts coral cover in *Acropora cervicornis* thickets: Potential relationships between density, growth, and polymorphisms. *Ecol. Evol.* **9**, 4518–4531 (2019).

47. Matz, M. V., Treml, E. A., Aglyamova, G. V. & Bay, L. K. Potential and limits for rapid genetic adaptation to warming in a Great Barrier Reef coral. *PLoS Genet.* **14**, 1–19 (2018).

48. van Woesik, R. Coral bleaching: The winners and the losers. 3–4 (2010).

49. Bronstein, O. & Loya, Y. Daytime spawning of *Porites rus* on the coral reefs of Chumbe Island in Zanzibar, Western Indian Ocean (WIO). *Coral Reefs* **30**, 441–441 (2011).

50. Gaither, M. R., Szabó, Z., Crepeau, M. W., Bird, C. E. & Toonen, R. J. Preservation of

corals in salt-saturated DMSO buffer is superior to ethanol for PCR experiments. *Coral Reefs* **30**, 329–333 (2011).

51. Chen, S. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. *iMeta* **2**, 1–5 (2023).
52. Celis, J. S. *et al.* Binning enables efficient host genome reconstruction in cnidarian holobionts. *Gigascience* **7**, 1–12 (2018).
53. Md, V., Misra, S., Li, H. & Aluru, S. Efficient architecture-aware acceleration of BWA-MEM for multicore systems. *Proc. - 2019 IEEE 33rd Int. Parallel Distrib. Process. Symp. IPDPS 2019* 314–324 (2019) doi:10.1109/IPDPS.2019.00041.
54. Broad Institute. Picard Toolkit. *2018*.
55. Jun, G., Wing, M. K., Abecasis, G. R. & Kang, H. M. An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Res.* **25**, 918–925 (2015).
56. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. Angsd. *BMC Bioinformatics* **15**, 1–13 (2014).
57. Skotte, L., Korneliussen, T. S. & Albrechtsen, A. Estimating individual admixture proportions from next generation sequencing data. *Genetics* **195**, 693–702 (2013).
58. Fifer, J. He. https://github.com/jamesfifer/JapanRE/blob/main/He.R (2021).
59. Barfield, S. heterozygosity_beagle.R. (2020).
60. Hijmans, R. geosphere: Spherical Trigonometry_. R. (2022).
61. Oksanen, J. *et al.* vegan. (2022).
62. Meirmans, P. G. The trouble with isolation by distance. *Mol. Ecol.* **21**, 2839–2846 (2012).
63. Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A. & Mayrose, I. Clumpak:

A program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* **15**, 1179–1191 (2015).

64. Barfield, S. J., Aglyamova, G. V., Bay, L. K. & Matz, M. V. Contrasting effects of Symbiodinium identity on coral host transcriptional profiles across latitudes. *Mol. Ecol.* **27**, 3103–3115 (2018).

65. Korneliussen, T. S. & Moltke, I. NgsRelate: A software tool for estimating pairwise relatedness from next-generation sequencing data. *Bioinformatics* **31**, 4009–4011 (2015).

66. Hedrick, P. P. W., Lacy, R. C. & Baker, C. S. Measuring relatedness between inbred individuals. *J. Hered.* **106**, 20–25 (2015).

67. Slatkin, M. Isolation by Distance in Equilibrium and Non-Equilibrium Populations. *Evolution (N. Y).* **47**, 264 (1993).

68. Foster, N. L. *et al.* Hurricane-Driven Patterns of Clonality in an Ecosystem Engineer: The Caribbean Coral *Montastraea annularis*. *PLoS One* **8**, (2013).

69. Stachowicz, J. J., Bruno, J. F. & Duffy, J. E. Understanding the effects of marine biodiversity on communities and ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **38**, 739–766 (2007).

70. Tisthammer, K. H., Forsman, Z. H., Toonen, R. J. & Richmond, R. H. Genetic structure is stronger across human-impacted habitats than among islands in the coral *Porites lobata*. *PeerJ* **2020**, 1–24 (2020).

71. Loreau, M. & Hector, A. Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**, 72–76 (2001).

72. Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N. & Vellend, M. Ecological consequences of genetic diversity. *Ecol. Lett.* **11**, 609–623 (2008).

73. Wells, F. H. Tropical Cyclones Affecting Guam (1671-1990).pdf. vol. 56 52 (1990).

74. Smith, J. M., Kennedy, A. B., Westerink, J. J., Taflanidis, A. A. & Cheung, K. F. Hawaii Hurricane Wave and Surge Modeling and Fast Forecasting. *Coast. Eng. Proc.* 8 (2012) doi:10.9753/icce.v33.management.8.

75. Sturm, A. B., Eckert, R. J., Carreiro, A. M. & Voss, J. D. Population genetic structure of the broadcast spawning coral, *Montastraea cavernosa*, demonstrates refugia potential of upper mesophotic populations in the Florida Keys. *Coral Reefs* **41**, 587–598 (2022).

76. Fifer, J. E., Yasuda, N., Yamakita, T., Bove, C. B. & Davies, S. W. Genetic divergence and range expansion in a western North Pacific coral. *Sci. Total Environ.* **813**, 152423 (2022).

77. Primov, K. D., Burdick, D. R., Lemer, S., Forsman, Z. H. & Combosch, D. J. Genomic data reveals habitat partitioning in massive *Porites* on Guam , Micronesia. *Sci. Rep.* 1–15 (2024) doi:10.1038/s41598-024-67992-w.

78. Torrado, H. *et al.* Evolutionary genomics of two co-occurring congeneric fore reef coral species in Guam (Mariana Islands). (2024).

79. Addison, J. A. & Hart, M. W. Spawning, copulation and inbreeding coefficients in marine invertebrates. *Biol. Lett.* **1**, 450–453 (2005).

80. Maier, E., Tollrian, R., Rinkevich, B. & Nürnberger, B. Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Mar. Biol.* **147**, 1109–1120 (2005).

81. Zayasu, Y. *et al.* Genome-wide SNP genotyping reveals hidden population structure of an acroporid species at a subtropical coral island: Implications for coral restoration. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **31**, 2429–2439 (2021).

82. Charlesworth, D. & Willis, J. H. The genetics of inbreeding depression. *Nat. Rev. Genet.* **10**, 783–796 (2009).

83. Jiang, Y., Bolnick, D. I. & Kirkpatrick, M. Assortative mating in animals. *Am. Nat.* **181**, (2013).

84. Wahlund, V. S. Zusammensetzung von Population und Korrelationserscheinung vom standpunkt der Vererbungslehre aus betrachtet. *Heriditas* 65–106 (1928).

85. Kawahigashi, K. Reproductive Behavior of *Gomphosus varius* (Labridae) in Relation to Current Patterns at a Spawning Aggregation Site: Implications for Larval Dispersal. (University of Guam, 2021).

86. Quek, Z. B. R. *et al.* A hybrid-capture approach to reconstruct the phylogeny of Scleractinia (Cnidaria: Hexacorallia). *Mol. Phylogenet. Evol.* **186**, 107867 (2023).

87. Kerr, A. M. Molecular and morphological supertree of stony corals (Anthozoa: Scleractinia) using matrix representation parsimony. *Biol. Rev. Camb. Philos. Soc.* **80**, 543–558 (2005).

88. Baird, A. H. The ecology of coral larvae: settlement patterns, habitat selection and the length of the larval phase. *J. Proc. R. Soc. New South Wales* **137**, 43–43 (2004).

89. Davies, S. W., Treml, E. A., Kenkel, C. D. & Matz, M. V. Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. *Mol. Ecol.* **24**, 70–82 (2015).

90. Nakabayashi, A. *et al.* The potential role of temperate Japanese regions as refugia for the coral *Acropora hyacinthus* in the face of climate change. *Sci. Rep.* **9**, 1–12 (2019).

91. Burdick, D. *et al.* Status of the coral reef ecosystems of Guam. 1–76 (2008) doi:10.13140/RG.2.1.2183.8960.

92. Colton, M. A. *et al.* Coral conservation in a warming world must harness evolutionary adaptation. *Nat. Ecol. Evol.* **6**, 1405–1407 (2022).

93. Walsworth, T. E. *et al.* Management for network diversity speeds evolutionary adaptation to climate change. *Nat. Clim. Chang.* **9**, 632–636 (2019).

94. Bellwood, D. R., Hughes, T. P., Folke, C. & Nyström, M. Confronting the coral reef crisis. *Nature* **429**, 827–833 (2004).

95. Muller, E. M., Bartels, E. & Baums, I. B. Bleaching causes loss of disease resistance within the threatened coral species *Acropora cervicornis*. *Elife* **7**, 1–20 (2018).

96. Reed, D. H. & Frankham, R. Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**, 230–237 (2003).

97. Montgomery, M. E. *et al.* Relationships between population size and loss of genetic diversity: Comparisons of experimental results with theoretical predictions. *Conserv. Genet.* **1**, 33–43 (2000).

98. Affre, L. & Thompson, J. D. Population genetic structure and levels of inbreeding depression in the Mediterranean island endemic *Cyclamen creticum* (Primulaceae). *Biol. J. Linn. Soc.* 527–549 (1997).

99. Poudel, R. C. *et al.* Low genetic diversity and high inbreeding of the endangered yews in central Himalaya: Implications for conservation of their highly fragmented populations. *Divers. Distrib.* **20**, 1270–1284 (2014).

100. Gingerich, D. P., Borsa, P., Suckling, D. M. & Brun, L. O. Inbreeding in the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) estimated from endosulfan resistance phenotype frequencies. *Bull. Entomol. Res.* **86**, 667–674 (1996).

101. Wright, S. Evolution in Mendelian Populations. *Genetics* **17**, 97–159 (1931).

102. Spieth, P. T. Gene flow and genetic differentiation. *Genetics* **78**, 961–965 (1974).

103. Schwartz, M. K. & Mills, L. S. Gene flow after inbreeding leads to higher survival in deer mice. *Biol. Conserv.* **123**, 413–420 (2005).

104. Nichols, S. *et al.* Genetic rescue attempt in a small, inbred population of a wild endangered passerine. *Biol. Conserv.* **290**, 110430 (2024).

105. Razgour, O. *et al.* Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 10418–10423 (2019).

Supplemental Publication Figures and Tables

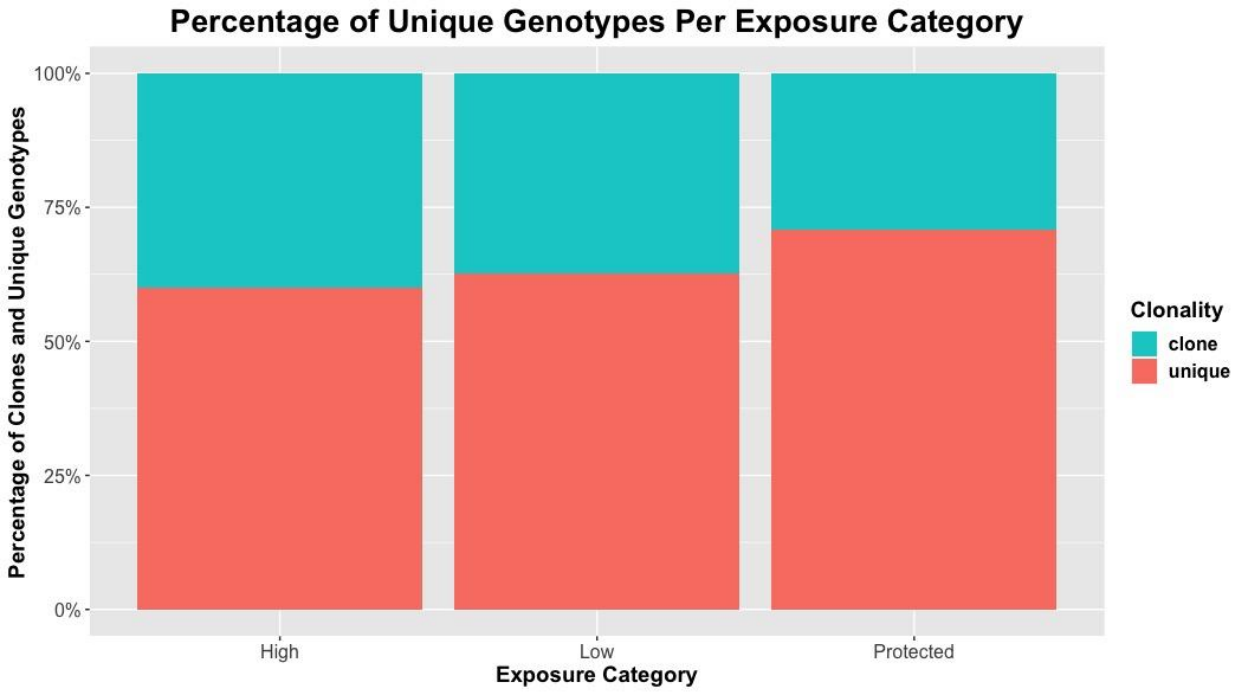
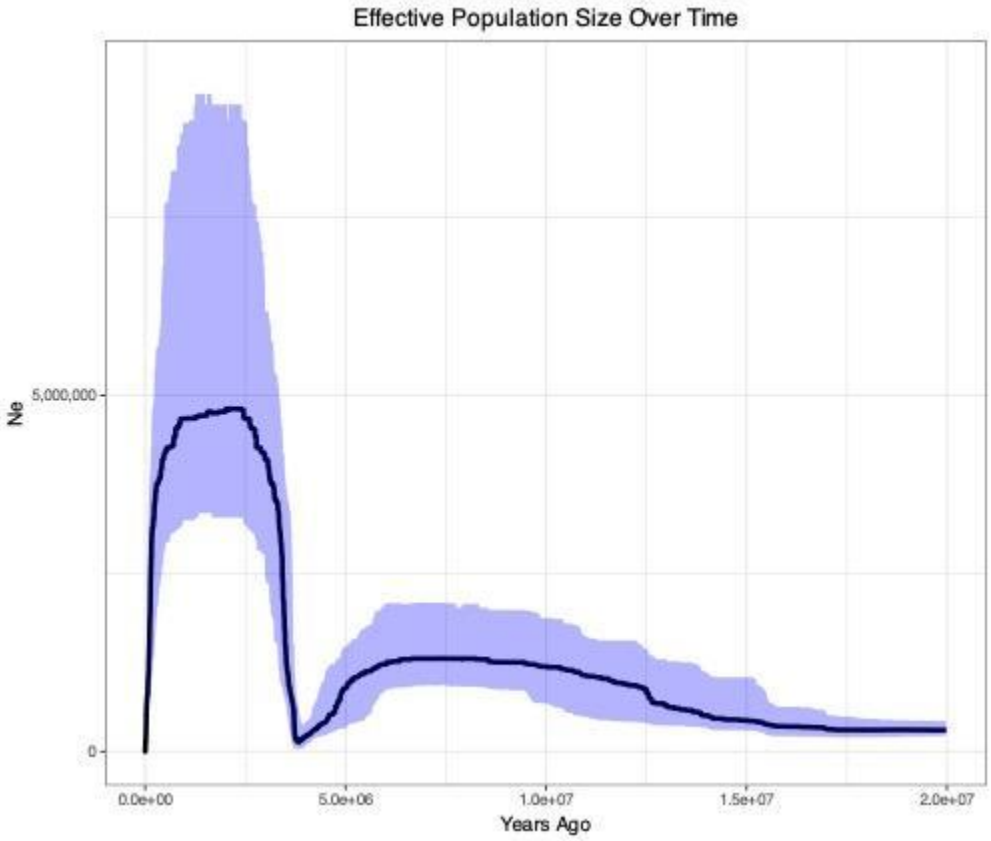


Figure S1: Clonality by estimated exposure. Wave exposure was estimated by classifying east side sites as high exposure, west side sites as low exposure, and sites with substantial physical barriers from wave action as protected. There were no significant differences in clonality between exposure categories based on a Fisher's exact test.

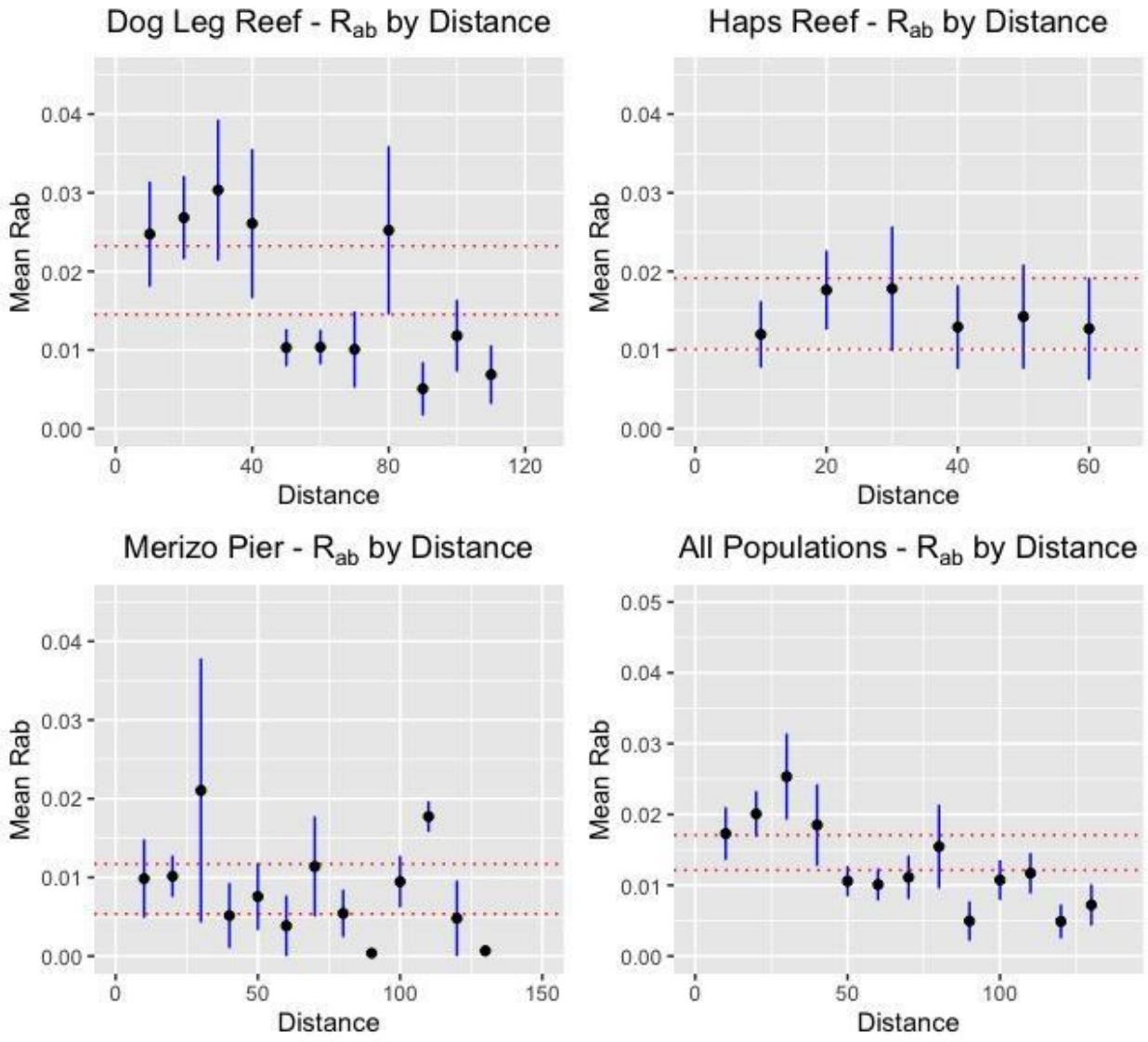
Table S1: Description of datasets

Dataset	Samples	Genomic sites	Analysis
1	All	SNPs	Determination of clonality, differences in clonality between islands
2	All except for low N subsites	SNPs	Relationship between clonality and density
3	No clones/technical replicates	All	F_{st}, IBD, H_O
4	No clones/technical replicates	1 in 10 subsampling	H_E
5	No clones/technical replicates	SNPs, 1 in 100 thinning	Admixture, relatedness, PCoA

Chapter 3: Supplemental Figures and Tables



Additional Figure 1: Effective population size over time. Effective population size over time was estimated using stairwayplot2, and results show a population expansion ~375,000 years ago. Results were plotted in R using ggplot2.



Additional Figure 2: RAB values by Distance. Rab values were calculated in ngsRelate and plotted against distance intervals in R. Mean Rab values at each distance interval at each individual site where samples were collected on transect, and at all three sites combined. Red dotted lines represent 95% confidence intervals. Results show that at Dog Leg Reef, samples within 40 m of each other were more related than samples farther away. Interestingly, Dog Leg Reef is the most protected of all sites shown here, because it is located in a large, deep lagoon (Apra Harbor). The same relationship is seen at all three sites combined, but is driven by the strong pattern at Dog Leg Reef.

Additional Table 1: Percent Variation Explained by each PCoA Axis. Results show that no PCoA axis explained a meaningful percentage of variation. PCoA was generated using a covariance matrix from ANGSD as described in the methods of the main text.

Axis	Variation Explained (%)	Axis	Variation Explained (%)	Axis	Variation Explained (%)
Axis 1	1.79	Axis 34	1.06	Axis 67	0.94
Axis 2	1.61	Axis 35	1.06	Axis 68	0.94
Axis 3	1.49	Axis 36	1.06	Axis 69	0.94
Axis 4	1.36	Axis 37	1.05	Axis 70	0.93
Axis 5	1.32	Axis 38	1.05	Axis 71	0.93
Axis 6	1.28	Axis 39	1.04	Axis 72	0.93
Axis 7	1.27	Axis 40	1.04	Axis 73	0.93
Axis 8	1.25	Axis 41	1.03	Axis 74	0.92
Axis 9	1.24	Axis 42	1.03	Axis 75	0.91
Axis 10	1.21	Axis 43	1.03	Axis 76	0.91
Axis 11	1.20	Axis 44	1.02	Axis 77	0.91
Axis 12	1.19	Axis 45	1.02	Axis 78	0.90
Axis 13	1.18	Axis 46	1.02	Axis 79	0.90
Axis 14	1.17	Axis 47	1.02	Axis 80	0.90
Axis 15	1.17	Axis 48	1.01	Axis 81	0.89
Axis 16	1.16	Axis 49	1.01	Axis 82	0.89
Axis 17	1.15	Axis 50	1.00	Axis 83	0.89
Axis 18	1.14	Axis 51	1.00	Axis 84	0.88
Axis 19	1.14	Axis 52	0.99	Axis 85	0.87
Axis 20	1.13	Axis 53	0.99	Axis 86	0.87
Axis 21	1.13	Axis 54	0.99	Axis 87	0.86
Axis 22	1.12	Axis 55	0.98	Axis 88	0.86
Axis 23	1.12	Axis 56	0.98	Axis 89	0.85
Axis 24	1.11	Axis 57	0.98	Axis 90	0.85
Axis 25	1.11	Axis 58	0.98	Axis 91	0.84
Axis 26	1.10	Axis 59	0.97	Axis 92	0.84
Axis 27	1.10	Axis 60	0.97	Axis 93	0.83
Axis 28	1.09	Axis 61	0.96	Axis 94	0.82

Axis 29	1.08	Axis 62	0.96	Axis 95	0.82
Axis 30	1.08	Axis 63	0.96	Axis 96	0.79
Axis 31	1.08	Axis 64	0.95	Axis 97	0.78
Axis 32	1.08	Axis 65	0.95	Axis 98	-0.13
Axis 33	1.07	Axis 66	0.95		