Hindawi Publishing Corporation
Journal of Marine Biology
Volume 2011, Article ID 839134, 17 pages
doi:10.1155/2011/839134

Research Article

Phylogeography of the Pacific Blueline Surgeonfish, *Acanthurus nigroris*, Reveals High Genetic Connectivity and a Cryptic Endemic Species in the Hawaiian Archipelago

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Received 16 June 2010; Accepted 11 October 2010

Academic Editor: Kim Selkoe

Understanding genetic connectivity is fundamental to the design of marine protected areas in the service of ecosystem-scale management. Here we evaluate such trends for a Pacific surgeonfish (*Acanthurus nigroris*; $N = 544$) at two spatial scales: (1) within the Hawaiian archipelago, and (2) across the entire species range from the central to southwest Pacific. The mtDNA cytochrome *b* data reveal genetic divergence ($d = 0.041$) between Hawaii and the rest of the Pacific range indicating a cryptic species pair, with one taxon endemic to Hawaii. Johnston Atoll, 1400 km SW of Hawaii, also has the Hawaiian species but is distinct from most Hawaiian locations in population genetic comparisons, indicating the limits of gene flow for this widespread reef species. No consistent population genetic differences were observed among Hawaiian sites or among the other Pacific island sites. We also detected a modest bias in gene flow from the southeast towards the northwest islands of the Hawaiian Archipelago, indicating that the Papahānaumokuākea Marine National Monument may be a recipient, rather than a source of propagules to replenish reef resources.

1. Introduction

Reef fishes have been subject to a number of genetic studies in the interest of understanding the dynamics of population connectivity and phylogeography [1–3]. Early molecular studies indicated that many fishes are genetically homogeneous across wide geographic scales owing to their potential for dispersal over substantial distances during the pelagic larval stages [4–7]. This traditional view has begun to shift, however, with increased genetic surveys and the advent of novel techniques (e.g., [8, 9]). Recent research has shown population genetic structure in marine fishes on the scale of tens to a few hundred kilometers (see [10–13]), challenging the prediction of vast panmictic populations based on potential dispersal during planktonic development [14–16]. Although it is clear that larval dispersal ability remains a predictor of population structure in some cases (e.g., [17]), mounting empirical evidence suggests that other factors such as biogeographic barriers [18], contemporary oceanographic patterns [19], larval behavior [12, 20], local adaptation [21], and the ecological requirements of each species [16, 22] may all play greater roles in shaping population connectivity (for review, see [23]).

Contemporary population genetic structure can also be reflective of historical episodes of isolation rather than recent patterns of connectivity. Factors such as population fragmentation, extinction and recolonization, and range expansion have the potential to influence genetic signatures in reef fish that persist for many generations ([24]). One example is the rapid sea level changes driven by glaciation cycles during the Pleistocene [25, 26]. Lowering of the sea by up to a 120 m below present levels during the Last Glacial

Maximum (~19 000 BP; [27]) exposed coral reef, altered the direction of sea surface currents, and even isolated entire oceans basins (e.g., formation of the Indo-Pacific Barrier; [28]). Such periodic changes in habitat availability have the potential to alter the range of reef fish species while producing cycles of population isolation, secondary contact, and subsequent merging or speciation [4, 11]. Repeated geological-climactic events in the Indo-West Pacific have also been invoked to explain geminate species pairs distributed in the Indian and Pacific Oceans, which display similar geographic (and genetic) boundaries [4, 18].

The study of genetic connectivity (i.e., gene flow) is particularly relevant in defining spatially explicit management regimes for reef fishes, like Marine Protected Areas (MPAs). The degree of interpopulation connectivity among geographic areas, or specific location of genetic breaks, sets the scale at which management strategies for marine species need to be applied to ensure that local extirpation is overcome by continued demographic exchange [3]. In order to promote species persistence and abundance, MPAs must be simultaneously self-sustaining and adequately linked via dispersal to other areas outside of the reserve boundaries (spillover effect; [29]). In the absence of genetic connectivity, isolated populations within a species can be identified by random changes in neutral genetic variation that accumulate over long periods of time [30].

Genetic management strategies are highly relevant to the unique ecosystems of the Pacific Islands. The Hawaiian archipelago is of particular interest given that it represents an isolated island chain and is characterized by some of the highest levels of tropical marine endemism in the world (i.e., 25% for shore fishes, [31]; 20% for molluscan fauna, [32]; 25% for algae, [33]). Endemism in other centrally located archipelagos in the Pacific is usually less than 2%, with one notable exception, the Marquesas Islands (12% for fishes; [34]). The Hawaiian Island chain, which includes both the geologically young Main Hawaiian Islands (MHI; 0.5 to 4.7 million years (my) old and the much older Northwestern Hawaiian Islands (NWHI; 7.3 to 29.8 my; [35]), extends 2600 km across the Central North Pacific (area = 341,360 km^2). The MHI start from the southeastern island of Hawaii (Big Island) through all eight inhabited Windward Islands ending at Kauai. The NWHI consist of 10 uninhabited islands extending from Nihoa to Kure Atoll (see Figure 1). Despite the large size of the archipelago, individual islands lie in close proximity to each other (mean separation 150 km), indicating that genetic connectivity may be high within Hawaii even with the overall isolation of the island chain (e.g., [36–39]).

The most immediate concern of marine resource managers in Hawaii is the extent of demographic linkages between the NWHI, which was declared a marine monument in June 2006 (the Papahānaumokuākea Marine National Monument) but mostly closed to fishing for decades, and the heavily fished MHI [40]. For example, if the MHI and the NWHI fish populations are connected, then stocks spanning the entire Hawaiian archipelago should be managed as a single unit. On the other hand, if NWHI populations are isolated from the MHI, management as separate units would be more appropriate. Although these islands clearly vary in terms of their level of fishing pressure, differences in oceanography and ecology further complicate the issue [40]. As one example, the NWHI are low lying atolls with modest freshwater runoff, whereas the MHI are high, mountainous islands with much greater runoff; this has the potential to influence sediment load over the surrounding coral reefs. Given that the objectives of a well-designed MPA should include fisheries enhancement [41] and the conservation of unique biodiversity [42], phylogeographic surveys assessing reef fish connectivity within the Hawaiian archipelago, as well as between Hawaii and other Pacific islands, are clearly mandated.

Here we focus on the Pacific Blueline Surgeonfish (*Acanthurus nigroris*), which provides an opportunity to examine the role of contemporary and historical factors in shaping present day patterns of genetic connectivity in the Pacific. *A. nigroris* is usually found in schools from a few to several hundred individuals and feeds primarily on plankton or filamentous algae [43]. This habitat generalist occupies lagoons, seaward reefs, mixed coral and rubble, and sand (depth range: 1 to 90 m; [43]) across the central and western Pacific and likely lives up to 25 years [44]. This colorful fish is also not fished or targeted by the aquarium trade [45]. Long-distance dispersal in *A. nigroris* presumably occurs during the pelagic larval stage that lasts approximately 55 to 60 days, based on estimates from related surgeonfish [46, 47]. Despite this potential for high levels of gene flow, slight morphological differences (fin rays and gill raker counts) in this species have been detected among Pacific populations, indicating that some regions may have been isolated on an evolutionary timescale [48].

In this study, we obtained samples from across much of the known distribution of *A. nigroris* in order to assess genetic structure using mtDNA sequence data (see Figure 1). Such sampling efforts also afforded a rare opportunity to consider genetic connectivity among sites in the Pacific Ocean and thus identify putative management units. Our objective is, therefore, to address the following questions: (1) Is there evidence of genetic structure within the Hawaiian archipelago (i.e., MHI versus NWHI) that would guide ecosystem-level management? (2) Is there genetic structure among other sampled Pacific populations? and (3) Is there evidence of recent or ongoing genetic exchange across the large stretches of open ocean separating Hawaii from the rest of the Central Pacific?

2. Methods

2.1. Collections. A total of 544 tissue samples of *A. nigroris* were collected with pole spears while scuba diving or snorkeling at 20 locations across the Hawaiian Archipelago (14 sampling sites in the NWHI and MHI), Johnston Atoll, and the Central Pacific (5 sampling sites: American Samoa, Line Islands, Marshall Islands, Society Islands, and Tokelau Islands) between 2004 and 2009 (see Figure 1). Specimens collected from the uninhabited NWHI were obtained on the NOAA Ship Hi'ialakai as part of an initiative to document and monitor resources in the Papahānaumokuākea Marine

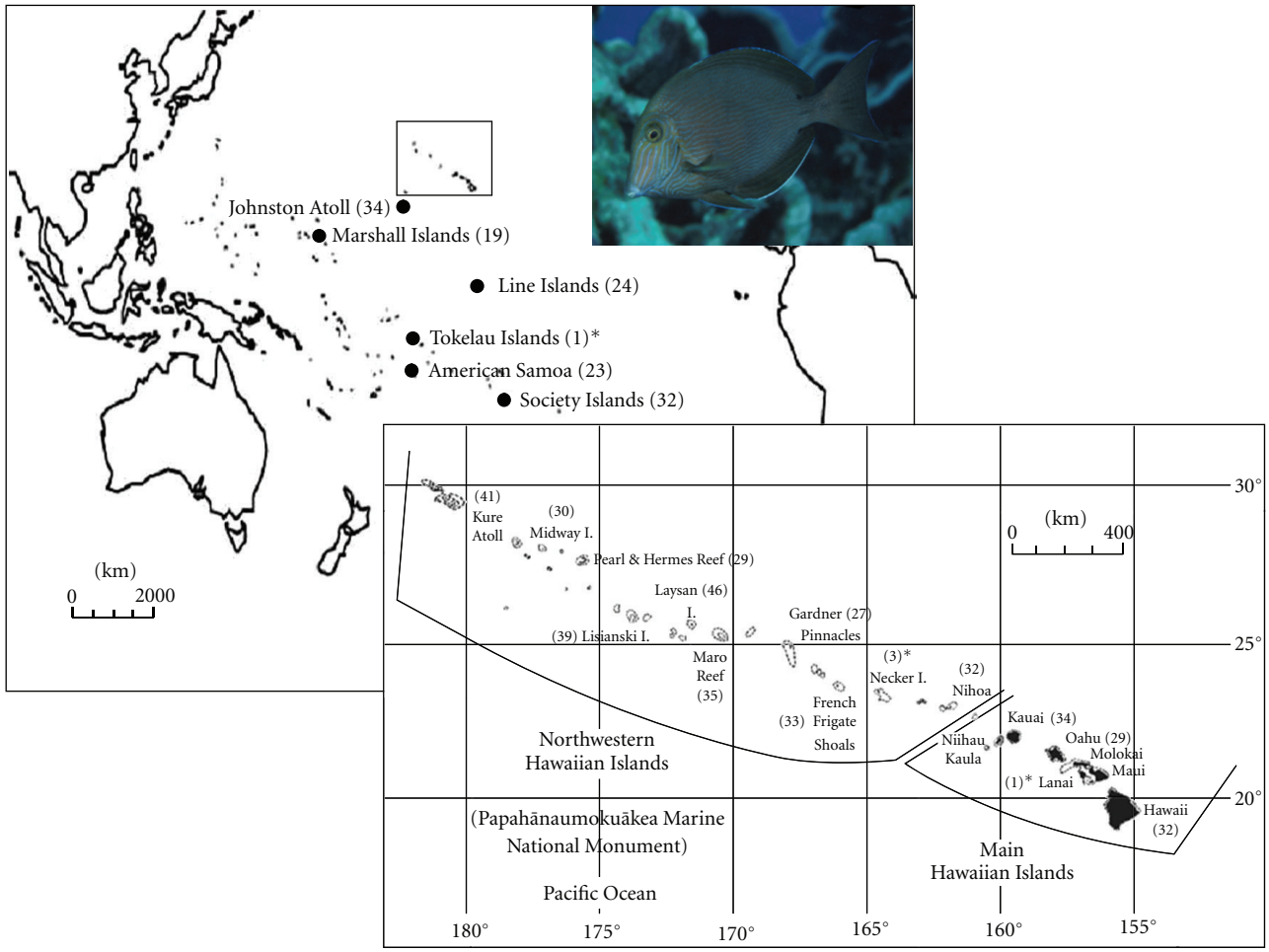

Figure 1: Scaled maps indicating the collection sites and sample sizes for *Acanthurus nigroris* in the Pacific Ocean. Locations marked with an asterisk were omitted from most population genetic analyses owing to low sample sizes (i.e., $N < 6$ in all cases) but were included when all (or only "Pacific") populations were pooled together, as well as in subsequent statistical parsimony networks. Map of the Hawaiian archipelago is courtesy of NOAA. Note that the boundaries of the Papahānaumokuākea Marine National Monument include only the Northwestern Hawaiian Islands (Photo credit: Jack Randall).

National Monument; all other samples were obtained during research expeditions by authors and colleagues. Tissue was stored in 70% ethanol or in a saturated salt-DMSO buffer at room temperature (25°C) until DNA extraction.

2.2. DNA Extraction, PCR, and Sequencing. Total genomic DNA was extracted from each tissue sample using a "HotSHOT" protocol [49] and subsequently stored at −20°C. A 797 base pair (bp) segment of the mtDNA cytochrome *b* (cyt *b*) gene was amplified using heavy-strand (5′-GTGACTTGAAAAACCACCGTTG-3′; [50]) and light-strand primers (5′-AATAGGAAGTATCATTCGGGTTTGATG-3′; [51]). Polymerase chain reaction (PCR) mixes were prepared following manufacturer's instructions using BioMixRED (Bioline Ltd., London, UK), 0.26 μM of each primer, and 5–50 ng template DNA in 15 μl total volume. Thermal cycling reactions used the following parameters: initial denaturing step at 95°C for 10 minutes, then 35 cycles of amplification (30 seconds of denaturing at 94°C, 45 seconds of annealing at 63°C, and 45 seconds of extension at 72°C), followed by a final extension at 72°C for 10 min.

PCR products were cleaned of excess oligonucleotides and unincorporated primers by incubating with exonuclease I and shrimp alkaline phosphatase (ExoSAP; USB, Cleveland, OH, USA) at 37°C for 60 min, followed by deactivation at 85°C for 15 min. All samples were then sequenced in the forward direction (and reverse direction for rare or questionable haplotypes, $N = 3$) with fluorescently labeled dye terminators following manufacturer's protocols (BigDye, Applied Biosystems Inc., Foster City, CA, USA) and analyzed using an ABI 3130XL Genetic Analyzer (Applied Biosystems) at the Hawaii Institute of Marine Biology EPSCoR Sequencing Facility. The sequences were aligned, edited, and trimmed to a common length using Geneious Pro *vers.* 4.8.4 DNA analysis software [52]. Variable sites were visually checked to ensure accuracy, and unique mtDNA cyt *b* haplotypes were deposited in GenBank (accession numbers: HM242298 to HM242393). jModelTest *vers.* 1.0.1 ([53]; but also see [54])

was used to determine the best nucleotide substitution model under Akaike information criterion (*AIC*); the Tamura-Nei model [55], with no gamma parameter, was here selected.

2.3. Population Genetic Analyses. *ARLEQUIN vers.* 3.1 software [56] was used to calculate haplotype (h) and nucleotide diversity (π) for cyt *b* sequences (as per [57]), as well as to test for genetic connectivity on several geographic scales: (1) within the Hawaiian archipelago, (2) among all other Central Pacific island groups (hereafter denoted "Pacific"), and (3) between Hawaiian and Pacific populations considered here. To test for genetic partitioning between regions, among populations within regions, and between all populations, we used Analysis of Molecular Variance (AMOVA; [58]); nonparametric permutation procedures ($N = 99999$ iterations) were used to construct null distributions and test the significance of variance components for each hierarchical comparison. Population pairwise Φ_{ST} statistics (a molecular analog of F_{ST} that includes information on mitochondrial haplotype frequency and genetic distance) were generated to identify genetic partitioning; significance was tested by permutation and *P* values adjusted according to the modified false discovery rate method (as per [59]). Genetic structure was also assessed with methods that do not make *a priori* assumptions about group identity (Spatial Analysis of Molecular Variance, *SAMOVA* 1.0; [60]) in order to confirm genetic patterns apparent with AMOVA. SAMOVA mitigates bias in group designation by implementing a simulated annealing process ($N = 100$ permutations) to randomly partitioned mtDNA sequences into K groups. We tested $K = 2$ to $K = 20$, and the configuration with the largest among group differentiation (Φ_{CT}) was retained. Moreover, pairwise genetic differences between populations (or regions) were calculated by dividing the average number of corrected nucleotides that differ between samples (as per [55]) by the total number of base pairs in that sequence (i.e., corrected sequence divergence, d).

Deviations from neutrality were assessed with Tajima's *D* [61] and Fu's *Fs* [62] for each population using *ARLEQUIN*; significance was tested with 99999 permutations. Negative (and significant) Tajima's *D* and Fu's *Fs* values indicate an abundance of rare haplotypes or recent mutations in nonrecombining sequences such as mtDNA, a signature of population expansion or background selection. As neutrality tests are sensitive to deviations from panmixia, we estimated these statistics both on the full data set and independently within each region identified as genetically distinct by SAMOVA (i.e., all Hawaiian Islands including Johnston Atoll versus all other Pacific islands). Samples collected from Johnston Atoll were initially grouped with Hawaiian samples given the pronounced overlap of inshore fish fauna between these two regions [63]. Moreover, three sites had low sample sizes (Necker Island, $N = 3$; Lanai, $N = 1$; Tokelau Islands, $N = 1$) and were, therefore, omitted from most population genetic analyses, although these specimens were included in parsimony networks and when all populations (or exclusively Hawaiian or Pacific populations) were pooled together.

Evolutionary relationships were estimated by constructing unrooted parsimony-based haplotype networks with the program *NETWORK vers.* 4.5.1.0 (http://www.fluxus-engineering.com/network_terms.htm). The haplotype network was generated using a median joining algorithm and default settings (as per [64]), and each haplotype divided into representative populations as reflected by the pie diagrams.

2.4. Coalescence Analyses. The historical demography of *A. nigroris* was analyzed by calculating mismatch distributions (the distribution of observed differences between haplotypes; [65]) and Harpending's raggedness index [66] with *ARLEQUIN*. We estimated such metrics for cyt *b* sequences from four different datasets: (1) all MHI populations ($N = 92$ individuals), (2) all NWHI populations ($N = 315$ individuals), (3) the entire Hawaiian archipelago (including Johnston Atoll, $N = 441$ individuals), and (4) all remaining Pacific populations ($N = 98$ individuals, excluding Tokelau). Populations that have been stable over time exhibit bimodal or multimodal mismatch distributions, whereas unimodal distributions or nonsignificant raggedness scores suggest recent (and rapid) population expansion (see [65]). All four datasets were also fitted with the population parameter τ in order to estimate the time to coalescence (as per [66, 67]). Time to coalescence (or population age) was calculated using the equation $\tau = 2\mu t$, where t is the age of the population in generations and μ is the mutation rate per generation for the sequence (μ = number of base pairs · divergence rate within a lineage · generation time in years). A range of mutation rate estimates were taken from previous work in fish (1% to 2%, based on 2% per million years between lineages or 1% within lineages, [5]; 1.55% per million years within lineages or 1.55×10^{-8} mutations per site per year, [68]), and while generation time is unknown for *A. nigroris*, we conditionally used 5 years based on estimates in a related surgeonfish (*Acanthurus nigrofuscus,* [44]). Although absolute values of time to coalescence should be interpreted with caution here owing to the approximation of mutation rate and generation time, comparisons between populations within this dataset provide useful estimates of within-species variation.

To further explore genetic connectivity of *A. nigroris* within the Pacific Ocean, we estimated coalescence-based migration rates (Nm, where N is the effective female population size and m is migration rate) with the program *MIGRATE vers.* 3.1.2 [69]. Estimates of gene flow generated in *MIGRATE* are not constrained by the assumption that a single ancestral population has split into two daughter populations [70] and appear to be robust to some common biases [71]. Although these approaches are sometimes sensitive to the presence of unsampled (i.e., ghost) populations [72], collecting *A. nigroris* from almost all of the Hawaiian islands as well as pooling the remaining Pacific samples for analysis likely reduced these effects. *MIGRATE* was therefore run: (1) among the MHI ($N = 92$ individuals), NWHI ($N = 278$ individuals), and Johnston Atoll ($N = 34$ individuals), (2) among all remaining Pacific populations ($N = 98$ individuals), and (3) between Hawaiian and Pacific populations by pooling all sampled individuals from each region (Hawaii, including Johnston Atoll, $N = 445$; Central Pacific, $N = 99$).

Estimates of migration rates based on coalescence theory provide not only an indication of the level of migration and population size, but also the directionality of gene flow in most cases. The maximum-likelihood (ML) approach implemented in *MIGRATE,* however, can sometimes provide unrealistic migration rate estimates and inflated confidence intervals (see [71, 73]). We therefore employed the recommended Bayesian inference search strategy of a single, replicated, 500,000 step chain with the first 20% discarded as burn in [74]. Each run was replicated ten times to ensure that the parameter space was widely sampled, and we took the average of all runs to calculate migration rates, thus accounting for variability between runs. Starting parameters for θ (theta) and M were estimated from F_{ST} [75], and initial runs were conducted with default exponential priors and an unrestricted migration model; posterior distributions for θ and M were used to inform priors for the final set of replicated runs. Only runs that produced normally distributed, unimodal posterior θ distributions were considered here.

Estimates of the number of migrants per generation were calculated by multiplying final estimates (mean 2.5%, and 97.5% quantile) of θ and M [76]. Given that we employed a single locus and make a number of simplifying assumptions regarding population history, we also regard these estimates as informative primarily for comparisons among populations *within* this dataset; comparisons with other species should be conducted with caution.

3. Results

3.1. Molecular Characteristics. We resolved 797 bp of mtDNA (cyt *b*) from 544 *A. nigroris* sampled at 20 locations across the Pacific Ocean (see Figure 1 and Table 1). There were no shared haplotypes between Hawaiian populations (including Johnston Atoll) and the remaining Pacific islands. In the Hawaiian samples, we observed 38 haplotypes (35 transitions, 1 transversion, and no indels). The most common and second most common haplotypes were detected at every site, and overall, the number of unique haplotypes per site was low. In the remaining Pacific islands, 58 haplotypes were observed (66 transitions, 3 transversion, and no indels), and while there were multiple haplotypes common to many of the sites, the majority of haplotypes were observed exclusively at single locations. Indeed, haplotype diversity was twice as high and nucleotide diversity almost an order of magnitude higher within Pacific sites ($h = 0.97$, $\pi = 0.0061$) compared to Hawaiian sites ($h = 0.52$, $\pi = 0.00080$; Table 1), despite a much greater sampling effort in the latter ($N = 441$ in Hawaii versus $N = 98$ in the Pacific). Note that the three sites that had low sample sizes (Necker Island, $N = 3$; Lanai, $N = 1$; Tokelau Islands, $N = 1$) shared common haplotypes with either Hawaiian or Pacific samples, thus justifying their inclusion in pooled analyses.

Negative and significant Tajima's D (or Fu's Fs) values in 7 out of the 13 (or 9 out of 13) Hawaiian samples (Tajima's $D = -1.99$ to -0.46; Fu's $Fs = -7.018$ to -0.73) and in 3 out of the 4 (or 4 out of 4) remaining Pacific samples (Tajima's $D = -1.64$ to -1.31; Fu's $Fs = -17.76$ to -7.54; Table 1) indicate past population expansion or selection within each region. These results were similar when both the Hawaiian and Pacific regions were analyzed separately (data not shown), indicating that our neutrality statistic estimates were robust to deviations from panmixia (see below).

3.2. Population Genetic Analyses. Grouping samples into Hawaiian (including Johnston Atoll) and the remaining Pacific locations with AMOVA revealed that most of the variability in mtDNA was explained by a significant break between these two regions ($\Phi_{CT} = 0.96$, $P < 0.001$; see Table 2). Moreover, variance explained by the among-populations-within-regions variance component ($\Phi_{SC} = 0.014$, $P = 0.035$) was an order of magnitude smaller than that between regions. This pattern held even when Johnston Atoll was excluded from AMOVA analysis altogether ($\Phi_{CT} = 0.96$, $P < 0.001$; $\Phi_{SC} = 0.010$, $P = 0.088$; $\Phi_{ST} = 0.96$, $P < 0.001$), and so the overall patterns were therefore not driven by its inclusion in the Hawaiian group. SAMOVA further confirms these genetic partitions (i.e., $K = 2$ maximally differentiated groupings) with all Hawaiian populations (including Johnston Atoll) being significantly different from the remaining Pacific populations ($\Phi_{CT} = 0.96$, $P < 0.001$).

Population pairwise tests provide insight into particular geographic regions or sites, where genetic partitioning is considerable, modest, or absent (Table 3). We found no significant genetic differentiation among sites within the Pacific (pairwise Φ_{ST} range: -0.0063 to 0.027), but comparisons among sampling locations in the Hawaiian archipelago revealed some genetic structure (pairwise Φ_{ST} range: -0.0015 to 0.19). Samples from Johnston Atoll were significantly different from all other Hawaiian locations except for French Frigate Shoals ($P = 0.043$), Kauai ($P = 0.049$), and Kure ($P = 0.24$). Removal of Johnston Atoll from the analysis eliminated significant (albeit marginal) genetic structuring within the Hawaiian archipelago (AMOVA with Johnston Atoll: $\Phi_{ST} = 0.019$, $P = 0.011$; AMOVA without Johnston Atoll: $\Phi_{ST} = 0.011$, $P = 0.073$).

A haplotype network based on statistical parsimony supports the genetic (and geographic) partitioning of *A. nigroris* into two clusters corresponding to the Hawaiian archipelago (including Johnston Atoll) and the remaining Pacific sampling sites (Figure 2). The characteristic "star phylogeny" for the two dominant Hawaiian haplotypes is consistent with low partitioning among samples [77], as well as a more recent population expansion of *A. nigroris* within Hawaii. Numerous low frequency haplotypes, on the other hand, were observed for the Pacific populations. We also found that the average corrected sequence divergence between Hawaiian and Pacific haplotypes was large ($d = 4.12\%$, based on 25 mutational steps), consistent with a long period of separation between the two lineages. Genetic divergence within each region, on the other hand, was much lower (Hawaii, average $d = 0.10\%$; Pacific, $d = 0.60\%$).

3.3. Coalescence Analyses. In order to resolve the relative timing of *A. nigroris* lineage divergence between Hawaii and the rest of the Pacific, as well as to infer putative population expansion events, we estimated pairwise mismatch distributions and coalescence times for individuals from:

Table 1: Sample size and molecular diversity indices for the studied *Acanthurus nigroris*.

Collection locality	N	H_N	H_U	Haplotype diversity ($h \pm$ SD)	Nucleotide diversity ($\pi \pm$ SD)	Tajima's D	Fu's Fs
Hawaiian Archipelago							
French Frigate Shoals	33	6	1	0.42 ± 0.10	0.00064 ± 0.00062	**−1.57**[a]	**−3.74**
Gardner Pinnacles	27	4	0	0.57 ± 0.061	0.00080 ± 0.00072	−0.46	−0.76
Hawaii (i.e., Big Island)	32	10	3	0.66 ± 0.085	0.0012 ± 0.00094	**−1.93**	**−7.018**
Johnston Atoll	34	4	1	0.22 ± 0.093	0.00037 ± 0.00044	**−1.75**	**−2.37**
Kauai	34	3	1	0.27 ± 0.092	0.00040 ± 0.00047	−0.69	−0.73
Kure Atoll	41	6	1	0.31 ± 0.093	0.00054 ± 0.00056	**−1.84**	**−4.059**
Laysan Island	46	9	5	0.58 ± 0.069	0.00086 ± 0.00074	**−1.74**	**−6.43**
Lisianski Island	39	8	3	0.58 ± 0.082	0.0010 ± 0.00083	**−1.99**	**−4.45**
Maro Reef	35	7	2	0.62 ± 0.064	0.00095 ± 0.00080	−1.34	**−3.57**
Midway Island	30	6	3	0.58 ± 0.080	0.00097 ± 0.00081	−1.41	**−2.47**
Nihoa	32	9	4	0.63 ± 0.091	0.00086 ± 0.00075	**−1.97**	**−7.35**
Oahu	29	5	1	0.54 ± 0.093	0.00082 ± 0.00073	−0.94	−1.81
Pearl and Hermes Reef	29	5	0	0.64 ± 0.057	0.00096 ± 0.00081	−0.66	−1.39
All of Hawaii[b]	441	38	25	0.52 ± 0.025	0.00080 ± 0.00069	**−2.38**	**-3.4×10^{38}**
Pacific							
American Samoa	23	16	7	0.94 ± 0.034	0.0054 ± 0.0031	**−1.61**	**−7.95**
Line Islands	24	16	10	0.95 ± 0.029	0.0053 ± 0.0031	−1.31	**−7.54**
Marshall Islands	19	19	15	1.00 ± 0.017	0.0065 ± 0.0037	**−1.64**	**−17.76**
Society Islands	32	25	15	0.98 ± 0.012	0.0073 ± 0.0040	**−1.53**	**−16.56**
All of Pacific[b]	98	58	47	0.97 ± 0.0081	0.0061 ± 0.0033	**−2.069**	**−25.54**

Abbreviations are as follows: N: sample size; H_N : number of haplotypes; H_U: number of unique haplotypes.
[a]Numbers in bold are significant, $P < 0.05$.
[b]Samples from Necker Island ($N = 3$), Lanai ($N = 1$), and the Tokelau Islands ($N = 1$) were omitted from these analyses.

Table 2: Genetic structuring (Analysis of Molecular Variance, AMOVA) of *Acanthurus nigroris* sampled at sites throughout the Pacific based on 797 bp of mtDNA cyt *b* sequence data ($N = 539$). All Hawaiian populations (including Johnston Atoll) and the remaining Pacific populations were divided into two separate groups to assess the relationship between these regions. Φ_{CT}: region variance component relative to total variance; Φ_{SC}: between population within region variance component divided by the sum of itself and within population variance; Φ_{ST}: sum of the variance due to region and population within region divided by the total variance.

Source	*d.f.*	*SS*	*Variance components*	% *variation*	Φ_{CT} Φ_{SC}	*P value*	Φ_{ST}	*P value*
Among regions (Hawaii versus Pacific)	1	2543.54	15.86	95.74	**0.96**[a]	< 0.001	**0.96**	< 0.001
Among populations (within regions)	15	15.11	0.0097	0.06	**0.014**	0.035		
Within populations	522	363.35	0.70	4.2				

[a]Numbers in bold are significant, $P < 0.05$.

(1) all MHI populations, (2) all NWHI populations, (3) the entire Hawaiian archipelago (including Johnston Atoll), and (4) the remaining Pacific populations (Figure 3). For all MHI populations, the unimodal mismatch distribution did not show a significant deviation from the simulated sudden demographic expansion null model (Harpending's raggedness index, $r = 0.11$, $P = 0.35$, Figure 3a); all other datasets, on the other hand, deviated from such a model despite being unimodally distributed (NWHI: Harpending's raggedness index, $r = 0.12$, $P = 0.005$, Figure 3b); Hawaiian archipelago: Harpending's raggedness index, $r = 0.11$, $P = 0.016$, Figure 3c; Pacific: Harpending's raggedness index, $r = 0.026$, $P = 0.004$, Figure 3d). Using the range of mutation rates (see Methods) and the population parameter τ, we identified markedly different coalescence dates in Hawaiian and Pacific populations (MHI: 20,953 to 41,905 years, $\tau = 0.67$; NWHI: 23,771 to 47,541 years, $\tau = 0.76$; Hawaiian archipelago: 22,178 to 44,356 years, $\tau = 0.71$; Pacific: 163,210 to 326,419 years, $\tau = 5.20$). Notably the coalescent estimates here reflect only the most recent population expansion in each region and not separation times between Hawaiian and Pacific *A. nigroris* (see above).

Our population genetic analyses were further supported by estimates of bidirectional, effective migration rates (Nm)

Table 3: Matrix of population pairwise Φ_{ST} values (below diagonal) and associated P values (above diagonal) based on 797 bp of mtDNA cyt b sequence data from *Acanthurus nigroris* sampled at sites across the Pacific ($N = 539$).

Location	Kure	Midway	P and H	Lisianski	Laysan	Maro	Gardner	FFR	Nihoa	Kauai	Oahu	Hawaii	JOH	AS	LI	MI	SI
Kure	—	0.016	0.00058	0.15	0.041	0.0035	0.0024	0.54	0.085	0.33	0.083	0.040	0.24	<0.001	<0.001	<0.001	<0.001
Midway	**0.071**[a]	—	0.44	0.54	0.51	0.69	0.70	0.20	0.44	0.15	0.52	0.94	0.0013	<0.001	<0.001	<0.001	<0.001
P and H	**0.14**	−0.0091	—	0.15	0.22	0.75	0.88	0.021	0.13	0.020	0.26	0.46	0.00047	<0.001	<0.001	<0.001	<0.001
Lisianski	0.013	−0.0078	0.021	—	0.95	0.18	0.15	0.92	0.99	0.69	0.81	0.95	0.023	<0.001	<0.001	<0.001	<0.001
Laysan	0.035	0.077	0.010	−0.014	—	0.28	0.26	0.42	0.88	0.40	0.88	0.93	0.0074	<0.001	<0.001	<0.001	<0.001
Maro	**0.10**	−0.016	−0.018	0.010	0.0050	—	0.99	0.091	0.24	0.091	0.37	0.45	0.0011	<0.001	<0.001	<0.001	<0.001
Gardner	**0.14**	−0.017	−0.026	0.018	0.0059	−0.026	—	0.063	0.21	0.052	0.34	0.40	0.00064	<0.001	<0.001	<0.001	<0.001
FFR	−0.0055	0.014	**0.064**	−0.014	−0.0040	0.037	0.059	—	0.68	0.77	0.50	0.46	0.043	<0.001	<0.001	<0.001	<0.001
Nihoa	0.021	−0.0052	0.023	−0.014	−0.013	0.0070	0.012	−0.012	—	0.60	0.84	0.88	0.023	<0.001	<0.001	<0.001	<0.001
Kauai	0.0024	0.024	**0.080**	−0.010	−0.0015	0.049	0.077	−0.019	−0.013	—	0.24	0.29	0.049	<0.001	<0.001	<0.001	<0.001
Oahu	0.034	−0.011	0.0093	−0.015	−0.017	−0.0038	0.0016	−0.0068	−0.016	0.0040	—	0.98	0.011	<0.001	<0.001	<0.001	<0.001
Hawaii	0.034	−0.018	−0.0050	−0.015	−0.015	−0.0067	−0.0047	−0.0055	−0.015	−0.0010	−0.022	—	0.012	<0.001	<0.001	<0.001	<0.001
JOH	0.0055	**0.12**	**0.18**	**0.043**	**0.080**	**0.15**	**0.19**	0.029	**0.043**	0.035	**0.078**	**0.060**	—	<0.001	<0.001	<0.001	<0.001
AS	**0.95**	**0.93**	**0.94**	**0.94**	**0.95**	**0.94**	**0.93**	**0.94**	**0.94**	**0.94**	**0.94**	**0.93**	**0.95**	—	0.095	0.54	0.10
LI	**0.95**	**0.93**	**0.93**	**0.94**	**0.94**	**0.94**	**0.93**	**0.94**	**0.94**	**0.94**	**0.93**	**0.93**	**0.94**	0.027	—	0.38	0.26
MI	**0.94**	**0.93**	**0.93**	**0.93**	**0.94**	**0.93**	**0.93**	**0.93**	**0.93**	**0.94**	**0.93**	**0.93**	**0.94**	−0.0063	0.00096	—	0.41
SI	**0.92**	**0.91**	**0.90**	**0.91**	**0.92**	**0.91**	**0.90**	**0.91**	**0.91**	**0.92**	**0.91**	**0.90**	**0.92**	0.020	0.0064	0.00040	—

AS: American Samoa; FFR: French Frigate Shoals; JOH: Johnston Atoll; LI: Line Islands; MI: Marshall Islands; P and H: Pearl and Hermes Reef; SI: Society Islands.
[a]Significant Φ_{ST} values (adjusted $P < 0.025$; as per [59]) are indicated in bold.

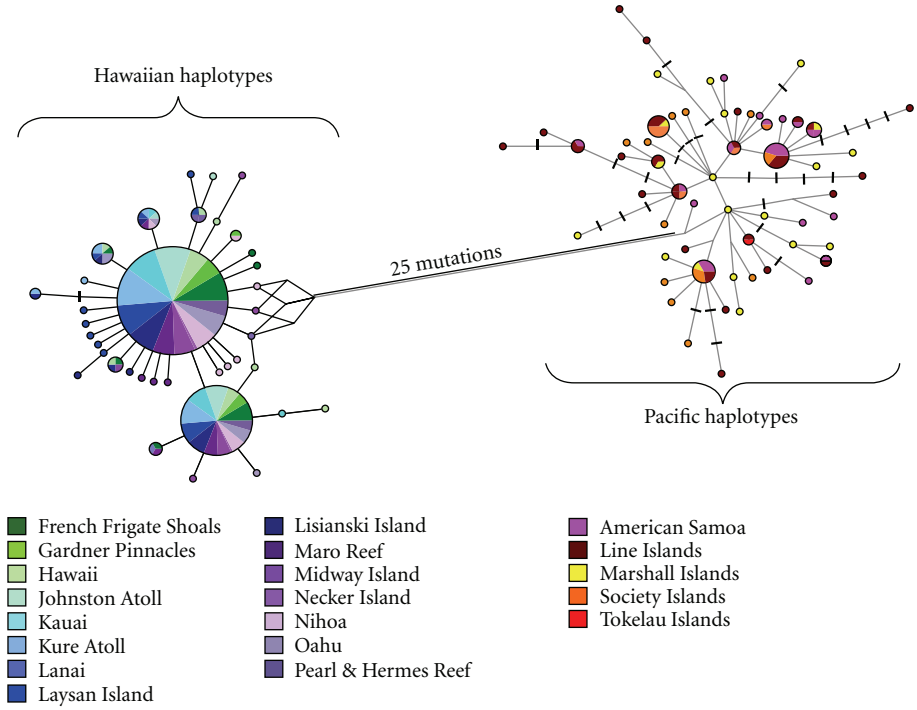

Figure 2: Median-joining statistical parsimony network based on 797 bp of mitochondrial cyt *b* sequence data ($N = 544$) from *Acanthurus nigroris* sampled across the Pacific. Each circle represents a haplotype, and its size is proportional to its total frequency. Branches represent a single nucleotide change and black crossbars indicate unsampled haplotypes; colors denote collection location as indicated by the embedded key. It should be noted that there were no shared haplotypes between Hawaiian (including Johnston Atoll) and the remaining Pacific populations, which form two distinct clades separated by 25 mutational steps (corrected sequence divergence, $d = 4.12\%$; [55]).

with Bayesian methods. Migration was by far the greatest within the Pacific and Hawaiian regions and not surprisingly low between regions (Table 4). Indeed, based on a genetic distance of 25 mutational steps, the number of estimated migrants from the Hawaiian archipelago to the rest of the Pacific (and vice versa) approaches zero. Within Hawaii in particular, there was a modest bias in migration from the MHI to the NWHI ($Nm = 35.19$, 95% CI = 0–162) versus from the NWHI to the MHI ($Nm = 22.99$, 95% CI = 0–132). Moreover, the number of migrants per generation moving from Hawaii to Johnston Atoll (from MHI: $Nm = 3.69$, 95% CI = 0–26.01; from NWHI: $Nm = 3.22$, 95% CI = 0–24.65) was more than an order of magnitude lower than migration from Johnston to Hawaii (to MHI: $Nm = 62.11$, 95% CI = 4.79–197; to NWHI: $Nm = 62.56$, 95% CI = 3.83–190), indicating that gene flow is biased towards rather than away from the Hawaiian archipelago. The posterior distributions for all parameters were also consistent over multiple runs, thus indicating sufficient convergence to interpret values [78].

4. Discussion

All genetic analyses outlined above support the conclusion that Hawaiian *A. nigroris* represents an ancient evolutionary separation from those sampled elsewhere in the Pacific Ocean. Genetic distance among these distinct groups is comparable to or greater than comparisons among other congeneric pairs of reef fishes [79–82], which indicates independence between regions. On the other hand, we found that with few exceptions, there were high levels of genetic connectivity within Hawaii as well as among all other sampled Pacific island populations. Johnston Atoll also has a significant population genetic distinction from many, but not all Hawaiian samples (see Table 3), with implications for the colonization of Hawaiian reefs.

4.1. Gene Flow in Hawaii. One objective in this study was to characterize genetic structure within the Hawaiian archipelago (2600 km from Kure Atoll to the island of Hawaii); throughout this region, we found little evidence for population genetic differentiation of *A. nigroris*. The existence of haplotypes shared across vast distances in Hawaii indicates that populations from each of these separate islands either freely exchange propagules or have done so in the recent past.

We are particularly interested in whether fish sampled in the MHI are connected to those in the NWHI, the largest marine conservation area under US jurisdiction. Based on our genetic results here, we cannot exclude this possibility. Although the short-term success of the NWHI monument in protecting regional biodiversity depends on enforcement within the reserve itself, long-term persistence of these reef fish populations also requires connectivity with other sites. The high genetic connectivity of *A. nigroris* detected within the Hawaiian archipelago indicates that movement of

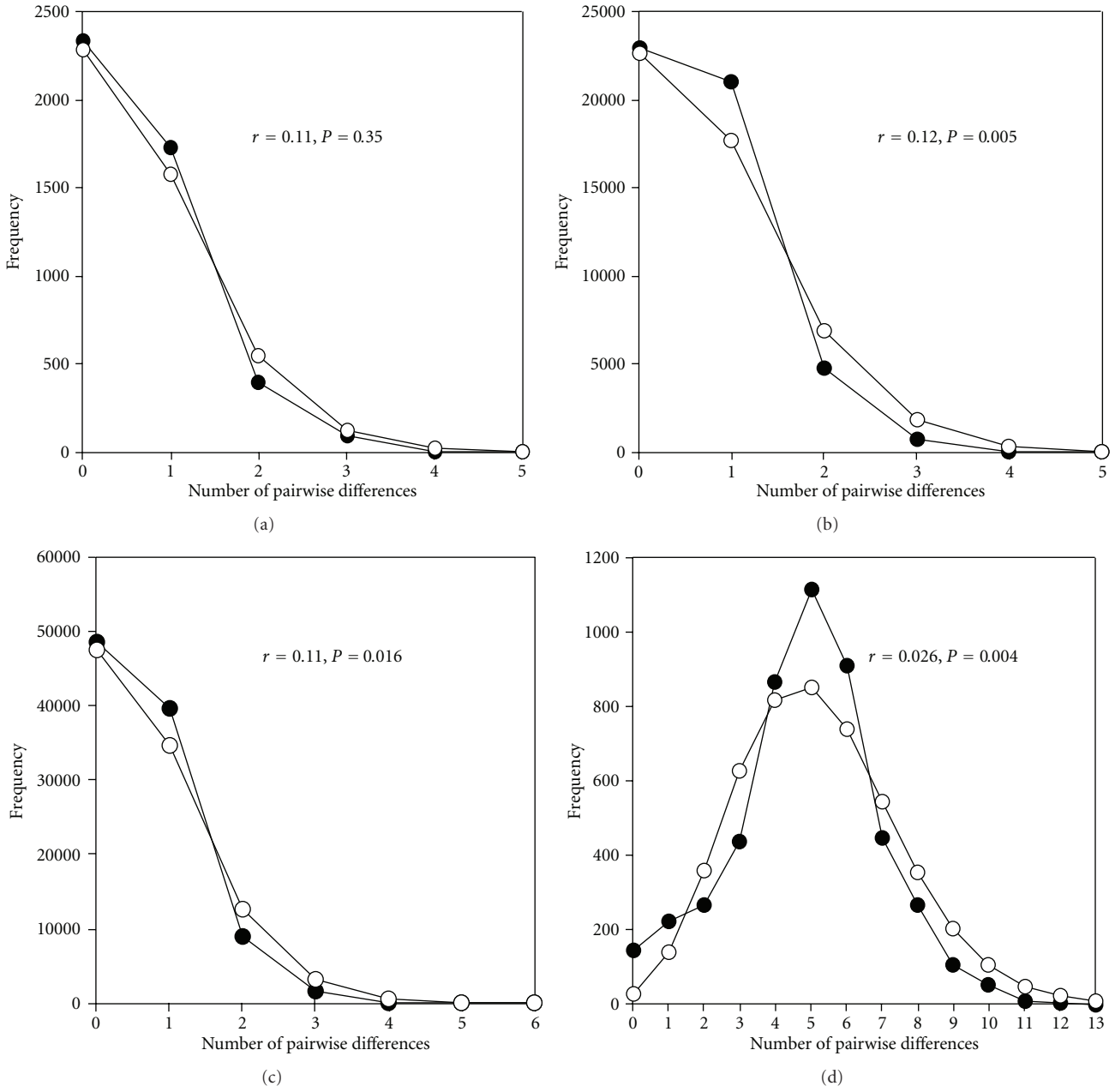

Figure 3: Mismatch distributions of mitochondrial cyt *b* sequence data from *Acanthurus nigroris* from (a) all Main Hawaiian Island (MHI) populations ($N = 92$ individuals), (b) all Northwest Hawaiian Island (NWHI) populations ($N = 315$ individuals), (c) the entire Hawaiian archipelago (including Johnston Atoll, $N = 441$ individuals), and (d) all remaining Pacific populations ($N = 98$ individuals). Observed and simulated pairwise differences calculated in *ARLEQUIN vers.* 3.1 are represented by lines drawn through black and open circles, respectively. Harpending's raggedness index (r) and associated P values are shown for each dataset.

fish larvae from the NWHI may supply adjacent fisheries, although high exploitation rates in the MHI could erode any such beneficial effects. High human population density, along with the immediate effects of urbanization (i.e., increased sediment, nutrients, and pollutants) and unregulated fishing, have depleted MHI fish fauna [40, 83]. Total fish biomass of large apex predators, a common indicator of healthy coral reef ecosystems, is also more than an order of magnitude higher in the uninhabited NWHI [40], as well as elsewhere in the Pacific [83–85].

If the lack of genetic structure observed throughout the Hawaiian archipelago is the result of life-history traits that differentially affect larval dispersal in reef fishes, comparisons among published genetic surveys might help resolve why some species appear to exchange propagules over long distances and others do not (Table 5). Here we show that 6 out of the 14 species surveyed throughout Hawaii display clear genetic breaks between sites in the NWHI versus MHI, but that there is no obvious correlation between genetic structure and pelagic larval duration (PLD), reproductive strategy,

Table 4: Strength and direction of gene flow for *Acanthurus nigroris:* (1) between the MHI ($N = 92$ individuals), NWHI ($N = 278$ individuals), and Johnston Atoll ($N = 34$ individuals), (2) among all remaining Pacific populations ($N = 98$), and (3) between Hawaiian and Pacific populations by pooling all sampled individuals (Hawaii, $N = 445$; Pacific, $N = 99$). Values are reported as the mean effective number of migrants (Nm) per generation taken from ten independent runs.

		Number of immigrants per generation into receiving population		
	Comparison	2.5% percentile	Mean	97.5% percentile
Among regions (Hawaii versus Pacific)	Hawaii into Pacific	0	0.71	3.19
	Pacific into Hawaii	0	0.34	1.63
Among populations[a] (within Hawaii)	MHI into NWHI	0	35.19	162.00
	NWHI into MHI	0	22.99	132.00
	MHI into JOH	0	3.69	26.04
	JOH into MHI	4.79	62.11	197.00
	NWHI into JOH	0	3.22	24.65
	JOH into NWHI	3.83	62.56	190.00
Among populations[a] (within Pacific)	AS into LI	0	11.94	54.80
	AS into MI	0	20.87	63.20
	AS into SI	0	22.91	66.00
	LI into AS	0	13.76	54.40
	MI into AS	0	7.14	37.60
	SI into AS	0	10.58	50.40
	LI into MI	0	16.17	53.60
	LI into SI	0	18.33	56.80
	MI into LI	0	6.40	35.60
	SI into LI	0	8.38	48.40
	MI into SI	0	9.18	39.60
	SI into MI	0	14.62	53.20

AS: American Samoa; JOH: Johnston Atoll; LI: Line Islands; MHI: Main Hawaiian Islands; MI: Marshall Islands; NWHI: Northwest Hawaiian Islands; SI: Society Islands.

[a]Samples from Necker Island ($N = 3$), Lanai ($N = 1$), and the Tokelau Islands ($N = 1$) were omitted from these analyses.

or habitat preference. Furthermore, there is no agreement among even closely related surgeonfish species with similar life-history characteristics. Indeed, one species (*Ctenochaetus strigosus*) shows genetic structure within Hawaii, whereas mtDNA of the other surgeonfish species considered, including *A. nigroris*, do not (*Acanthurus nigrofuscus* and *Zebrasoma flavescens* [38]; see [39]). Clearly connectivity between the MHI and NWHI needs to be evaluated on a case-by-case basis, incorporating the relevant facets of life history and ecology where possible (e.g., [86]); other factors not considered here (i.e., timing of reproduction, larval behavior, and ecological requirements) warrant further investigation. Indeed, several recent reports have drawn links between reef fish ecology and dispersal, phylogeography, and speciation [16, 86–89].

The estimates of gene flow generated here and elsewhere are useful in the design of marine reserves. Such gene flow estimates based on F (or Φ) statistics, however, are subject to several caveats [90]. Rare dispersal may be sufficient to ensure genetic homogeneity over evolutionary time scales, and so AMOVA analyses cannot distinguish whether genetic similarity among sampling sites is due to ongoing gene flow or incomplete lineage sorting (i.e., recent isolation). In some cases, supplementation with physical tagging can solve this problem [12, 29], but such treatments are outside the scope of this study. We instead turn to Bayesian estimation of migration rates using coalescent procedures in MIGRATE [69], which clearly show elevated migration within the Hawaiian and Pacific regions in comparison to that between regions (Table 4). MIGRATE results also support an emerging trend for northwestward flow of larvae from the depleted reefs of the MHI into the healthy NWHI (i.e., existing MPA acts as a sink rather than a source; [91, 92]). Although *MIGRATE*-based estimates of gene flow based on a single molecular marker should be treated with caution, we are here interested in relative differences between regions and not absolute values. Therefore, these findings, along with complimentary data based on multi-disciplinary research in other taxa (e.g., genetics, mark-recapture, and oceanographic modeling), should be considered by managers in the design of future marine reserves in the MHI.

4.2. Johnston Atoll and the Colonization of Hawaii. Another objective in this study was to test for recent or ongoing genetic exchange between Hawaii and the rest of the Central Pacific. Indeed, the Hawaiian archipelago is separated from

Table 5: Surveys of intraspecific genetic structure (Φ_{ST} or F_{ST}) in reef fishes sampled within the entire Hawaiian archipelago (i.e., Main Hawaiian Islands and Northwest Hawaiian Islands). Species common (and scientific) name, marker type (and mtDNA gene or number of nuclear loci), genetic structure, ecological differences (pelagic larval duration, PLD; Habitat type) between the study species, and references are listed here.

Species	Marker type	Genetic structure?	Genetic break	Global (or pairwise) Φ_{ST} or F_{ST}	P value	PLD (days)	Reproduction	Habitat type	Reference
Bigscale soldierfish (*Myripristis berndti*)	mtDNA (Cyt *b*)	No	N/a	0.00040	> 0.05	55	N/a	Subtidal reef flats to outer reef slopes	Craig et al. [36]
Blueline surgeonfish (*Acanthurus nigroris*)	mtDNA (Cyt *b*)	No	N/a	0.011	0.073	55 to 60	Group, broadcast spawning	Reef and rubble (1 to 90 m)	This study
Blue-striped butterflyfish (*Chaetodon fremblii*)	mtDNA (Cyt *b*)	No	N/a	−0.014	0.41	N/a	Pair, broadcast spawning	Shallow reef	Craig et al. [37]
Brown surgeonfish (*Acanthurus nigrofuscus*)	mtDNA (Cyt *b*)	No	N/a	−0.0060	> 0.05	55 to 60	Broadcast spawning	Reef and rubble	Eble et al. [38]
Hawaiian gregory (*Stegastes fasciolatus*)	Allozymes (8)	No	N/a	0.0010 to 0.0050	N/a	25	Demersal eggs	Reef and rock	Shaklee and Samollow [129]
Hawaiian gregory (*Stegastes fasciolatus*)	mtDNA (control)	Yes	NWHI versus MHI	0.093 to 0.10	< 0.05	25	Demersal eggs	Reef and rock	Ramon et al. [130]
Hawaiian grouper (*Epinephelus quernus*)	mtDNA (control)	Yes	NWHI versus MHI	−0.007 to 0.043	0.01	40	Group, broadcast spawning	Reef and rubble	Rivera et al. [131]
Milletseed butterflyfish (*Chaetodon miliaris*)	mtDNA (Cyt *b*)	No	N/a	−0.0050	0.66	N/a	Group, broadcast spawning	Shallow reef	Craig et al. [37]
Milkfish (*Chanos chanos*)	Allozymes (9)	Yes	Oahu versus Hawaii	0.000080 to 0.0041	N/a	14 to 21	Group, broadcast spawning	Shallow reef/ estuarine (1 to 30 m)	Winans [87]
Pebbled butterflyfish (*Chaetodon multicinctus*)	mtDNA (Cyt *b*)	No	N/a	−0.0080	0.82	N/a	Pair, broadcast spawning	Shallow reef	Craig et al. [37]
Pink snapper (*Pristipomoides filamentosus*)	Allozymes (5)	No	N/a	0.0010 to 0.011	N/a	60 to 120	Group, broadcast spawning	Reef and rock (180 to 270 m)	Shaklee and Samollow [129]
Spotted surgeonfish (*Ctenochaetus strigosus*)	mtDNA (Cyt *b*)	Yes	P and H and Maro versus rest of islands	0.034 to 0.074	0.041 to 0.008	55 to 60	Group/pair, broadcast spawning	Shallow reef	Eble et al. [38]
Undulated moray (*Gymnothorax undulatus*)	mtDNA (Cyt *b* and COI)	Yes	Maro versus Hawaii and Oahu	0.060 to 0.10	< 0.05	Up to 730	Pair mating	Reef and rock (1 to 100 m)	Reece et al. [88]
White-spotted damselfish (*Dascyllus albisella*)	mtDNA (control)	Yes	NWHI versus MHI	0.033 to 0.72	< 0.05	27	Pair, broadcast spawning	Shallow reef	Ramon et al. [130]

Table 5: Continued.

Species	Marker type	Genetic structure?	Genetic break	Global (or pairwise) Φ_{ST} or F_{ST}	P value	PLD (days)	Reproduction	Habitat type	Reference
Yellow-edged moray (*Gymnothorax flavimarginatus*)	mtDNA (Cyt *b* and COI)	No	N/a	−0.070 to 0.030	> 0.05	Up to 730	Pair mating	Reef and rock (1 to 100 m)	Reece et al. [88]
Yellow tang (*Zebrasoma flavescens*)	mtDNA (Cyt *b*)	Yes	Hawaii	0.077 to 0.17	0.034 to 0.001	55 to 60	Group, broadcast spawning	Reef and rubble (1 to 80 m)	Eble et al. [38]
Yellow tang (*Zebrasoma flavescens*)	Microsatellite (14)	Yes	Multiple	−0.010 to 0.042	< 0.001	55 to 60	Group, broadcast spawning	Reef and rubble (1 to 80 m)	Eble et al. [132]

Abbreviations: MHI: Main Hawaiian Islands; NWHI: Northwest Hawaiian Islands; P and H: Pearl and Hermes Reef.

other Central Pacific archipelagos by a minimum deep water gap of *ca.* 1400 km. The Line Islands directly south of Hawaii represent the closest archipelago, and so this island chain has been suggested as a source of colonizing fishes. Gosline [63] proposed that at low sea level stands associated with glaciations periods, the North Equatorial Current (and Countercurrent) may have been deflected by the Line Islands, providing greater opportunities for colonization into Hawaii. Johnston Atoll has also been forwarded as a key gateway (i.e., stepping stone) for larvae dispersing into Hawaii given its intermediate location (minimum distance of 865 km) and east/west prevailing current system [63, 93]. In support of this possibility, our *MIGRATE* analysis indicates an order of magnitude greater gene flow from Johnston to Hawaii, rather than in the opposite direction (Table 4). Subtle (but overlapping) meristic differences between fish collected from Johnston versus all Hawaiian locations [48], however, along with concordant population-level isolation observed in this study, indicate that Johnston may simply serve as the southernmost outpost of Hawaiian *A. nigroris* [94]. Indeed, Johnston is genetically divergent from most (i.e., 9 out of 12 comparisons significantly different; pairwise Φ_{ST} range: 0.043 to 0.19) but not all Hawaiian sampling sites (pairwise Φ_{ST} range: 0.0055 to 0.029).

The transport of larval fishes from the West Pacific via the Kuroshio extension of the North Pacific Equatorial Current has also been suggested as an alternative dispersal corridor into Hawaii [94, 95]. This possibility is supported by the discovery of some West Pacific fishes in the NWHI, including the Japanese angelfish (*Centropyge interruptus*; [96, 97]) and the splendid perch (*Grammatonotus macrophthalmus*; [98]). Indeed, 4.6% (57 species) of the Hawaiian fish fauna have ranges restricted to the Western North Pacific and Hawaiian Islands [98]. Using phylogenetic methods, Craig et al. [37] also showed that two endemic Hawaiian butterflyfish (*Chaetodon fremblii* and *Chaetodon miliaris*) groups with ancestral species in the West Pacific. We feel that this alternative colonization pathway is unlikely for *A. nigroris,* however, because this species is absent from the Pacific region west of Micronesia (i.e., China, Indonesia, Japan, Philippines, and Taiwan). Hence a South Pacific origin, as proposed by Gosline [63], seems more likely in this case. That said, our analysis is based on extant populations only, with the observed phylogenetic separation of Hawaiian and Pacific fish being too old to provide clues; the conclusion of a southern colonization pathway into Hawaii must therefore be regarded as provisional.

4.3. Endemism in Hawaii. The possibility of an endemic surgeonfish species in Hawaii is not surprising given that this Pacific archipelago supports many endemic reef fishes (25%; [31]). Although nuclear loci were not considered here, high levels of mtDNA differentiation between Hawaiian and Pacific *A. nigroris* (Table 2) is concordant with differences in morphology. More than half a century ago, Randall [48] noted variation in dorsal fin rays (Hawaii, range =23 to 26; Pacific, range =24 to 27) and anal soft rays (Hawaii, range =22 to 24; Pacific, range =22 to 25), as well as nonoverlapping gill raker counts (Hawaii, range =26 to 31; Pacific, range =21 to 25) between regions, although this morphological difference was not used to distinguish species at that time due to identical coloration. With the new genetic information provided here, a reclassification of this species is proposed in a companion paper, which includes a Hawaiian endemic (*A. nigroris*) and a widespread Pacific form (resurrected *A. nigros*; [99]). Given that other members of the genus *Acanthurus* show no genetic differentiation between Hawaii and elsewhere in the Pacific [39], what then might explain the apparent isolation of *A. nigroris*?

Several factors may contribute to the generation of endemic shore fishes. One conventional possibility is allopatric speciation, in this case by marine barriers that differentially impact larval dispersal [100]. Larvae must travel vast distances to either colonize or disperse outside the Hawaiian archipelago [94]. While some species may readily overcome these large stretches of open ocean, rare colonization events by a few individuals, coupled with natural selection (i.e., local adaptation, which may inhibit further gene flow; [101]) or the genetic consequences of variance in reproductive success (sweepstakes recruitment; [102]), could prompt rapid speciation [103]. Mesoscale eddy formation, upwelling zones, and sustained wind patterns may then enhance the local retention of fish larvae at oceanic islands and thus promote isolation between incipient species.

Several studies have highlighted the genetic distinctiveness of Hawaiian fishes relative to other locations in the Pacific Ocean ([104–107] but see [39]). This is in contrast to reef fish populations within the Hawaiian archipelago that exchange propagules across the relatively short distances between neighboring reefs. High genetic connectivity between adjacent reefs may also explain the lack of adaptive radiation among Hawaiian fishes [108]. For example, few closely related marine species (i.e., sister taxa) cooccur in Hawaii [109, 110], and so most Hawaiian endemics are paired with widespread Indo-Pacific taxa in phylogenetic analyses (e.g., [37]).

The alternative hypothesis of speciation along ecological boundaries has been applied to explain the extremely high biodiversity on coral reefs [16, 82, 111]. While natural selection for habitat preference and other life-history traits is undoubtedly influencing the evolutionary pathways of Hawaiian fauna, we feel that ecological components are probably not driving speciation within *A. nigroris*. The evidence from comparisons of Johnston Atoll and Hawaiian locations indicate that 865 kilometers is near the limit of larval dispersal for this species. Indeed, the closest Hawaiian island, French Frigate Shoals, shares two common haplotypes with Johnston and is not genetically different, a pattern also apparent in other reef fishes [100]. The nearest alternate sites for dispersal and colonization are found in the Northern Line Islands (Kingman Reef and Palmyra Atoll), a minimal distance of 1385 km (see [93]). That said, our sample of *A. nigroris* from the Line Islands has been isolated from the Hawaiian population for approximately 2 my based on conventional mutation rates. Although divergence time estimates from single-locus data should be interpreted with caution (see [112]), our approximation is consistent with phylogenetic studies of closely related reef fishes that diverged in the last one to five million years (e.g., [113]). Our approximation is also consistent with the emerging trend for most Hawaiian marine biota to be much younger than the formation of the Hawaiian archipelago itself [35, 37]. Thus, the observed genetic pattern for *A. nigroris* in Hawaii likely reflects a long history of rare colonization and peripatric isolation resulting in divergence from ancestral Pacific populations.

Regardless of the process that generates endemic species, there are many unrecognized reef fishes awaiting discovery. As we have shown here, genetic tools are instrumental in identifying unique evolutionary significant units (ESUs, *sensu* [114]), which provide a phylogenetic framework for specifying taxa with highly restricted gene flow at the level of the species. Genetic methods have uncovered cryptic evolutionary lineages in other reef fish families in Hawaii (*Canthigaster coronata,* [115]*; Cirrhitops fasciatus,* [116]*; Halichoeres ornatissimus,* [117]), in addition to elsewhere in the Indo-Pacific (*Discotrema monogrammum* and *Discotrema chrinophylum*, [118]; *Amphiprion melanopus*, *Cirrhilabrus punctatus*, *Labroides dimidiatus*, and *Pomacentrus moluccensis*, [119]; *Chaetodontoplus poliourus*, [120]; *Pictichromis dinar*, [121]; *Scarus ghobban*, [122]) and Eastern Pacific (*Epinephelus quinquefasciatus,* [123]). In the tropical Atlantic Ocean, 8 out of 15 surveyed reef fishes showed cryptic evolutionary partitions [124]. Cryptic species are aptly named because they often develop barriers to fertilization despite a lack of accompanying divergence in other aspects of morphology or ecology (for review see [125]). This therefore stresses the importance of conducting range-wide genetic surveys for existing species to identify marine biodiversity that may have been overlooked (e.g., [126]).

In conclusion, we identified genetic isolation and independent evolutionary trajectories of Hawaiian and Pacific *A. nigroris*, despite high connectivity within each region. The Pacific Blueline Surgeonfish can readily traverse the tens to hundreds of kilometers between reef habitats in the Hawaiian Archipelago (average distance =150 kilometers) and the hundreds of kilometers between reef habitats elsewhere in the Pacific (maximum distance =800 kilometers; [127]). This species appears to rarely disperse between Johnston Atoll and the Hawaiian Archipelago (average distance =1250 kilometers), and there is effectively no migration between the Hawaiian Islands and other locations in the Pacific that are *ca.* 1400 km away or more (i.e., Line Islands). While such dispersal is impressive (and daunting from a management perspective), several other groups of reef fishes can even exceed this, including pygmy angelfishes (genus *Centropyge*; [127]), unicornfishes (genus *Naso*; [7]), soldierfishes (genus *Myripristis*; [36]), and moray eels (genus *Gymnothorax*; [88]). The Pacific BlueLine Surgeonfish therefore joins a growing list of reef fishes (Table 5) for which high dispersal is coupled with broad habitat and feeding preferences, as well as a large geographic range. These dispersive species present a special challenge to wildlife managers because they exhibit connectivity on a scale that far exceeds the boundaries of any single jurisdiction. We suggest that genetic connectivity in the less dispersive corals and other reef architects may provide guidelines for regional ecosystem-level management [128], but the more dispersive reef fishes demonstrate the need for international cooperation.

Acknowledgments

This research was supported by the National Science Foundation Grants OCE-0453167 and OCE-0929031 to B. W. Bowen, as well as NOAA National Marine Sanctuaries Program MOA no. 2005-008/66882. It was also funded in part by a Natural Sciences and Engineering Research Council of Canada (NSERC) postgraduate fellowship to J. D. DiBattista. The views expressed herein are those of the authors and do not necessarily reflect the views of these agencies. For specimen collections, the authors thank Kim Andersen, Paul H. Barber, J. Howard Choat, G. Concepcion, Toby S. Daly-Engel, Joshua A. Drew, John L. Earle, Kevin Flanagan, Michelle R. Gaither, Brian D. Greene, Matthew Iacchei, Stephen A. Karl, Randall K. Kosaki, Carl G. Meyer, Yannis P. Papastamatiou, David Pence, Richard Pyle, Joshua Reece, D. Ross Robertson, Laurie Sorensen, Jennifer K. Schultz, Derek Skillings, Derek Smith, Zoltan Szabo, Kim Tenggardjaja, Tonatiuh Trejo-Cantwell, Bill Walsh, Ivor Willliams, Jill P. Zamzow, and the crew of the R. V. *Hi'ialakai*. The authors also thank Robert Toonen, Serges Planes, Ben Victor, Hawaii Department of Land and Natural Resources, the Coral Reef

Research Foundation, and the Papahānaumokuākea Marine National Monument, US Fish and Wildlife Service, and members of the ToBo lab for logistic support; Jack Randall for providing photographs; Jeff Eble and three anonymous reviewers for useful comments on the paper; Amy Eggers, Rajesh Shrestha, Lauren Valentino, and Mindy Mizobe of the HIMB EPSCoR core facility for their assistance with DNA sequencing. This is contribution no. 1417 from the Hawai'i Institute of Marine Biology and no. 8061 from the School of Ocean and Earth Science and Technology.

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