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Large-scale introduction of the Indo-Pacific damselfish *Abudefduf vaigiensis* into Hawai'i promotes genetic swamping of the endemic congener *A. abdominalis*

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Abstract

Hybridization in the ocean was once considered rare, a process prohibited by the rapid evolution of intrinsic reproductive barriers in a high-dispersal medium. However, recent genetic surveys have prompted a reappraisal of marine hybridization as an important demographic and evolutionary process. The Hawaiian Archipelago offers an unusual case history in this arena, due to the recent arrival of the widely distributed Indo-Pacific sergeant (*Abudefduf vaigiensis*), which is hybridizing with the endemic congener, *A. abdominalis*. Surveys of mtDNA and three nuclear loci across Hawai'i (N = 396, *Abudefduf abdominalis* and N = 314, *A. vaigiensis*) reveal that hybridization is significantly higher in the human-perturbed southeast archipelago (19.8%), tapering off to 5.9% in the pristine northwest archipelago. While densities of the two species varied throughout Hawai'i, hybridization was highest in regions with similar species densities, contradicting the generalization that the rarity of one species promotes interspecific mating. Our finding of later generation hybrids throughout the archipelago invokes the possibility of genetic swamping of the endemic species. Exaptation, an adaptation with unintended consequences, may explain these findings: the endemic species has transient yellow coloration during reproduction, whereas the introduced species has yellow coloration continuously as adults, in effect a permanent signal of reproductive receptivity. Haplotype diversity is higher in Hawaiian *A. vaigiensis* than in our samples from the native range, indicating large-scale colonization almost certainly facilitated by the historically recent surge of marine debris. In this chain of events, marine debris promotes colonization, exaptation promotes hybridization, and introgression invokes the possible collapse of an endemic species.

Keywords: hybridization, marine debris, NEWHYBRIDS, Papahānaumokuākea, population density, STRUCTURE

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Introduction

Hybridization between members of different species (or genetic lineages) is an important evolutionary force that can act as a source of genetic diversity, genetic innovations and, in some cases, new species (Seehausen 2004). Interspecific hybridization is prominent in the evolution of plants and is responsible for major diversification events (Soltis & Soltis 2009). More than 25% of plants and 10% of animals are reported to hybridize in the wild (Mallet 2005). The consequences of hybridization

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are well documented and typically lead to poorly adapted gene combinations that reduce fitness (outbreeding depression) (Gardner 1997; Córdoba-Aguilar 2009; Rasmussen *et al*. 2010). A smaller number of studies report hybrids with heterosis (hybrid vigour): an increase in fitness and survivorship compared to one or both parental species (Gardner 1997; Córdoba-Aguilar 2009; Schierenbeck 2011). Additionally, hybridization with backcrossing can lead to hybrid swarms (Rhymer & Simberloff 1996; Allendorf *et al.* 2001; Schierenbeck 2011): a population with widespread introgression or complete admixture of parental strains potentially leading to the loss of one or both parental species through genetic swamping (Schierenbeck 2011). Hybridization without backcrossing can produce self-sustaining species that do not interbreed with parental strains, although these are thought to be rare in animals (Larsen *et al.* 2010). In other cases, introgression at the boundary between species ranges can produce stable hybrid zones that persist for decades or centuries (Hobbs *et al.* 2009; Abbott *et al.* 2013).

Hybridization was once considered rare in the sea. The high-dispersal potential of marine organisms, relative to freshwater and terrestrial fauna, was believed to promote intrinsic reproductive barriers that preclude hybridization (Palumbi 1992). However, recent work indicates that these events are more common than previously recognized (Schwartz 2001; Hobbs *et al.* 2009; DiBattista *et al.* 2012). Recently, a hybridization hotspot has been discovered in the eastern Indian Ocean: a region where Pacific and Indian Ocean biota overlap, bringing sister taxa into sympatry (Hobbs *et al.* 2009). Certain taxonomic families of marine fishes seem prone to hybridization, including butterflyfishes (family Chaetodontidae) (Montanari *et al.* 2012) and angelfishes (family Pomacanthidae) (Hobbs *et al.* 2009; DiBattista *et al.* 2012). Despite these recent advances, hybridization in the sea is still poorly documented and little understood. The conditions that promote hybridization in a high-dispersal medium, and the consequences for biodiversity and evolutionary processes remain ambiguous.

Here, we investigate an unusual case of marine hybridization in the natural evolutionary laboratory of the Hawaiian Islands, between the endemic Hawaiian sergeant, *Abudefduf abdominalis*, and the recently arrived Indo-Pacific sergeant, *A. vaigiensis* (family Pomacentridae). *Abudefduf vaigiensis* has a very broad distribution from Africa and the Red Sea to the central Pacific. However, the first record of this conspicuous reef fish in Hawai'i was in 1991, on the island of Maui in the southeast (inhabited) end of the archipelago (Severns & Fiene-Severns 1993; Randall 2007). How *A. vaigiensis* colonized Hawai'i is not known with certainty but it is believed to have arrived by rafting; juvenile damselfishes, especially *Abudefduf* spp., are routinely observed among abandoned fishing nets and marine debris (Gooding & Magnuson 1967; Hunter & Mitchell 1967; Jokiel 1990; Mundy 2005; Carlton & Eldredge 2009). *Abudefduf vaigiensis* quickly became established, and suspected hybrids with the Hawaiian endemic congener *A. abdominalis* (based on intermediate coloration) were documented soon after their arrival (Randall 1996) (Fig. 1).

Members of the genus *Abudefduf* are benthic spawners with males guarding clutches of 100s to 1000s of eggs deposited by multiple females. Field observations show that *A. abdominalis* and *A. vaigiensis* form heterospecific social groups and mating pairs (Maruska & Peyton 2007). Embryos collected from heterospecific mating

Fig. 1 *Abudefduf* species and hybrid: (top) *A. abdominalis*, (middle) *A. abdominalis* × *A. vaigiensis* hybrid, (bottom) *A. vaigiensis*. *Photo credit: KeokiStender*.

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events demonstrate normal embryonic development and viable larvae. To date, nothing is known regarding the frequency of hybridization in Hawai'i, whether the hybrids are fertile, or if introgression is occurring between the two species. The introduced *A. vaigiensis* has colonized the full 2600 km of the Hawaiian Archipelago including the Papahānaumokuākea Marine National Monument in the Northwest Hawaiian Islands (NWHI), a United Nations Educational, Scientific and Cultural Organization (UNESCO) world heritage site and the largest marine protected area administered by the United States.

Abudefduf abdominalis and *A. vaigiensis* co-occur throughout the Hawaiian Archipelago, and genetic analysis across the range should reveal the distribution of individuals with hybrid ancestry. To determine whether the evolutionary integrity of the endemic Hawaiian sergeant, *A. abdominalis*, is at risk, we must first assess whether hybrid offspring are surviving to adulthood, reproducing and backcrossing. Here, we employ mitochondrial and nuclear DNA to resolve whether the phenotypic intermediates are F_1 hybrids, and to describe the nature and extent of hybridization across the Hawaiian Archipelago including the protected waters of the NWHI and the populated Main Hawaiian Islands (MHI).

Materials and methods

Taxon sampling

Between 2009 and 2012, 396 tissue samples of *A. abdominalis* and 314 *A. vaigiensis* samples were collected from throughout the Hawaiian Archipelago, using pole spears with SCUBA or snorkelling (Fig. 2). Sampling was initially conducted as part of a broader geographic study, and suspected hybrids were not specifically targeted. As part of the current study, 7 putative F_1 hybrids were collected from Maui ($N = 3$) and O'ahu ($N = 4$) to confirm that the intermediate phenotype (Fig. 1) was indicative of a hybrid. Tissues were preserved in salt-saturated DMSO buffer (Amos & Hoelzel

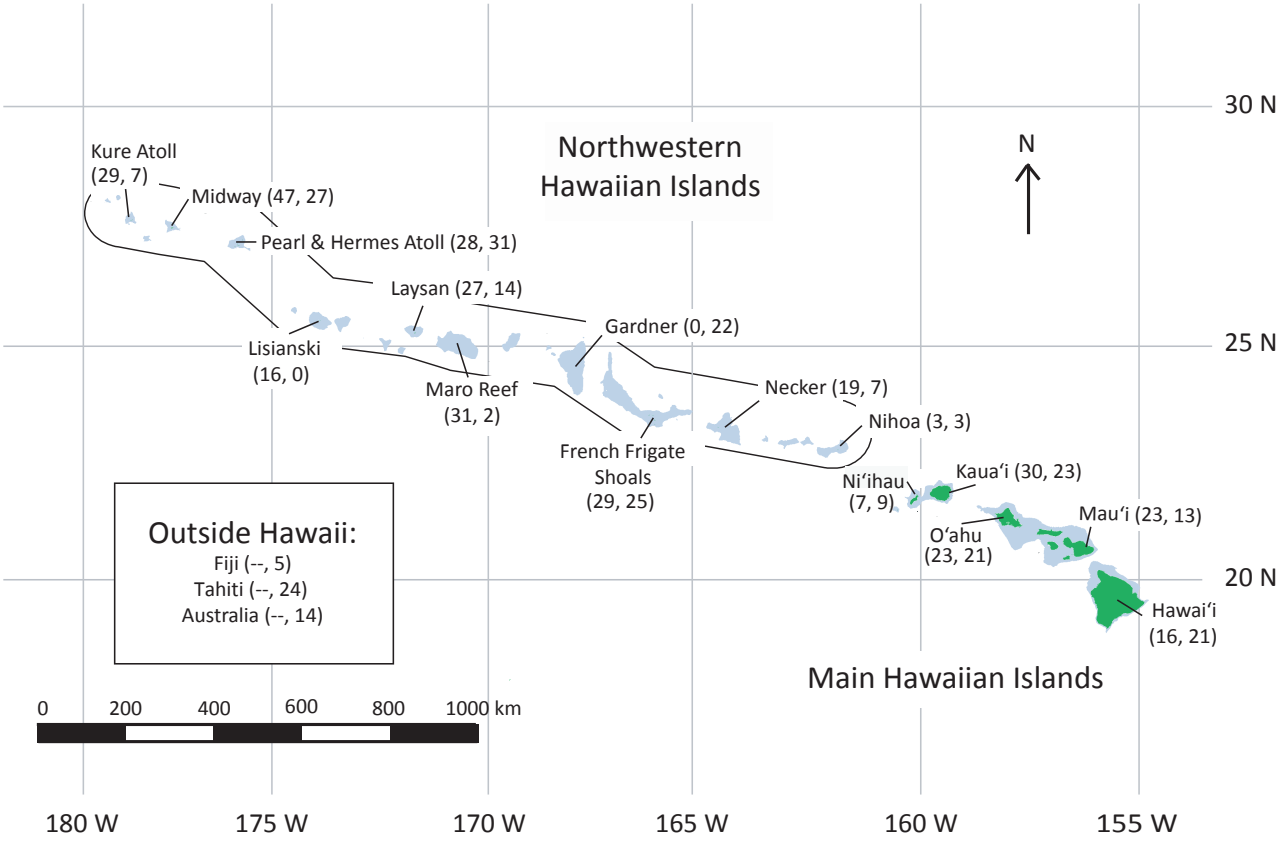

Fig. 2 Collection locations and sample sizes of *Abudefduf abdominalis* and *A. vaigiensis*. Solid line designates the Northwestern Hawaiian Islands which, in 2006, was designated the Papahānaumokuākea Marine National Monument. Filled darker areas represent current coastlines while light areas represent the maximum historical above-water island area. Sample sizes for each species are in parentheses (*A. abdominalis*, *A. vaigiensis*). Additional samples of *A. vaigiensis* were collected at locations outside of Hawai'i where *A. abdominalis* is absent (see inset).

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1991) and stored at room temperature. To resolve the genetic composition of pure *A. vaigiensis*, 43 specimens of *A. vaigiensis* were collected from three locations outside of Hawai'i (where *A. abdominalis* does not occur): Tahiti, Australia and Fiji (Fig. 2). Samples of *A. abdominalis* from locations where few *A. vaigiensis* occurred were used to represent the parental *A. abdominalis*.

DNA manipulations

Total genomic DNA was isolated from preserved tissue following the HotSHOT method (Meeker *et al.* 2007) and stored at −20 °C. A 574-bp fragment of the mtDNA cytochrome *b* (cyt *b*) gene was amplified to identify the maternal lineage of each individual using the forward primer (5′-GTGACTTGAAAAACCACCGTTG-3′) (Song *et al.* 1998) and H15573 (5′-AATAGGAAGTATCATTCG GGTTTGAT-3′) (Taberlet *et al.* 1992). PCRs were performed in 10 μL reactions containing 10–15 ng of DNA, 5 μL of premixed BioMixRed™ (Bioline Inc., Springfield, NJ, USA), 0.26 μM of each primer and nanopure water (Thermo Scientific, Barnstead, Dubuque, IA, USA) to volume and using the following conditions: 4 min at 94 °C, then 35 cycles of denaturing for 60 s at 94 °C, annealing for 30 s at 50 °C, and extension for 45 s at 72 °C, and a final extension for 10 min at 72 °C.

Eight individuals of each species were screened at sixteen nuclear loci (Table S1, Supporting information). Of these, three amplified consistently and provided diagnostic characters (fixed differences) between species: intron 3 of the gonadotropin-releasing hormone 3 (GnRH3-3), intron 1 of the S7 ribosomal protein (S7) and intron 2 of glyceraldehyde-3-phosphate dehydrogenase (Gpd2). We amplified 120 bp of GnRH3-3 using the primers GnRH3F and GnRH3R (Hassan *et al.* 2002), 361 bp of S7 using the primers S7RPEX1F and S7RPEX2R (Chow & Hazama 1998), and 169 bp of Gpd2 using primers modified from Hassan *et al.* (2002); Gpd2F5 (5′-AGCCTGGAGGCACGACGACA-3′) and Gpd2R5 (5′AGGCAGAACGGATGATGCAGGA-3′). For each intron, PCRs were performed using the same reaction mixture as described for cyt *b* but using the following temperature conditions: 5 min at 94 °C, then 35 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 58 °C, and extension for 30 s at 72 °C, and a final extension for 10 min at 72 °C.

PCR products were visualized using a 1.5% agarose gel with GelStar™ (Cambrex Bio Science Rockland, Inc., Rockland MA, USA) and then purified by incubating with 0.75 units of exonuclease and 0.5 units of shrimp alkaline phosphatase (ExoSAP; USB, Cleveland, OH, USA) per 7.5 μL of PCR product for 30 min at 37 °C, followed by 15 min at 85 °C. DNA sequencing was performed using fluorescently labelled dideoxy terminators on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the University of Hawai'i Advanced Studies of Genomics, Proteomics and Bioinformatics sequencing facility or at the Hawai'i Institute of Marine Biology's Evolutionary Core Lab.

Data analysis – mtDNA

Sequences were aligned and edited using the program SEQUENCHER v.4.8 (Gene Codes, Ann Arbor, MI, USA). Molecular diversity indices including haplotype and nucleotide diversity were calculated for all loci using ARLEQUIN v.3.11 (Excoffier *et al.* 2005) and DNASP v.5.10 (Librado & Rozas 2009) for each species (after hybrids were identified and removed from the data set). To confirm that only *A. abdominalis* and *A. vaigiensis* specimens were collected (*A. sordidus* is also found in the Hawaiian Islands), a phylogenetic reconstruction of the genus *Abudefduf* was conducted using available cyt *b* sequences obtained from GenBank and rooted with the Mango Tilapia, *Sarotherodon galilaeus* (family Cichlidae) (Table S2, Fig. S1, Supporting information). A model for DNA sequence evolution and model parameters were selected using the program JMODELTEST v.2.1 (Guindon & Gascuel 2003; Darriba *et al.* 2012). The best fit model of HKY+I+Γ was identified by the Akaike information criterion and used for phylogenetic reconstructions. A maximum-likelihood tree was created using the program PHYML v.3.0.1 (Guindon *et al.* 2010) with clade support assessed after 1000 nonparametric bootstrap replicates. Bayesian inference was conducted using the program MRBAYES v.3.1.2 (Huelsenbeck *et al.* 2001; Ronquist 2004), and a pair of independent searches was run for 1 million generations with trees saved every 1000 generations with the first 250 sampled trees of each search discarded as burn-in. A haplotype network was reconstructed for each species with NETWORK v.4.6.1.1 (http://www.fluxus-engineering.com/network_terms.htm) using a median-joining algorithm (Bandelt *et al.* 1999) and default settings.

Data analysis – nuclear DNA

Allelic states of nuclear sequences with more than one heterozygous site were estimated using the Bayesian program PHASE v.2.1 (Stephens & Donnelly 2003) as implemented in DNASP v.5.10. Three separate runs, each of 100 000 repetitions after a 10 000 iteration burn-in, were conducted for each locus. All runs returned consistent allele identities. The Microsoft Excel Microsatellite Toolkit add-in (Park 2001) was used to format data files, and the program CONVERT v.1.31 (Glaubitz 2004) was used to produce input files for STRUCTURE v.2.3.2 (Pritchard *et al.* 2000; Hubisz *et al.* 2009). We tested for Hardy–Weinberg equilibrium (HWE) within popula-

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tions and linkage disequilibrium between nuclear loci using ARLEQUIN. False discovery rates were controlled and maintained at $\alpha = 0.05$ total among all pairwise tests (Yoav & Yekutieli 2001; Narum 2006).

Hybrid identification

To identify hybrids, we employed two methods: the assignment test of STRUCTURE and NEWHYBRIDS v.1.1 (Anderson & Thompson 2002). Only individuals for which we were able to amplify all four gene regions were included in hybrid analyses. STRUCTURE is a Bayesian method that estimates ancestry and categorizes individuals into discrete populations. The simulation was run for 1 million generations with the first 100 000 discarded as burn-in. Twenty replicates of each simulation from $K = 1$ to 10 genetic clusters were run, and hybrid identification was compared across all replicates. Default parameters were utilized, which incorporated an admixture model and assuming that allele frequencies were correlated. We determined the most likely number of genetic clusters (K) based on the results of STRUCTURE HARVESTER v.0.6.93 (Earl & von Holdt 2012). Estimated membership coefficients for each individual (Q) were determined from the STRUCTURE analysis. To ensure no hybrids were included in the parental populations, we followed criterion 3 of Burgarella *et al.* (2009) for distinguishing between purebreds and hybrids, which assumes that all individuals with Q ≥ threshold value (Tq) are purebred and that all others are hybrids. To determine the most appropriate Tq for our data set, we simulated parental and hybrid classes using HYBRIDLAB v.1.0 (Nielsen *et al.* 2006) following Hasselman *et al.* (2014). Using this procedure, we determined that the lowest number of misassigned purebreds (0.009) and hybrids (0.028) occurred when $Tq = 0.920$.

NEWHYBRIDS uses Markov chain Monte Carlo simulations in a Bayesian setting to calculate posterior probabilities, P, of an individual belonging to one of six genotypic frequency classes: Parental 1 (P_1), Parental 2 (P_2), F_1 ($P_1 \times P_2$), F_2 ($F_1 \times F_1$), $P_1 \times F_1$ backcross (B_{x1}) or $P_2 \times F_1$ backcross (B_{x2}). Analyses were run using default settings for 100 000 generations with the first 10 000 discarded as burn-in. Following the steps described above, we determined that the lowest number of misassigned purebreds (0.009) and hybrids (0.025) occurred when $Tq = 0.880$ (the optimal Tq) for NEWHYBRIDS. NEWHYBRIDS has difficulty distinguishing among hybrid genotypic classes. Therefore, all individuals with $Tq < 0.880$ were classified as hybrids and separate hybrid genotypic classes were not considered. Individuals with $Tq > 0.880$ for one of the six genotypic classes were classified accordingly. In our analyses, we were unable to discriminate between F_2 and backcrosses (B_x); therefore, individuals with a total combined $Tq > 0.880$ for the F_2 and B_x classes were binned into a F_2/B_x group. If individuals had $Tq < 0.880$ for a specific genotypic class and they did not qualify as a F_2/B_x, then the individual was categorized as simply a 'hybrid' irrespective of a specific genotypic class. Hence, individuals were categorized into two parental types and two hybrid classes: F_1 hybrids and F_2/B_x hybrids.

Regional comparisons

A Mann–Whitney calculation was conducted to compare hybridization frequency between the MHI and the NWHI. Population densities for each species across the Hawaiian Archipelago were available from the National Oceanic and Atmospheric Administration (NOAA) Coral Reef Ecosystem Division for the years 2008 to 2012. Islands with combined samples sizes <10 were removed from the analysis. A Kruskal–Wallis one-way analysis of variance was conducted using the software MINITAB v.16 (www.minitab.com) to compare population densities between the MHI and the NWHI.

Results

mtDNA and nuclear introns

Our phylogenetic reconstruction for the genus *Abudefduf* (Fig. S1, Supporting information) recovered two strongly supported monophyletic lineages among our samples that correspond with *A. abdominalis* and *A. vaigiensis,* confirming that only individuals from these two species were included in our analyses. Our data indicate the two species are 4% divergent at cyt *b*. Using a provisional molecular clock of 2% per million years between lineages (Bowen *et al.* 2001; Reece *et al.* 2010), we estimate that these two species diverged approximately 2 Myr. Notably, they are not sister species in the phylogeny (Quenouille *et al.* 2004).

Molecular diversity indices for cyt *b* and the nuclear introns are presented in Table 1. The recent arrival *A. vaigiensis* demonstrated higher mtDNA diversity in Hawai'i ($h = 0.760$) than the endemic *A. abdominalis* ($h = 0.524$). Surprisingly, Hawaiian *A. vaigiensis* also demonstrated higher mtDNA diversity than our samples of the native range ($h = 0.629$). However, the opposite was true among nuclear loci with each locus having higher levels of diversity in the native range. In comparing nuclear DNA diversity within Hawai'i, the native *A. abdominalis* shows greater genetic diversity than *Abudefduf viagiensis* in GnRH and S7, but less in Gpd2. Nuclear markers were in Hardy–Weinberg equilibrium and linkage equilibrium after controlling for

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Table 1 Molecular diversity indices for cytochrome *b* and three nuclear introns amplified from *Abudefduf abdominalis* and *A. vaigiensis* (hybrids removed)

Species	cyt*b* (574 bp)					GnRH (120 bp)					Gpd2 (169 bp)					S7 (361 bp)				
	n	*Nh*	*S*	π	*h*	*n*	*Nh*	*S*	π	*h*	*n*	*Nh*	*S*	π	*h*	*n*	*Nh*	*S*	π	*h*
Abudefduf abdominalis	321	35	47	0.00129	0.524	320	7	4	0.00493	0.445	319	8	7	0.00022	0.036	332	36	29	0.00462	0.808
A. vaigiensis	221	30	49	0.00473	0.801	187	6	5	0.00101	0.113	163	15	13	0.00487	0.498	184	36	33	0.00443	0.796
A. vaigiensis (native range)	37	10	13	0.00308	0.629	37	5	4	0.00137	0.135	13	8	7	0.01085	0.782	33	10	9	0.00527	0.743
A. vaigiensis (Hawai'i)	184	27	47	0.00459	0.760	151	4	3	0.00091	0.108	160	13	11	0.00427	0.462	151	31	30	0.00409	0.741
All samples	542	63	67	0.02085	0.797	507	10	7	0.00554	0.580	482	21	19	0.00664	0.519	487	63	55	0.00678	0.894

Fragment length (bp), number of individuals (*n*), number of haplotypes (*Nh*), number of polymorphic sites (*S*), nucleotide diversity (*π*) and haplotype diversity (*h*) are given for each species for all populations. Sample sizes reported in summary statements are larger than any individual sample size here because not all specimens worked in all assays.

false discovery rates (corrected α was 0.009 for both *A. abdominalis* and *A. vaigiensis)*.

Hybridization analyses

All seven specimens that were identified as hybrids in the field based on intermediate morphology were confirmed to be hybrids. Using the program STRUCTURE, we identified two genetic clusters ($K = 2$, Fig. S2, Supporting information) that corresponded with *A. abdominalis* and *A. vaigiensis*. STRUCTURE identified 37 hybrids (Table 2, Fig. 3) among 535 individuals or 6.92% of the combined *A. abdominalis* and *A. vaigiensis* population. Hybrid frequency varied across the archipelago with no hybrids found at the islands of Nihoa, Gardner, Maro, Laysan and Lisianski while nearly a quarter of the individuals sampled at Maui (23.1%) were hybrids. When reviewing hybrid frequency by region, we found that 14.8% of individuals sampled in the MHI were hybrids with proportions ranging from 6.25% at Ni'ihau to 23.1% at Maui. In the NWHI, hybrid frequency was low and had a narrower range from 0% at several locations to 6.67% at Maro Reef.

Analyses using NEWHYBRIDS identified a slightly higher number of hybrids compared to STRUCTURE (Fig. 4, Table 2). This program identified 57 hybrids among 535 individuals or 10.7% of the *Abudefduf* sampled in Hawai'i. Ni'ihau, again, had the lowest proportion of hybrids (6.25%) in the MHI with Maui (30.8%) and Hawai'i Island (21.9%) having the highest. NEWHYBRIDS detected 2 hybrids at both Nihoa and Laysan, which STRUCTURE did not. For this analysis, hybrid frequency in the NWHI was highest at Nihoa (33.3%), a figure attributed to low sample size at this location. The next highest hybrid frequency was at Maro Reef (13.3%), double the number of hybrids identified using STRUCTURE. When comparing hybrid frequency between the MHI and NWHI, we found a significantly higher proportion of hybrids in the MHI (Mann–Whitney, $W = 77.0$, d.f. = 17, $P = 0.01$) (Table 2).

Using NEWHYBRIDS, we detected 7 (1.30%) F_2 and 25 (4.39%) F_2/B_x hybrids, but no F_1 hybrids were detected. Based on the parameters we assigned, we found an additional 25 uncategorized hybrids. For statistical comparisons, F_2 individuals were pooled with B_x hybrids into a group referred to as F_2/B_x. When broken down by region, the MHI had a F_2/B_x hybrid frequency of 12.6%. The single individual identified as a hybrid at Ni'ihau was classified as a F_2/B_x hybrid. Among the islands with the highest hybrid frequency, Maui and Hawai'i Island, F_2/B_x hybrids accounted for more than half of all hybrids, with 18.0% and 15.6% of all fishes examined falling into this hybrid class, respectively. The NWHI had a lower F_2/B_x hybrid frequency

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Table 2 Population density of each species (*A. abdominalis*, Aab; *A. vaigiensis*, Ava), number of hybrids detected and per cent of individual screened that were hybrids are listed by geographic location

		Population density (ind./m^2)		STRUCTURE		NEWHYBRIDS			
	Total	Aab	Ava	Hybrids, *n*	% hybrids	Hybrids, *n*	% hybrids	F_2 /B_x, *n*	% F_2/B_x
MHI									
Hawai'i	32	0.079	0.004	7	21.88	7	21.88	5	15.63
Maui	39	0.039	0.041	9	23.08	12	30.77	7	17.95
O'ahu	42	0.107	0.268	5	11.9	7	16.67	4	9.52
Kaua'i	53	0.031	0.037	5	9.43	9	16.98	6	11.32
Ni'ihau	16	0.008	0.012	1	6.25	1	6.25	1	6.25
NWHI									
Nihoa	6	0	0	0	0	2	33.33	0	0
Necker	26	0	0	1	3.85	1	3.84	0	0
French Frigate Shoals	53	0.156	0.228	3	5.66	3	5.66	2	3.77
Gardner	20	0	9.53	0	0	0	0	0	0
Maro Reef	30	0.231	0.044	2	6.67	4	13.33	1	3.33
Laysan	40	0.799	0.140	0	0	2	5.00	1	2.50
Lisianski	16	0.485	0.107	0	0	0	0	0	0
Pearl & Hermes	56	0.667	0.149	2	3.57	5	8.93	2	3.57
Midway	71	1.39	0.015	1	1.41	3	4.23	2	2.81
Kure	35	0.285	0.051	1	2.86	1	2.86	1	2.86
MHI	182	0.063	0.088	27	14.84	36	19.78	23	12.64
NWHI	353	0.542	0.328	10	2.83	21	5.94	9	2.23
TOTAL	535	0.417	0.273	37	6.92	57	10.65	32	5.79

MHI, Main Hawaiian Islands; NWHI, Northwestern Hawaiian Islands.
Results for STRUCTURE (Pritchard *et al.* 2000; Hubisz *et al.* 2009) and NEWHYBRIDS (Anderson & Thompson 2002) analyses are shown.

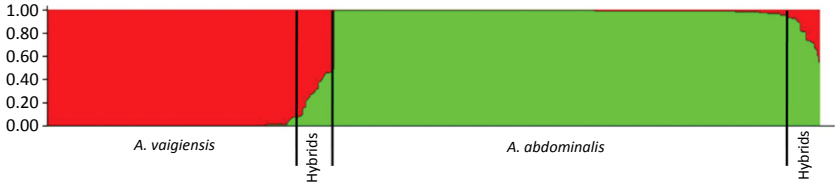

Fig. 3 STRUCTURE bar plot for *Abudefduf vaigiensis* and *A. abdominalis*. Each bar represents the membership coefficient, Q, (y-axis) per individual to estimate the identity of the parental species. Each shade corresponds to an inferred evolutionary cluster; mixed shading ($0.1 < Q < 0.9$) indicates hybrids.

(2.23%). No F_2/B_x hybrids were detected at Nihoa, Necker, Gardner or Lisianski. The NWHI islands with the highest F_2/B_x hybrids were Pearl and Hermes and French Frigate Shoals, 3.57% and 3.77%, respectively. Similar to the overall hybrid frequency, we detected a significantly higher frequency of F_2/B_x hybrids in the MHI compared to the NWHI (Mann–Whitney, $W = 60.5$, d.f. = 17, $P = 0.01$).

Among hybrids, the mtDNA of both parental species was present at similar levels (*A. abdominalis*, 45%; *A. vaigiensis*, 55%). This ratio is similar between regions, indicating interspecific mating is reciprocal and hybridization is not a result of unidirectional mating.

Population density

Based on the survey data provided by NOAA Coral Reef Ecosystem Division, we found significantly higher population densities for both species in the NWHI compared to the MHI with the former harbouring over three times the population abundance (Kruskal–Wallis, H = 17.16, d.f. = 1, $P < 0.001$) (Table 2). In the MHI, *A. vaigiensis* had a slightly higher, though not statistically significant, population density (0.088 individuals/m^2) than *A. abdominalis* (0.063 individuals/m^2). In contrast, the NWHI had significantly higher population densities of *A. abdominalis* (0.54 individuals/m^2) than *A. vaigiensis*

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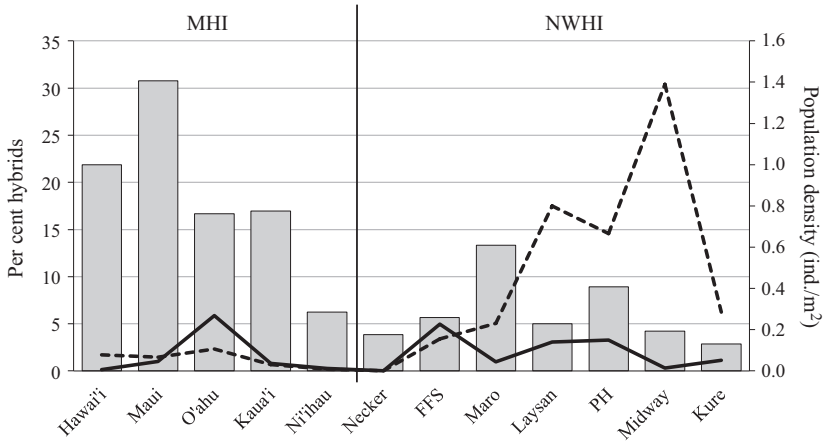

Fig. 4 Per cent hybrids based on NEWHYBRIDS (Anderson & Thompson 2002) analysis and population density by species for each island where hybrids are found. Solid lines separates Main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI); bars, hybrids identified by NEWHYBRIDS; thick solid line, *A. vaigiensis* population density; dashed line, *A. abdominalis* population density. Islands with combined sample sizes < 10 are removed. FFS, French Frigate Shoals; PH, Pearls & Hermes.

(0.33 individuals/m^2) (Kruskal–Wallis, H = 17.35, d.f. = 1, $P < 0.001$). Moreover, if we remove one island (Gardner) with a very skewed ratio (9.53 *A. vaigiensis* and 0 *A. abdominalis*/m^2), the *A. vaigiensis* NWHI population density drops to 0.092 individuals/m^2, revealing a strong difference in density.

Discussion

Abudefduf vaigiensis was first reported in Hawaiian waters in 1991 (Severns & Fiene-Severns 1993; Randall 2007; Carlton & Eldredge 2009). Approximately twenty years later, this species had colonized the entire 2600-km archipelago including the Papahānaumokuākea Marine National Monument in the uninhabited NWHI. The pioneering work of Maruska & Peyton (2007) previously documented successful interspecific spawning between *A. vaigiensis* and *A. abdominalis*. Here, we confirm hybridization between this recent migrant and the endemic *A. abdominalis* and document the first record of viable, fertile hybrids among damselfish of the genus *Abudefduf*. It is not known how *A. vaigiensis* arrived in Hawai'i, but hitchhiking along with marine debris is very likely (Mundy 2005; Carlton & Eldredge 2009). Population densities of the recent arrival are now comparable to the native *A. abdominalis* in the MHI, even exceeding the native species on the island of O'ahu. However, the native species still outnumbers the new migrant by nearly 2:1 in the NWHI where population densities of *A. abdominalis* reach their peak at 1.4 individuals per m^2 at Midway Atoll (Fig. 4). Here, we record hybrids throughout the Hawaiian Islands, but their proportions are much higher where population densities of both species are similar in the MHI. Our data demonstrate that hybridization is common between these two species and their offspring are fertile, raising concerns over the integrity and survival of the endemic Hawaiian sergeant.

Introduction into Hawai'i

Prior to dissecting our results, we address a prominent caveat: Did *A. vaigiensis* arrive in the last few decades or merely escape detection in reef fish surveys? It is certainly possible that *A. vaigiensis* existed in Hawai'i in low densities prior to first detection. Invaders can persist for decades before population explosions, presumably during a process of fine-tuning to the new environment (Gaither *et al.* 2012). However, Hawai'i is perhaps the best studied reef habitat in the Pacific, and there are no records of *A. vaigiensis* in Hawai'i prior to 1991. Further, collections prior to 1990 in the Bernice Pauahi Bishop Museum in Honolulu revealed no specimens of *A. vaigiensis* ($N = 36$ *Abudefduf* individuals). We conclude that the recent recognition of *A. vaigiensis* in Hawai'i is not an artefact of scientific coverage, but the product of a recent colonization.

Results from the programs STRUCTURE and NEWHYBRIDS were congruent and identified the same individuals as hybrids. However, NEWHYBRIDS identified 20 additional hybrids, consistent with the comparison of these methods by Vaha & Primmer (2006), who concluded that NEWHYBRIDS is more sensitive for hybrid detection. However, we were unable to confidently assign these individuals to specific genotypic classes using NEWHYBRIDS. Surveys of additional nuclear loci would be necessary to confidently identify hybrids (Boecklen & Howard 1997). However, the assignment of individuals to the combined F_2/B_x category confirms that *A. abdominalis* × *A. vaigiensis* hybrids are fertile and introgression is ongoing.

The haplotype diversity in the introduced range of *A. vaigiensis* ($h = 0.760$) is higher than our (admittedly limited) sample from the native range ($h = 0.629$), a phenomenon observed in other introduced species, and one that has yet to be fully explained (Kolbe *et al.* 2004; Roman 2006; Gaither *et al.* 2012; Ghabooli *et al.* 2013).

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Based on the number of haplotypes detected, our data demonstrate that at least 27 female *A. vaigiensis* have colonized Hawai'i (and at least one male); however, the actual number of colonizers is likely much higher. This observation begs the question of why species that have maintained separate ranges for 2 million years have suddenly come into sympatry in the remote Hawaiian Archipelago with a seeming flood of colonists. A human element to this phenomenon is strongly implicated. Members of this genus are frequently observed with drifting garbage and flotsam, most especially the 'ghost nets' in the North Pacific Gyre that routinely enter Hawaiian waters. An estimated 52 metric tons of this material accumulate every year in the NWHI (Dameron *et al.* 2007). This invokes a strong conservation concern about marine debris, with the new dimension that species introduced via debris can corrupt the genetic integrity of native species.

Causes of hybridization

Many processes can facilitate interspecific spawning including overlapping resources and environmental heterogeneity (Gardner 1997; Montanari *et al.* 2012). The formation of heterospecific social groups may be attributed to similarities in diet and habitat preference. Both species inhabit coral-dominated coastal reefs and are planktivores that feed in the water column (Tyler & Stanton 1995; Frédérich *et al.* 2009). Like most members of the family Pomacentridae, *A. abdominalis* and *A. vaigiensis* are benthic spawners and *A. abdominalis* has synchronized spawning events throughout the year that peak at 1-month intervals (Tyler & Stanton 1995). In Hawai'i, these species spawn at the same time and in similar habitats.

While interspecific spawning can be explained by habitat choice and timing, the causes for differing levels of hybridization across the archipelago are not clear. Hybridization levels vary among islands (Fig. 4), and the MHI has higher levels of hybridization than the NWHI (Table 2). What are the mechanisms driving the differences in hybridization levels between the MHI and NWHI? The first potential mechanism is the difference in population densities between the species. Population density imbalance is often used to explain hybridization in birds (Randler 2002; McCracken & Wilson 2011), a group where one in ten species is known to hybridize (McCarthy 2006). When population densities are highly skewed, the rarer species is more likely to mate with heterospecifics (Hubbs 1955; Gardner 1997). Interspecific mating is often unidirectional, with the female of the rare species more likely to mate with a male of the common species, although the generality of this pattern is still debated (Wirtz 1999; Randler 2002).

Our results do not conform to this prevailing principle of hybridization in areas of unequal population densities. While we observed significant differences in hybridization rates between the MHI and the NWHI, the MHI with low but similar population densities had higher rates of hybridization. In the NWHI, population density was significantly imbalanced yet demonstrated lower rates of hybridization. Perhaps at higher population densities, such as in the NWHI, the abundance of mates in both species is above a threshold sufficient to assure the predominance of conspecific mating. Conversely, lower population densities of both species in the MHI may promote hybridization.

A second possible explanation for higher hybrid densities in the MHI is that this region may be the area where the introductions of *A. vaigiensis* first occurred and hybrids, including various backcrosses, have had sufficient time to accumulate in high densities. This is supported by the fact that Maui in the MHI is the first-reported location of *A. vaigiensis* in Hawaiian waters and is the site of the highest hybrid density. It may be a matter of time before the hybrid densities found in Maui are observed across the entire archipelago.

A third potential mechanism is the degree of anthropogenic disturbance in the MHI compared to the NWHI. The MHI are heavily populated with approximately 1.4 million permanent residents and more than 8 million annual visitors. In contrast, the NWHI is relatively pristine, does not support a substantial human population (<150 individuals at a given time) and is thus removed from much of the locally generated anthropogenic influences that affect the MHI. Anthropogenic environmental changes have been correlated with increased hybridization among a broad array of species that occur in sympatry (Gilman & Behm 2011). For example, lake whitefish (*Coregonus* spp., family Salmonidae) in Lake Huron began hybridizing after fishing pressure, and increased predation reduced the population density of one of the parental species (Todd & Stedman 1989). Sympatric *Banksia* species (flowering plants, family Proteaceae) hybridize in areas of human development but not in neighbouring undisturbed areas (Lamont *et al.* 2003). Hybridization among African cichlids increased due to eutrophication and reduced visibility that interfered with mate selection (Seehausen *et al.* 1997). Stickleback fishes (genus *Gasterosteus*) in southwestern British Columbia collapsed into a hybrid swarm after premating barriers were removed by habitat alteration and decreased water clarity (Taylor *et al.* 2006; Behm *et al.* 2010).

While the pattern of increased hybridization in disturbed habitat is documented in Hawaiian *Abudefduf*, a corresponding explanation remains elusive. Unlike the above cases, these species were only recently found in sympatry. Based on a previous phylogeny of the genus

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Abudefduf, the two species are not sister taxa (Quenouille *et al.* 2004). Mating behaviour and mate selection cues in *A. abdominalis* and *A. vaigiensis* developed in allopatry for over 2 million years, with no need for specific premating barriers to maintain species integrity (Metz & Palumbi 1996; Palumbi *et al.* 1997). Part of the explanation may be exaptive traits that facilitate interspecific reproduction, including male nuptial coloration. During courtship, the endemic *A. abdominalis* turns pale blue with yellow bars (Helfrich 1958). The congener *A. vaigiensis* also turns pale blue, but adults of both sexes have yellow coloration continuously, regardless of reproductive state (Allen & Erdmann 2012). Thus, the newcomer to Hawai'i has coloration that may signal reproductive receptivity to the endemic species, an example of an adaptive trait with unintended consequences (exaptation). In an observational study of interspecific spawning event, Maruska & Peyton (2007) record successful heterospecific courtship with the male *A. abdominalis* displaying nuptial coloration. Although courting involves additional factors such as behavioural cues, coloration may be sufficient to initiate interspecific spawning.

Premating barriers can certainly evolve in allopatry, but in this case, and suggested by the presence of similar nuptial coloration, there has been insufficient time for isolating mechanisms to become established. In terrestrial and freshwater systems, hybridization generally occurs between species with mtDNA coding regions that differ by < 2% (Tilley *et al.* 1990; Coyne & Orr 1997; Montalvo & Ellstrand 2001; Edmands 2002; Harushima *et al.* 2002; Mallet 2005) with some deeper divergences (2-6+%) also noted among aquatic and terrestrial species (Karl *et al.* 1995; Riley *et al.* 2003; Mallet 2005; Crow *et al.* 2007; Montanari *et al.* 2012). In our study, *A. abdominalis* and *A. vaigiensis* differ by 4% at cyt *b*. Due to the limited number of studies focusing on hybridization in the marine realm, there is no approximate yardstick for the depth of divergences that preclude marine hybrids.

While we cannot definitively conclude the mechanisms responsible for differences in hybridization between regions, similar population densities seem to be the driving force for high levels in the MHI. If the entry point was in the MHI (as the original identification indicates), then that might explain the relative scarcity of *A. vaigiensis* in newly colonized habitat in the NWHI. Another factor is the allopatric distribution of the two species over the last few million years, putting these species in a naïve state regarding reproductive isolation.

Finally, the generalizations about terrestrial and freshwater hybrids examined above (uneven densities and disturbed habitat) do not readily fit this case. This raises the recent theme that evolutionary processes in the sea may operate at a different tempo than on land and freshwater, due in part to the extensive dispersal potential of species like *A. vaigiensis* (Bowen *et al.* 2013). It is almost inconceivable that a vertebrate entering a terrestrial system could spread across 2600 km in 30 years and induce hybridization in every corner of the range of the endemic species.

Extinction by hybridization

The recent arrival of *A. vaigiensis* in Hawai'i, an isolated biogeographic province where 25% of the inshore fish fauna are endemic (Randall 2007; Briggs & Bowen 2012), has potential consequences for the endemic Hawaiian sergeant including reduced survivorship or genetic swamping (Ryan *et al.* 2009). Hybridization is estimated to be responsible for up to 38% of all fish extinctions in the United States (Schierenbeck 2011). This can occur through the formation of hybrid swarms, when species freely interbreed leaving no genetically 'pure' individuals (Allendorf & Leary 1988; Allendorf *et al.* 2001) or by the replacement of one or both parental species by hybrids of greater fitness (heterosis) (Perry *et al.* 2001; Schierenbeck 2011).

Our ability to track the status of *A. abdominalis* is hindered by the difficulty of identifying hybrids in the field. As a result, state and federal monitoring efforts do not record suspected hybrids, but categorize specimens as one of the parental species. During our collection efforts, we found that most hybrids were originally identified as *A. vaigiensis,* which is likely due to the striking yellow coloration found in both the hybrids and *A. vaigiensis* but not in *A. abdominalis* (Fig. 1). Despite robust survey data from NOAA for the past decade, the available data are insufficient to determine whether hybridization frequency has changed over recent years. Our study does indicate that estimates of *A. vaigiensis* densities in the Hawaiian Islands are slightly elevated as hybrids are often misidentified as *A. vaigiensis*.

Ultimately, the extent of hybridization recorded here relative to the time of the first recording of *A. vaigiensis* in Hawai'i (1991) is a cause for alarm and a concern for the evolutionary integrity of *A. abdominalis*. Certainly, there is no refuge remaining for the endemic *A. abdominalis*, and if hybridization levels continue or even escalate, this species can quickly approach a scenario of genomic extinction.

Implication of future introductions into Hawai'i

Our data indicate that the observed recruitment of *A. vaigiensis* at Maui in the MHI may not have been a

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rare colonization event but instead was the first record of a continuing (probably ongoing) recruitment event. Our mtDNA haplotype network (Fig. S3, Supporting information) shows no shared haplotypes between Hawaiian *A. vaigiensis* and Tahiti, indicating that the introductions are not coming from the South Pacific, but likely from a closer source. In addition, Hawaiian *A. vaigiensis* has a higher genetic diversity than our samples from the native range, indicating that Hawaiian *A. vaigiensis* has not experienced a genetic bottleneck, a result found in other introduced fishes in Hawai'i (Gaither *et al.* 2010, 2013). As mentioned previously, *A. vaigiensis* probably arrived by rafting with marine debris. This vector is responsible for transporting alien species across vast distances (Bryan *et al.* 2012; Williams *et al.* 2013), and juvenile *A. vaigiensis* are commonly observed alongside debris in the offshore waters of Hawai'i. Between 1996 and 2009, over 650 metric tons of marine debris was collected in the waters of the NWHI (National Oceanic & Atmospheric Administration (NOAA) 2013) with each item potentially acting as a vector for such introductions (Barnes 2002; Choong & Calder 2013).

The implication of continued introductions of exotic species into Hawaiian waters extends far beyond the uncertain evolutionary pathway of *A. abdominalis*. In Hawai'i, ecological and evolutionary interactions between alien and endemic species have the potential to disrupt a balance that has existed for millennia. Introduced species are known vectors for introducing parasites into Hawaiian waters and into endemic species (Vignon *et al.* 2009; Gaither *et al.* 2013) adding to the growing concern. With the continued accumulation of debris in the ocean, the prospect of introductions into Hawai'i and other oceanic islands is likely to accelerate. Although the consequences of such introductions are unknown, the extensive hybridization between *A. abdominalis* and *A. vaigiensis* may be a harbinger of future evolutionary and ecological consequences for formerly isolated marine communities.

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R.J.T. and F.S. designed the research project. R.R.C., M.R.G. and B.K. generated the molecular data. R.R.C. conducted the analyses and led the writing of the manuscript. The genotyping was conducted in the laboratory of R.J.T. and B.W.B.

Data accessibility

Sequence data for cyt b and S7 are deposited in GenBank under Accession nos KM579706-KM580024. Online supplemental materials (DRYAD doi:10.5061/dryad.sp17k) include:

Sequence fasta files are available in the following files: 'Aab_cytb', 'Aab_GnRH', 'Aab_Gpd2', 'Aab_S7', 'Ava_cytb, 'Ava_GnRH', 'Ava_Gpd2, 'Ava_S7'.

Input files for NEWHYBRIDS and STRUCTURE analyses are available in the following files: 'Abudefduf_NEWHYBRIDS' and 'Abudefduf_STRUCTURE'.

Excel file ('Coleman_etal_Abudefduf_09.25.14.suppl.xlsx) with all other supplementary materials in the following sheets: 1 – *Abudefduf abdominalis* sample ID and corresponding collection location; 2 – *A. vaigiensis* sample ID and corresponding collection location; 3 – Table S1 (Supporting information); 4 – Table S2 (Supporting information); 5 – Fig. S1 (Supporting information); 6 – Fig. S2 (Supporting information); 7 – Fig. S3 (Supporting information); 8 – *Abudefduf* Population Density – Hawaii.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Maximum likelihood tree for the genus *Abudefduf* based on cytochrome *b* sequences.

Fig. S2 Evanno graph from STRUCTURE HARVESTER (Earl & von Holdt 2012).

Fig. S3 Median-joining network (Bandelt *et al.* 1999) for cytochrome *b* constructed using NEWHYBRIDS v.4.6.1.1 (http://www.fluxus-engineering.com/network_terms.htm) for (a) *Abudefduf abdominalis* and (b) *A. vaigiensis*.

Table S1 Summary of loci screened but not used in this study.

Table S2 List of species and corresponding GenBank accession numbers for cytochrome *b* sequences used in *Abudefduf* phylogenetic analyses.

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