ISSN 0962-1083

VOLUME 19
NUMBER 6
MARCH
2010

MOLECULAR ECOLOGY

Published by
Wiley-Blackwell

MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 1107–1121 doi: 10.1111/j.1365-294X.2010.04535.x

Genetic consequences of introducing allopatric lineages of Bluestriped Snapper (*Lutjanus kasmira*) to Hawaii

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Abstract

A half century ago the State of Hawaii began a remarkable, if unintentional, experiment on the population genetics of introduced species, by releasing 2431 Bluestriped Snappers (*Lutjanus kasmira*) from the Marquesas Islands in 1958 and 728 conspecifics from the Society Islands in 1961. By 1992 *L. kasmira* had spread across the entire archipelago, including locations 2000 km from the release site. Genetic surveys of the source populations reveal diagnostic differences in the mtDNA control region (d = 3.8%; ϕ_{ST} = 0.734, P < 0.001) and significant allele frequency differences at nuclear DNA loci (F_{ST} = 0.49; P < 0.001). These findings, which indicate that source populations have been isolated for approximately half a million years, set the stage for a survey of the Hawaiian Archipelago (N = 385) to determine the success of these introductions in terms of genetic diversity and breeding behaviour. Both Marquesas and Society mtDNA lineages were detected at each survey site across the Hawaiian Archipelago, at about the same proportion or slightly less than the original 3.4:1 introduction ratio. Nuclear allele frequencies and parentage tests demonstrate that the two source populations are freely interbreeding. The introduction of 2431 Marquesan founders produced only a slight reduction in mtDNA diversity (17%), while the 728 Society founders produced a greater reduction in haplotype diversity (41%). We find no evidence of genetic bottlenecks between islands of the Hawaiian Archipelago, as expected under a stepping-stone model of colonization, from the initial introduction site. This species rapidly colonized across 2000 km without loss of genetic diversity, illustrating the consequences of introducing highly dispersive marine species.

Keywords: alien species, bottleneck, introductions, invasion biology, mtDNA, Marquesas, nuclear DNA, Papahānaumokuākea, Society Islands

Received 1 September 2009; revision received 8 November 2009; accepted 24 November 2009

Introduction

The Bluestriped Snapper, *Lutjanus kasmira* (Forsskål, 1775), is a widely distributed coral reef fish with a natural range from South Africa to the Marquesas and Line Islands in the central Pacific. This natural range does not include the Hawaiian Islands, which has only a subset of the Indo-Pacific flora and fauna, lacking many taxa such as most shallow water snappers and groupers (Randall 2007). In an effort to fill a perceived empty ecological niche, and to enhance local fisheries, the Hawaii Division of Fish and Game (HDFG) introduced *L. kasmira*, among other reef fishes, to the Hawaiian Islands (Oda & Parrish 1982; Randall 1987). The introduction of *L. kasmira* was conducted in two discrete events. In preparation for introduction, juvenile fish (approximately 100–120 g) were caught using hand lines and transferred to floating pens. When sufficient fish had been captured, the fish were transferred to the

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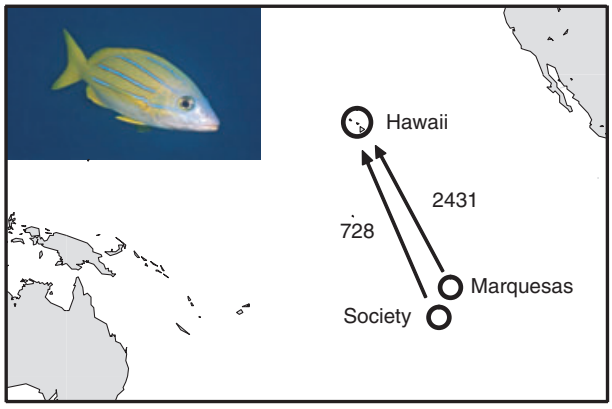

Fig. 1 Map of the Pacific Ocean. *Lutjanus kasmira* were introduced to the Hawaiian Island of Oahu from two source locations: Nuku Hiva in the Marquesas Islands and Moorea in the Society Islands. The number of fish introduced from each location is shown. (Photo credit: Keoki Stender.)

bait wells of the transport vessel and brought to Hawaii for transplant. In 1958, 2431 fish from Nuku Hiva in the Marquesas Islands, and, in 1961, 728 fish from Moorea in the Society Islands were released on Oahu (Fig. 1; 3.4: 1 ratio; HDFG records). *L. kasmira* quickly spread through the archipelago at a rate of about 60 km per year (Oda & Parrish, 1982; Randall 2007). In 1992, just 34 years after the initial introduction, *L. kasmira* was recorded at the far reaches of the archipelago at Midway Atoll (Randall *et al.* 1993) over 2000 km from the release site. This successful introduction provides a number of research opportunities relating to understanding founder/colonization processes.

The Marquesas and Society populations of *L. kasmira* are phylogenetically distinct with diagnostic differences in the mitochondrial genome (average sequence divergence for cytochrome $b = 0.53\%$; Gaither *et al.* 2010). The introduction of *L. kasmira* to the Hawaiian Islands from two genetically divergent populations, resulted in the sympatric distribution of lineages that have been separated for about a quater to a half a million years (Gaither *et al.* 2010). The genetic divergence between these two populations is a result of the phylogenetic distinction of the Marquesas population relative to other Indo-Pacific populations (Gaither *et al.* 2010). The Marquesas have the third highest level of endemism among shorefishes (11.6%) in Oceania (Randall 2001) and high levels of genetic differentiation in three of five species of non-endemic reef fishes examined to date (Planes & Fauvelot 2002; Craig *et al.* 2007; Schultz *et al.* 2007; Gaither *et al.* 2010). The distinction of the Marquesas shorefish fauna has been attributed to a combination of geographic isolation (enhanced by the westerly Southern Equatorial Current) and adaptation to unusually variable sea temperatures (Randall 2001; Gaither *et al.* 2010). Because geographic isolation and ecological divergence may both promote speciation in fishes (Rogers & Bernatchez 2006; Rocha & Bowen 2008), we ask whether *L. kasmira* populations separated for half a million years can freely interbreed in sympatry. The genetic distinctiveness of the two source populations provides an opportunity to identify the descendants of the Hawaiian introductions, to assess their relative success in the archipelago, and to determine if the two genetic lineages are mixing.

Colonization events, including human-mediated introductions, often involve a severe reduction in population size and isolation from the larger parental population. The dramatic decrease in effective population size that accompanies such founder events is expected to lead to decreased genetic diversity (Nei *et al.* 1975). However, the accumulation of data indicates that genetic bottlenecks in introduced populations are not an invariable outcome (Bossdorf *et al.* 2005; Wares *et al.* 2005; Roman & Darling 2007). Interpreting patterns of genetic diversity in introduced populations is confounded by the fact that in most cases the source population and the number of founding individuals are unknown. Under these conditions, researchers must reconstruct the history of introductions by combining molecular and geographic data to identify source populations (Wares *et al.* 2005). In some cases high genetic diversity in the introduced range can be attributed to admixture of genetically divergent populations (Kolbe *et al.* 2004; Genton *et al.* 2005; Carmeron *et al.* 2008; Rosenthal *et al.* 2008). In cases where only a single source population can be identified, high genetic diversity in the introduced range is often attributed to either a large number of colonizers, rapid population expansion following the founder event, or both (Hassan *et al.* 2003; Stepien *et al.* 2005). Discussions concerning the effect of founder events on genetic diversity would be greatly informed if more empirical data concerning the effects of founder population size on genetic diversity were available. Intentional and well documented introductions, where the source population and founder population size are confidently known, offer powerful test cases.

The intentional introduction of *L. kasmira* to the Hawaiian Islands provides a rare opportunity to directly evaluate the effects of founder population size on genetic diversity in recently established populations. Here we capitalize on two unique aspects of the introduction of *L. kasmira* to Hawaii: (i) the introduction occurred in two well documented events with known numbers of founders and source populations; (ii) the source populations (Nuka Hiva in the Marquesas Islands and Moorea in the Society Islands) are geneti-

cally distinct, allowing us to identify their descendents. We employ both mitochondrial and nuclear sequence data to ask the following questions (i) Did fish from both source populations become established in the Hawaiian Islands? If so, (ii) how are their descendents distributed in the archipelago? (iii) Were fish from both source populations equally successful at reproducing and colonizing the islands? (iv) Are these genetically divergent populations interbreeding in the Hawaiian Islands? (v) Is there evidence of genetic bottlenecks at the introduction site or as the fish spread throughout the archipelago? The circumstances of this study offer unprecedented opportunities to study species introductions and invasions, pertinent to management of marine resources including the Papahānaumokuākea Marine National Monument (PMNM) that traverses 2000 km of the north-western (NW) Hawaiian Islands. At least 350 alien marine species occupy the inhabited Main Hawaiian Islands (Eldredge & Smith 2001) and few studies have addressed the threat these aliens pose to the uninhabited (and nearly pristine) ecosystems of the NW Hawaiian Islands. Hence an ongoing concern is the level of connectivity between the Main Hawaiian Islands and the NW Hawaiian Islands (see Eble *et al.* 2009). Here we document an extreme scenario of rapid colonization into the NW Hawaiian Islands, in numbers that are sufficient to retain the genetic diversity of parent populations.

Materials and methods

Study species

Lutjanus kasmira has broad habitat preferences, occupying hard substrata from shallow waters to at least 265 m (Randall 1987) and has a generalized predatory diet that includes fish, crustaceans, and cephalopods (Randall & Brock 1960; Oda & Parrish 1982; Schumacher & Parrish 2005). This species reaches sexual maturity at 1–2 years (Rangarajan 1971; Morales-Nin & Ralston 1990) and engages in mass spawning (Suzuki & Hioki 1979). Long-distance movement between isolated patches of adult habitat (reefs) occurs during a highly dispersive pelagic larval phase that, in other species of *Lutjanus*, lasts 20–44 days (Zapata & Herron 2002; Denit & Sponaugle 2004).

Collections

A total of 385 specimens of *Lutjanus kasmira* were collected from 10 locations across the Hawaiian Archipelago by scuba divers using polespears (Table 1, Fig. 2). Specimens from the uninhabited NW Hawaiian Islands were obtained during research expeditions on the NOAA R/V Hi'ialakai, as part of an initiative by the Papahānaumokuākea Marine National Monument (http://hawaiireef.noaa.gov/) to monitor and characterize this vast protected area. Tissue samples (fin clips or gill filaments) were preserved in either 95% ethanol (EtOH) or saturated NaCl solution (Seutin *et al.* 1991), and stored at room temperature. Fifty *L. kasmira* samples from each of the Marquesas and Society source populations, previously analysed in Gaither *et al.* (2010), were also used in this study.

DNA extraction, PCR amplifications, and sequencing

All DNA extraction, PCR cycling, cloning, and sequencing protocols used here are identical to those in Gaither *et al.* (2010). The growth hormone (GH) and adenine nucleotide transporter translocase (ANT) intron sequences obtained from each of the Marquesas and Society populations in Gaither *et al.* (2010) were used in this study [GenBank accession numbers FJ754178–FJ754184 (GH intron), FJ754157–FJ754177 (ANT intron)]. All 385 specimens of *L. kasmira* collected from the Hawaiian Islands were sequenced at these two loci. Additionally, approximately 215 bp of the third intron in the gonadotropin-releasing hormone 3 (GnRH3-3) were amplified using the primers GnRH3F (5′-GCCCAAACCCAAGAGAGACTTAGACC-3′) and GnRH3R (5′-TTCGGTCAAAATGACTGGAATCATC-3′) (Hassan *et al.* 2002) and approximately 575 bp of the mitochondrial control region were amplified using the primers Lutjf1 (5′-GCACTCTGAAATGTCAAGTGAAAGG-3′) and CRA (5′-TTCCACCTCTAACTCCCAAAGCTAG-3′) (Lee *et al.* 1995) in all 484 samples (Hawaii = 385, Marquesas = 50, and Society = 49). PCR protocols and cycling conditions for both the GnRH3-3 intron and the mtDNA control region were carried out as described in Gaither *et al.* (2010) using an annealing temperature of 60 °C. Due to the presence of multiple indels at the GnRH3-3 locus, that would require extensive cloning to phase alleles, analysis of this locus was restricted to the presence or absence of a 10 bp indel near the reverse priming site. The presence of the indel was confirmed by cloning ten individuals and comparing alleles to direct sequences. The allelic state of the remaining individuals was inferred by direct sequencing.

Sequences for each locus were aligned and edited using SEQUENCHER 4.8 (Gene Codes, Ann Arbor, MI, USA) and trimmed to a common length. The mtDNA control region contained multiple indels which varied from 1 to 3 bp in length. Alignment of the mtDNA sequences was confirmed using default parameters in CLUSTAL W 1.81 (Thompson *et al.* 1994). Unique mtDNA haplotypes and nuclear alleles were identified with the merge taxa option in MACCLADE 4.05 (Maddison &

Table 1 Molecular diversity indices for the mitochondrial control region sequences for the two source populations of *Lutjanus kasmira* and ten populations across the introduced range. Number of specimens (N), number of haplotypes (N_h), number of singletons (N_s), haplotype diversity (h), and nucleotide diversity (π) as reported by ARLEQUIN 3.11 are listed. Numbers in parenthesis are standard deviations. Values for the entire data set (All Data) and for each mitochondrial lineage (Marquesas Lineage and Society Lineage) are listed. See Fig. 2 for locations of Hawaiian Islands (FFS = French Frigate Shoals)

	All data					Marquesas lineage					Society lineage				
	N	N_h	N_s	h	π	N	N_h	N_s	h	π	N	N_h	N_s	h	π
Source populations															
Marquesas	50	47	45	0.997 (0.005)	0.019 (0.010)	50	47	45	0.997 (0.005)	0.019 (0.010)	—	—	—	—	—
Society	49	31	23	0.970 (0.012)	0.017 (0.009)	—	—	—	—	—	49	31	23	0.970 (0.012)	0.017 (0.009)
Introduced range															
Oahu	50	40	30	0.992 (0.006)	0.033 (0.017)	40	33	26	0.991 (0.008)	0.023 (0.012)	10	7	4	0.933 (0.062)	0.018 (0.010)
Kona	50	41	35	0.989 (0.007)	0.038 (0.019)	28	26	25	0.992 (0.013)	0.019 (0.010)	22	15	10	0.957 (0.028)	0.018 (0.009)
Hilo	51	38	30	0.985 (0.008)	0.037 (0.018)	33	28	24	0.989 (0.011)	0.020 (0.010)	18	10	6	0.915 (0.041)	0.019 (0.010)
Maui Nui	39	32	28	0.985 (0.011)	0.034 (0.017)	29	24	21	0.980 (0.017)	0.018 (0.010)	10	8	7	0.933 (0.077)	0.019 (0.011)
Kauai	36	30	25	0.989 (0.010)	0.035 (0.018)	25	23	21	0.993 (0.013)	0.020 (0.011)	11	7	4	0.909 (0.066)	0.016 (0.009)
Necker	49	38	31	0.986 (0.008)	0.035 (0.018)	34	29	25	0.989 (0.010)	0.018 (0.010)	15	9	6	0.905 (0.054)	0.016 (0.009)
Maro	21	18	16	0.981 (0.023)	0.036 (0.018)	15	15	15	1.000 (0.024)	0.019 (0.011)	6	3	1	0.733 (0.155)	0.016 (0.010)
FFS	40	38	36	0.997 (0.006)	0.033 (0.017)	31	30	29	0.998 (0.009)	0.021 (0.011)	9	8	7	0.972 (0.064)	0.013 (0.008)
Midway	40	33	28	0.989 (0.009)	0.046 (0.023)	28	26	24	0.995 (0.011)	0.029 (0.015)	12	7	4	0.894 (0.063)	0.034 (0.018)
Kure	9	9	9	1.000 (0.052)	0.034 (0.019)	7	7	7	1.000 (0.076)	0.023 (0.013)	2	2	2	1.000 (0.500)	0.008 (0.009)
All Hawaii specimens	385	172	92	0.990 (0.001)	0.037 (0.018)	270	142	80	0.993 (0.001)	0.021 (0.011)	115	30	12	0.930 (0.011)	0.019 (0.010)
All specimens	484	218	123	0.991 (0.001)	0.037 (0.018)	320	170	99	0.993 (0.001)	0.021 (0.010)	164	48	24	0.946 (0.007)	0.018 (0.009)

Maddison 2002). All control region haplotypes and nuclear alleles unique to Hawaii were deposited in GenBank [accession numbers: GU123931–GU124148 (control region), GU192444–GU192447 ANT intron)]

Data analysis

Mitochondrial control region. Summary statistics including mtDNA haplotype diversity (h), and nucleotide diversity (π) were estimated using algorithms in Nei (1987) as implemented in ARLEQUIN 3.11 (Excoffier *et al.* 2005). A statistical parsimony network was constructed using the program TCS 1.21 (Clement *et al.* 2000). The resulting network was simplified using standard tie-breaking rules. In keeping with the cytochrome *b* data in Gaither *et al.* (2010) the control region sequences in the Marquesas and Society samples fell into two distinct lineages. Average percent difference between populations was calculated by dividing the average number of nucleotides (corrected; Tamura & Nei 1993) that differ between the two source populations (as calculated in ARLEQUIN) by the total number of base pairs. The average percent difference between populations is reported here as sequence divergence (d).

The number of individuals from the Hawaiian Islands that grouped with either the Marquesas or the Society mtDNA lineage was calculated, and deviations from the initial introduction ratio of 3.4 Marquesas:1.0 Society were tested using Fisher's exact test (Sokal & Rohlf 1995). To test for the loss of genetic diversity in the introduced range, while controlling for unequal sample sizes (Leberg 2002), we estimated haplotype richness using rarefaction analysis. For this method we determined the haplotype frequency distribution for the

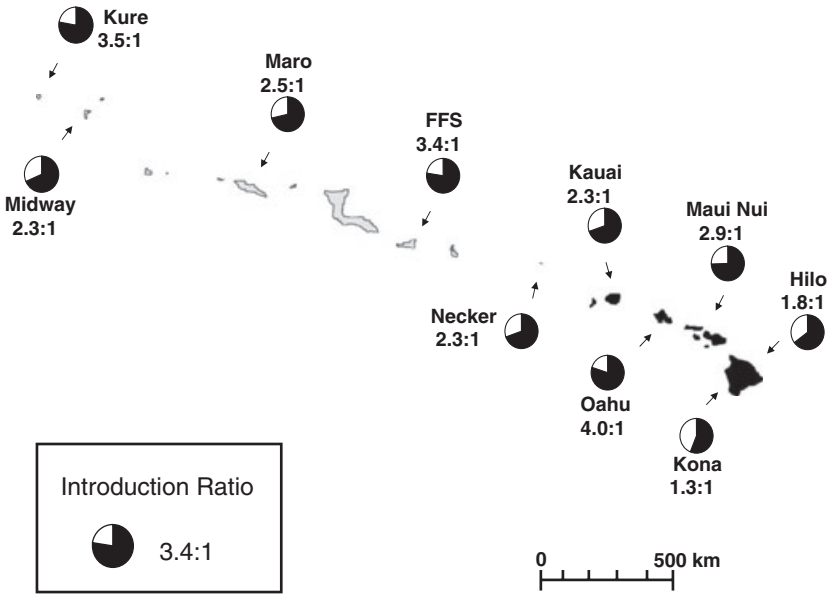

Fig. 2 Map of the Hawaiian archipelago. Pie chart in bottom left corner depicts the 3.4:1 introduction ratio of Marquesas fish (black) to Society fish (white). Pie charts for each sample location in Hawaii show the ratio of *Lutjanus kasmira* in either the Marquesas or Society lineage (see Fig. 3). Hilo and Kona are locations on opposite sides of Hawaii Island. The figure demonstrates that fish from both source populations are found at each sample location and are in roughly the same ratio as the original introduction ratio of 3.4:1. Abbreviation: FFS = French Frigate Shoals.

largest sample in the comparison. From this larger sample we randomly subsampled haplotypes (size of subsample = N of smaller sample) with replacement 10 000 times to estimate the number of haplotypes that would occur in the smaller sample. We compared the distribution of the subsamples with the number of haplotypes found in the smaller sample. *P*-values were calculated based on the number of times in 10 000 subsamples that as many or more haplotypes were found in the larger sample as found in the smallest. Rarefaction curves plotting the number of individuals sampled against the expected number of mitochondrial haplotypes were constructed using ANALYTIC RAREFACTATION 1.4 (UGA Stratigraphy Lab website; http://www.uga.edu/~strata/software/).

The Akaike Information Criterion in MODELTEST 3.7 (Posada & Crandall 1998) was used to determine the mutational model that best fit the control region data. The best fit model is TVM+I+G with equal rates for all sites and a Ti/Tv ratio of 10.13. Because this model is not implemented in ARLEQUIN (Excoffier *et al.* 2005), the most similar model available (Tamura & Nei 1993) was employed using a gamma value of 0.77, a transversion weighting of 10.13 and a transition and deletion weight of 1.0. To test for population genetic structure in Hawaii an *L. kasmira*, an analysis of molecular variance (AMOVA) was performed in ARLEQUIN using 20 000 permutations. An analogue of Wright's F_{ST} (ϕ_{ST}), which incorporates a model of sequence evolution, was calculated for the entire data set and for pairwise comparisons among all locations. We maintained $\alpha = 0.05$ among all pairwise tests by controlling for the false discovery rate as recommended by Benjamini & Yekutieli (2001) and reviewed by Narum (2006).

Nuclear introns. Observed (H_O) and expected (H_E) heterozygosities were calculated for each locus and an exact test of Hardy-Weinberg equilibrium (HWE) using 100 000 steps in a Markov chain was performed using ARLEQUIN. Additionally, average H_E was calculated for the multi-locus data set. Linkage disequilibrium between the three nuclear loci was assessed using the likelihood ratio test with 20 000 permutations in ARLEQUIN. F_{ST} was calculated for the entire data set and for pairwise comparisons between locations. The false discovery rate among multiple comparisons was controlled as described above.

Tests for loss of genetic diversity in the introduced range were conducted with BOTTLENECK 1.2.02 (Piry *et al.* 1999) using the infinite alleles mutation (IAM) model (Kimura & Crow 1964). The loss of rare alleles was evaluated using the mode-shift test (Piry *et al.* 1999). The Wilcoxon signed rank test, which assumes that populations in mutation-drift equilibrium have an equal probability of heterozygote excess or deficit, was used to detect genetic bottlenecks (Cornuet & Luikart 1996).

Interbreeding. To determine if *L. kasmira* Hawaiian descendents from the Marquesas and Society Islands are interbreeding we employed the genealogical-frequency and individual-assignment methods of Nason *et al.* (2002). This method assigns individuals to one of six genealogical categories using multi-locus diploid data. Individuals are classified as either pure parental (P_1 and P_2), crosses between pure parentals (F_1), crosses between F_1 individuals (F_2), or backcrosses (BP_1 and BP_2). The program uses maximum-likelihood estimates to assign each individual to one of the six genealogical classes while providing estimates of statistical power

for correct classification. For comparison, the Bayesian statistical model developed by Anderson & Thompson (2000), which computes the posterior probability that an individual belongs to each of the hybrid classes (P_1, P_2, F_1, F_2, BP_1, and BP_2) was employed using the program default settings. A third method of testing for interbreeding utilized the chi-square (χ^2) goodness of fit (Sokal & Rohlf 1995) to test whether the nuclear alleles at each locus were randomly distributed among the two mitochondrial lineages in the introduced range.

Results

Distribution of descendents of the two source populations

Mitochondrial control region. We resolved a 521 bp segment of the mtDNA control region in 484 individuals yielding 218 haplotypes, with a few common haplotypes, 123 haplotypes observed in single individuals, and 41 haplotypes observed in two individuals. The number of specimens (N), the number of haplotypes (N_h), the number of haplotypes observed in single individuals (N_s), h, and π per location are listed in Table 1. There were no shared haplotypes between the two source populations (Marquesas and Society Islands). A statistical parsimony network demonstrated that samples from the two source populations fell into distinct lineages separated by 22 steps (Fig. 3; d = 3.8% between source populations). Haplotypes observed in one or two specimens (singletons and doublets) were found at every sample site and exclusion of these haplotypes from the parsimony network did not change the overall structure (Fig. 3). Haplotypes observed in the introduced range (Oahu, Kona, Hilo, Maui Nui, Kauai, Necker, French Frigate Shoals (FFS), Maro, Midway, and Kure) are grouped with either the Marquesas or Society lineages (Fig. 3).

The overall ratio of the number of *L. kasmira* samples from the Hawaiian Islands that fell into the Marquesas lineage to those that fell into the Society lineage was 2.3:1. This value was significantly different than the introduction ratio of 3.4:1 (Fisher's exact text, P = 0.027). Among the ten sample locations scattered across the Hawaiian Archipelago only Kona on Hawaii Island, differed significantly from the 3.4:1 introduction ratio, with a ratio of 1.3:1 (Fisher's exact text, P = 0.036) (Fig. 2). Once these specimens were removed from the analysis the overall ratio (2.6:1) was not significantly different than the introduction ratio (Fisher's exact text, P = 0.131).

Nuclear introns. We resolved 148 bp of the GH intron in 482 specimens and 168 bp of the ANT intron in 471 specimens (Table 2). Three polymorphic sites yielded four alleles at the GH locus and 13 polymorphic sites yielded 15 alleles at the ANT locus. The GnRH3-3 intron was scored for the presence or absence of a 10 bp indel in 480 specimens. Summary statistics are listed in Table 2.

When all locations, from the native and introduced ranges, were grouped together there was a significant

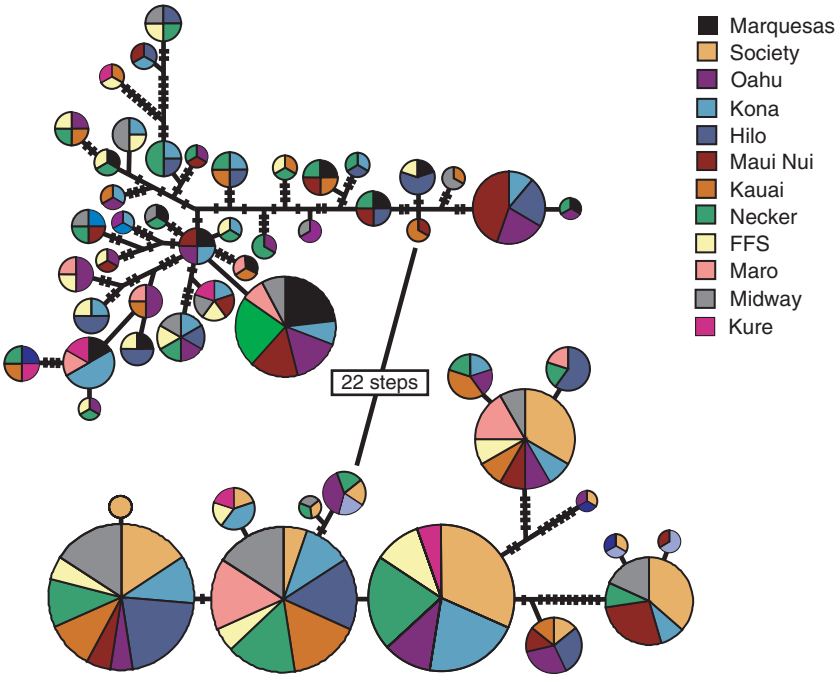

Fig. 3 Statistical parsimony network for 484 control region sequences of *Lutjanus kasmira* constructed using TCS 2.21 (Clement *et al.* 2000). Each circle represents one mitochondrial haplotype with the area of each circle is proportional to number of that particular haplotype in the data set; dashes represent hypothetical haplotypes; colours represent collection location (see key). There were no shared haplotypes between the two source populations (Marquesas and Society) which formed two distinct lineages that are separated by 22 steps (average percent sequence divergence = 3.8% between source populations). For clarification, singletons and doublets (164 haplotypes) were omitted. Singletons and doublets were found at every sample site and inclusion of these haplotypes did not change the pattern of the parsimony network. Abbreviation: FFS = French Frigate Shoals.

Table 2 Number of specimens (N), number of alleles (N_a), heterozygosity observed (H_O), heterozygosity expected (H_E), and the corresponding P-value for an exact test of Hardy–Weinberg equilibrium (HWE) are listed for each nuclear intron. N_a and average H_E are listed for the multi-locus data set (FFS = French Frigate Shoals)

	GH intron					ANT intron					GnRH3-3 intron					Multi-locus	
	N	N_a	H_O	H_E	P	N	N_a	H_O	H_E	P	N	N_a	H_O	H_E	P	N_a	H_E
Source populations																	
Marquesas	49	3	0.66	0.59	0.57	49	6	0.29	0.37	0.05	50	2	0.16	0.18	0.39	17	0.38
Society	50	3	0.10	0.10	1.00	47	5	0.31	0.38	0.03	50	2	0.37	0.49	0.14	16	0.32
Introduced range																	
Oahu	49	4	0.59	0.58	0.73	49	8	0.59	0.57	0.95	50	2	0.56	0.48	0.37	23	0.54
Kona	50	4	0.74	0.60	0.14	50	9	0.64	0.64	0.39	50	2	0.54	0.47	0.37	23	0.57
Hilo	52	3	0.50	0.61	0.09	47	4	0.62	0.56	0.94	51	2	0.33	0.43	0.11	24	0.54
Maui Nui	39	3	0.56	0.61	0.48	39	6	0.62	0.59	0.22	39	2	0.54	0.50	0.75	19	0.57
Kauai	35	3	0.60	0.58	0.57	36	5	0.57	0.51	1.00	36	2	0.39	0.43	0.69	19	0.50
Necker	50	3	0.60	0.59	0.75	48	7	0.58	0.59	0.31	45	2	0.49	0.49	1.00	28	0.56
Maro	20	3	0.60	0.61	0.90	20	5	0.35	0.36	0.35	21	2	0.52	0.49	1.00	14	0.49
FFS	40	3	0.50	0.56	0.43	38	6	0.61	0.60	0.98	39	2	0.51	0.50	1.00	22	0.55
Midway	39	3	0.62	0.58	0.57	40	7	0.75	0.69	0.72	40	2	0.50	0.49	1.00	22	0.58
Kure	9	3	0.22	0.54	0.03	9	3	0.89	0.66	0.12	9	2	0.33	0.50	0.49	10	0.57
All Hawaii specimens	383	4	0.58	0.59	0.68	375	12	0.61	0.59	0.85	380	2	0.48	0.48	0.83	59	0.53
All specimens	482	4	0.54	0.58	0.02	471	15	0.55	0.61	0.02	480	2	0.44	0.46	0.23	65	0.53

deviation from HWE (Hardy–Weinberg equilibrium) expectations at the GH and ANT loci ($P = 0.02$ for each) (Table 2). In each case an excess of homozygotes was detected. When samples were divided by archipelago (Marquesas, Society, and Hawaiian Islands) the Marquesas and Society populations deviated from HWE expectations, with an excess of homozygotes, at the ANT locus ($P = 0.044$ and $P = 0.032$ respectively). No evidence of linkage disequilibrium between pairs of nuclear loci was detected ($P > 0.05$) within populations from each of the three archipelagos.

The number of nuclear alleles at each locus was similar for the two source populations (Tables 2, S1) however; there were strong shifts in allele frequencies between the Marquesas and Society Islands (Table S1). Populations in the introduced range had allele frequencies intermediate between the two source populations (Table S1). Three putative private alleles are found in each source population (Table S1; Marquesas = GH3, A1, A11; Society = GH4, A4, A5). The presence of these alleles at widely separated locations in the introduced range (Table S1) provides additional evidence that descendents of both source populations spread throughout the archipelago. As expected for introduced populations of mixed lineages, many of the Hawaiian samples had a greater number of nuclear alleles and higher heterozygosities (H_O and H_E at ANT and GnRH3-3 loci) than either of the source populations (Table 2).

Population structure. Pairwise comparisons indicate significant population structure between the two source populations (Marquesas and Society Islands) with mtDNA $\phi_{ST} = 0.734$ ($P < 0.001$) and nDNA $F_{ST} = 0.49$ ($P < 0.001$) (Table 3). The Marquesas and Society populations were also significantly different than each of the ten Hawaiian populations (Oahu, Kona, Hilo, Maui Nui, Kauai, Necker, FFS, Maro, Midway, and Kure) (Table 2). In the introduced range, there was no population structure detected in the mtDNA (overall $\phi_{ST} = 0.001$, $P = 0.38$) or nDNA ($F_{ST} = 0.001$, $P = 0.30$) data sets (Table 3).

Interbreeding of the two populations in the Hawaiian Islands. The likelihood model of Nason *et al.* (2002) indicated that approximately 31% of the individuals from the Hawaiian Islands were F_1 X F_1 crosses (F_2 genealogical class) while the remainder (~69%) were F_2 X P_1 backcrosses (BP_1 genealogical class). The program did not assign any individual from the introduced range to either pure parental class (P_1 or P_2) or to the P_1 X P_2 cross (F_1 geneological class). This indicates that all assayed specimens of *L. kasmira* in the Hawaiian Islands are of mixed Marquesas and Society descent. The Anderson & Thompson (2002) model indicated similar results to the Nason model (data not shown).

The chi-square test corroborated the findings of the Nason *et al.* (2002) model, demonstrating that nuclear allele frequencies at the GH and ANT loci were not significantly different than expected if the alleles were

Table 3 Pairwise *F*-statistics for the two source populations of *Lutjanus kasmira* and ten populations across the introduced range. Pairwise ϕ_{ST} values for control region data are below diagonal and pairwise F_{ST} values for the multi-locus nuclear data set are above diagonal. We maintained an alpha value of 0.05 among all pairwise tests by controlling for the false discovery rate as recommended by Benjamini & Yekutieli (2001) and reviewed by Narum (2006)

	Source Populations						Introduced Range					
Sample Location	Marquesas	Society	Oahu	Kona	Hilo	Maui Nui	Kauai	Necker	FFS	Maro	Midway	Kure
Marquesas	—	**0.490**	**0.089**	**0.089**	**0.067**	**0.102**	**0.039**	**0.104**	**0.144**	**0.060**	**0.114**	**0.163**
Society	**0.734**	—	**0.295**	**0.248**	**0.293**	**0.303**	**0.356**	**0.266**	**0.233**	**0.434**	**0.250**	**0.184**
Oahu	**0.081**	**0.525**	—	−0.005	−0.003	−0.003	0.001	−0.004	0.002	0.009	−0.002	0.007
Kona	**0.224**	**0.339**	0.042	—	−0.009	−0.007	0.002	−0.009	−0.004	0.020	−0.007	−0.014
Hilo	**0.187**	**0.395**	0.021	0.003	—	−0.002	−0.005	−0.002	0.007	0.016	−0.004	−0.000
Maui Nui	**0.107**	**0.503**	−0.006	0.022	0.008	—	0.008	0.001	0.001	0.007	−0.006	0.010
Kauai	**0.149**	**0.466**	0.005	0.011	−0.011	0.000	—	0.005	0.025	−0.001	0.011	0.025
Necker	**0.130**	**0.455**	−0.003	0.005	0.002	−0.007	−0.006	—	0.000	0.018	−0.006	−0.014
FFS	**0.083**	**0.527**	−0.011	0.033	0.019	−0.004	0.003	−0.011	—	**0.047**	−0.008	−0.020
Maro	**0.117**	**0.532**	−0.006	0.013	0.009	−0.004	0.003	−0.019	−0.025	—	0.028	0.066
Midway	**0.125**	**0.401**	0.004	0.005	−0.005	−0.007	−0.005	−0.007	0.003	−0.006	—	−0.014
Kure	**0.119**	**0.575**	−0.037	−0.004	−0.032	−0.036	−0.054	−0.037	−0.042	−0.041	−0.041	—

Values in bold are significant at the corrected $\alpha = 0.010$. FFS = French Frigate Shoals.

randomly distributed between the Marquesas ($\chi^2 = 0.227$, $P = 0.99$; $\chi^2 = 0.157$, $P = 0.99$ respectively) and Society ($\chi^2 = 0.256$, $P = 0.96$; $\chi^2 = 0.803$, $P = 0.79$) mitochondrial lineages in Hawaii. The Society mitochondrial lineage in Hawaii deviated significantly from a random distribution of alleles at the GnRH3-3 locus ($\chi^2 = 4.718$, $P = 0.03$) while the Marquesas mitochondrial lineage did not ($\chi^2 = 1.729$, $P = 0.19$).

Genetic consequence of founder event. The Marquesas and Society populations had high mtDNA haplotype diversity ($h = 0.997$ and 0.970 respectively). All 10 populations in the introduced range (Oahu, Kona, Hilo, Maui Nui, Kauai, Necker, FFS, Maro, Midway, and Kure) had similarly high h values ($h = 0.985$–1.000; 'All Data' Table 1). Using the parsimony network in Fig. 3 we divided the Hawaiian samples into either the Marquesas or Society mitochondrial lineage (Table 1). The mtDNA haplotype diversity values in the Hawaiian Islands ranged from 0.989 to 1.00 for the Marquesas lineage and from 0.733 to 1.00 for the Society lineage.

Due to the lower sensitivity of heterozygosity to losses of genetic diversity (Nei *et al*. 1975) we restricted our statistical comparisons of diversity loss to haplotype richness which we compare at the archipelago level. We observed 47 haplotypes in the Marquesas ($N = 50$) and 31 haplotypes in the Society Islands ($N = 49$) (Table 4). By creating haplotype frequency distributions for the larger Hawaiian sample sets and by randomly subsampling (10 000 times with replacement) these populations we found evidence of a small but significant decrease (17%) in haplotypes from the Marquesas lineage in Hawaii which had a mean of 39.0 haplotypes per subsample ($N = 50$, P-value <0.001). A greater decrease (41%) was detected in the Society lineage in Hawaii with a mean of 18.3 haplotypes ($N = 49$, P-value <0.001). The difference in loss of haplotypes (17% vs. 41%) was marginally significant (Fisher's exact test, $P = 0.058$) (Table 4).

Table 4 Results of rarefaction analyses. Number of specimens (N), number of haplotypes (N_h), and mean number of haplotypes (H) (±standard deviation) estimated from 10 000 random subsamples (N = number of individuals sampled in the source population) of the Hawaiian lineages are listed. The % lost is the reduction in haplotypes when comparing the source population to the corresponding Hawaiian lineage. *P*-values reflect the number of times in 10 000 subsamples that as many (or more) haplotypes, that were found in the source population, were also found in the Hawaiian lineage. The difference in loss of haplotypes (17% vs. 41%) was marginally significant (Fisher's exact test, $P = 0.058$)

Population	N	N_h	H	% lost	*P*-value
Marquesas					
Source	50	47			
Hawaiian lineage	270	142	39.0 ± 2.50	17.0%	<0.001
Society					
Source	49	31			
Hawaiian lineage	115	30	18.3 ± 2.06	41.0%	<0.001

Rarefaction curves, that plotted the number of individuals sampled against the expected number of mitochondrial haplotypes, were constructed (Fig. 4).

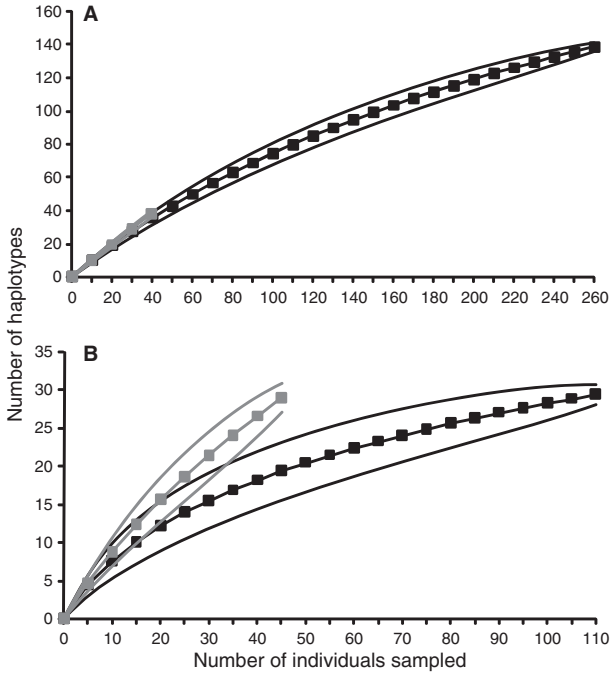

Fig. 4 Rarefaction curves plotting the number of individuals sampled against the expected number of mitochondrial haplotypes were calculated using the Analytic Rarefactation1.4 software available at the UGA Stratigraphy Lab website (http://www.uga.edu/~strata/software/). Samples belonging to the Marquesas (A) and Society (B) lineages are plotted separately. Grey lines represent data for source populations, black lines represent data from the introduced range, and solid lines are 95% confidence intervals. The Society lineage in the introduced range is significantly different than the source population indicating a loss of rare haplotypes.

Samples from the introduced range were separated by mitochondrial lineage and compared with their respective source population (Fig. 4). Due to the large confidence intervals (95%) there was no significant difference between expected number of mtDNA haplotypes in the native and introduced ranges at low sample sizes. However, as sample size increased ($N > 40$) the curves no longer over-lapped and a loss of mtDNA haplotypes in the introduced range became evident in the Society, but not the Marquesas lineage (Fig. 4).

Using the nuclear allele frequency data (Table S1) there was no evidence of a genetic bottleneck in any of the introduced populations using the Wilcoxon signed rank test or the mode shift test implemented in BOTTLENECK. However, it should be noted that three loci do not provide high levels of power for these analyses and due to the presence of shared alleles between the source populations and admixture of lineages in the introduced range we did not attempt to statistically compare allelic richness values using the nuclear data set.

Discussion

Establishment and spread of L. kasmira *throughout the Hawaiian Islands*

The introduction of *L. kasmira* to the Hawaiian Islands from two populations with diagnostic differences in the mitochondrial genome (Fig. 3) and several private nuclear alleles (Table S1) enables us to trace the fate of their descendents in the introduced range. Individuals from both source populations became established in the Hawaiian Islands and both mtDNA lineages are found on every island and atoll of the archipelago (Table S1, Figs 2 and 3). The mtDNA data (Fig. 2) indicate that the overall ratio of the two lineages across the Hawaiian Islands is less than the 3.4:1 introduction ratio. Although one Hawaii Island population had a ratio significantly less than 3.4:1, the overall ratio among the remaining populations (2.6:1) was not significant, albeit with a tendency in the same direction.

Hawaii Division of Fish and Game (HDFG) records show that the initial 2431 Marquesas fish began to reproduce and spread very soon after the initial release. When the 728 Society fish were introduced, 3 years after the Marquesas fish, the former had already spread to Hawaii Island about 500 km to the southeast of Oahu. This, in combination with the fact that maturity occurs at 1–2 years of age, indicates that the ratio at the time of the introduction of Society fish likely was greater than 3.4:1. After the 3 years head-start and rapid spread of the Marquesas lineage, it might be predicted that its descendents now would be proportionately more numerous and widespread than those of the Society lineage. This is not the case, however. Instead the data indicate that numerically the Society lineage was able to 'catch up' with, and even surpass, the Marquesas lineage, most notably on Hawaii Island. Given the estimated time of divergence between these two source populations (approximately half a million years) and the differences in their native environments (Gaither *et al*. 2010) it is possible that population specific adaptations endowed Society-lineage fish with a higher fitness in the Hawaiian environment. However, such an advantage would be quickly lost by interbreeding. Another likely advantage the Society lineage had over the Marquesas lineage at the time of introduction was the presence of an established population when the former were released. For the Society lineage this could have alleviated many of the adverse consequences associated with small population size, such as difficulty in finding suitable mates.

The genetic data indicate that there is now a single population of *L. kasmira* in the Hawaiian Islands. We found no population structure in the mitochondrial data

set across the archipelago, and only one of 45 pairwise comparisons of the nuclear data set was significant after control for false discovery rate. The lack of genetic structure coupled with the maintenance of genetic diversity across the archipelago implies that there was little or no loss of genetic lineages, as would be expected under a stepping stone model of colonization, as the fish spread through the islands. Instead our data indicate that either *L. kasmira* colonized each island in large enough numbers to capture most of the standing genetic diversity, or gene flow between the islands is sufficient to homogenize the geographic distribution of the genetic diversity, or both.

The success of *L. kasmira* in Hawaii, as indicated by HDFG catch records and corroborated here by our genetic data, is especially notable because most other introductions of reef fishes to the Hawaiian Islands have failed. In the 1950s the Hawaiian Division of Fish and Game introduced 11 non-native snappers and groupers (Oda & Parrish 1982; Randall 1987). Six of the eleven species were introduced in numbers greater than 1500 individuals (HDFG records) but 50 years later only three are regularly recorded in Hawaiian waters. Besides *Lutjanus kasmira*, these include the Blacktail Snapper (*Lutjanus fulvus*) and the Peacock Grouper (*Cephalopholus argus*). Notably, neither *L. fulvus* nor *C. argus* has colonized north of French Frigate Shoals (FFS; Fig. 2). While *L. fulvus* is not a common fish in the lower Hawaiian Islands, *C. argus* is more common there than in its natural range (Meyer 2008).

In the field of invasion biology, an intense debate revolves around the factors that promote successful colonization of new habitat (Kolar & Lodge 2001; Allendorf & Lundquist 2003), particularly the genetic factors (Frankham 2005; Golani *et al.* 2007; Zayed *et al.* 2007). Two of the primary factors that are thought to contribute to invasion success are large founder populations and multiple introduction events (Lockwood *et al.* 2005; Colautti *et al.* 2006). Certainly these conditions apply to the introduction of *L. kasmira* in Hawaii, and it is possible that introductions of two genetically distinct populations have yielded a more robust fish than either parental stock (hybrid vigour; Allendorf & Luikart 2007). Other traits that might apply specifically to the invasion success of *L. kasmira* include mass spawning (Suzuki & Hioki 1979), broad habitat preference (2–265 m depth; Randall 1987), and a generalist diet (Randall & Brock 1960; Oda & Parrish 1982). Range-wide mtDNA surveys also indicate that this species has a much more dispersive larval stage than either *Lutjanus fulvus* or *Cephalopholus argus* (Gaither *et al.* 2010; unpublished data) which may explain why *L. kasmira* has swiftly colonized the entire archipelago, while the other two species have not.

Interbreeding and outbreeding

The Marquesas and Society source populations of *L. kasmira* demonstrate an average mitochondrial control region sequence divergence of $d = 3.8\%$. The control region appears to evolve at 3–10% per million years in shore fishes (Bowen *et al.* 2006; Lessios *et al.* 2008). Using this range as a first order approximation we estimate that these two populations have been separated for about half a million years (380 000–1 300 000 years), a value that overlaps the estimate from cytochrome *b* data from the same samples (265 000–530 000 years; Gaither *et al.* 2010). This time interval is sufficient to produce gamete incompatibility in allopatric populations of sea urchins (Lessios 1984; Palumbi & Metz 1991). However, 3–4 Myr is insufficient to prevent gamete compatibility in geminate species of gobies (Rubinoff & Rubinoff 1971) or to prevent hybridization in trumpetfish species (Bowen *et al.* 2001). Populations which diverge sufficiently in allopatry might resume mating upon secondary contact but resulting offspring could have lower fitness than purebred offspring. Reinforcement theory predicts that due to lower fitness of hybrids, natural selection will favour the evolution of prezygotic isolating mechanisms to maximize fitness, and therefore drive further diversification (Coyne & Orr 2004). We see no evidence for reinforcement in the hybridization tests we performed here. Notably, our study was conducted approximately thirteen generations after the initial introduction (see Materials and methods for references). If reproductive barriers existed or preferential mating occurred during initial contact of these two lineages, the genetic signature has been lost. Furthermore, the mass spawning behaviour of this species (Suzuki & Hioki 1979) may have reduced the potential for assortative mating by eliminating active mate choice.

Genetic consequences of founding events

Contrary to expectations, alien species often retain high levels of genetic diversity in their introduced range (Bossdorf *et al.* 2005; Wares *et al.* 2005; Roman & Darling 2007). In cases where individuals from genetically divergent source populations are introduced to the same region (admixture), there may actually be an increase in genetic diversity in the introduced range (Kolbe *et al.* 2004; Genton *et al.* 2005; Roman & Darling 2007). This is the case for *L. kasmira* in the Hawaiian Islands. At the archipelago level, *L. kasmira* in Hawaii exhibit similar or slightly higher heterozygosities and a similar or greater number of nuclear alleles than either source population. The only possible exception to this pattern (Kure) may simply be an artefact due to small sample size ($N = 9$).

Introductions that involve a large number of individuals (high propagule pressure) are less likely to suffer the loss of rare alleles and heterozygosity associated with founder events (Lockwood *et al*. 2005). What is unclear is how many individuals are required to prevent such a loss of genetic diversity. The answer to this question is dependent on both the genetic diversity of the taxa involved and patterns of survivorship following the founder event. Dlugosch & Parker (2008) reviewed 80 surveys of molecular variation in introduced species. These include 11 cases of intentional introduction where the number of individuals is confidently known and derived from a single source population (Table 5). In these eleven cases, which cover a variety of taxa, a loss of genetic diversity was detected in all but one case involving the introduction of less than 250 individuals. The introduction of 2385 Peacock Groupers (*Cephalopholis argus*) to the Hawaiian Islands (Planes & Lecaillon 1998) is the only example in this review that involved a founder population of greater than 250 individuals (Dlugosch & Parker 2008) and for this species the authors found no loss of genetic diversity in the introduced range. As with *C. argus*, we found that 2431 *L. kasmira* were sufficient to prevent a major loss of mtDNA haplotypes (17%) (Marquesas Lineage, Table 1). In contrast, with a founder population size of 728 individuals (Society Lineage, Table 1), we detected a larger decrease in haplotype richness (41%) indicating, that at least for *L. kasmira* introduced to Hawaii, this founder size is at the level where we begin to detect substantial losses of genetic diversity. This conclusion should be tempered with the recognition that

Table 5 Table is modified from Dlugosch & Parker (2008). Studies of molecular variation in eleven intentionally introduced species. Only cases in which all individuals were derived from a single source population and the number of individuals released is confidently known are listed. Locations indicate the regions that served as the source (S) and introduced (I) areas. Number of individuals introduced (N_I) and marker type (number of loci is in parentheses) are listed. Values for allelic richness (A) and expected heterozygosity (H_E) are averages per locus and population

Organism	Location (S/I)	N_I	Marker	A (S/I)	H_E (S/I)	Reference
Birds						
Common Myna *Acridotheres tristis*	India/Australia	~250	allozymes (21)	1.43/1.30	0.06/0.06	Baker & Moeed 1987
Eurasian Tree Sparrow *Passer montanus*	Germany/United States	20	allozymes (39)	1.50/1.33	0.101/0.078	St. Louis & Barlow 1988
Reptiles						
Jamaican Anole *Anolis grahami*	Jamaica/Bermuda	71	allozymes (24)	1.75/1.50	0.078/0.064	Taylor & Gorman 1975
Mammals						
Red-necked Wallaby *Macropus rufogriseus*	Australia/New Zealand	6–10	microsatellites (5)	8.4/4.6	0.767/0.586	Le Page *et al*. 2000
Caribou *Rangifer tarandus*	Norway/Iceland	35	allozymes (1)	8.0/3.0	0.729/0.332	Roed *et al*. 1985
Javan Rusa Deer *Cervus timorensis russa*	New Caledonia/Australia	7	microsatellites (10,24)	7.60/2.29	0.595/0.467	Bonnet *et al*. 2002 Webley *et al*. 2004
Insects						
Mountain Butterfly *Erebia epiphron silesiana*	E/W Sudetans (Czechia)	50*	allozymes (17)	1.59/1.47	0.100/0.116	Schmitt *et al*. 2005
Amphibians						
Marsh Frog *Rana ridibunda*	Hungary/Britain	12	microsatellites (5)	3.2/2.2	0.522/0.484	Zeisset & Beebee 2003
Crustaceans						
Signal Crayfish *Pacifastacus leniusculus*	Canada (Pitt Lake)/ Sweden	~200	allozymes (4)	1.50/1.25	0.177/0.079	Agerberg & Jansson 1995
Fish						
Peacock Grouper *Cephalopholis argus*	Society/Hawaii	2385†	allozymes (9)	4.00/3.78	0.046/0.045	Planes & Lecaillon 1998
European Grayling *Thymallus thymallus*	NW Europe/ Norway	'a small number'	microsatellites (17)	3.75/1.90	0.435/0.170	Koskinen *et al*. 2002a, b

*50 inseminated females were translocated. This species is known to engage in multiple inseminations and to store sperm which would render that effective population size higher than expected from the census size.
†The number of individuals released is reported in this reference as 571 (released in 1956). This number has been corrected here to include the 1814 fish that were released in 1961 from the same source population (HDFG records).

even a small number of reproductive adults can retain genetic diversity over the short term (Spencer *et al*. 2000) and that even very large populations may not retain genetic diversity if there is a high variance in reproductive success (Hedgecock 1994), as is generally the case for marine fishes (Grant & Bowen 1998; 2006).

Conclusion

The introduction of *L. kasmira* to the Hawaiian Islands is a remarkable case study for two reasons. First, the introduction of this species occurred in two discrete and well documented events with known chronology, numbers, and source populations. Second, this species was introduced from two genetically distinct populations at the Marquesas and Society Islands, allowing us to trace the fate of their descendents in the introduced range. Using mitochondrial and nuclear sequence data we determined that individuals from both source populations became established in the archipelago, interbreed, and their descendents have colonized each island and atoll surveyed (Figs 2 and 3). We found that 2431 *L. kasmira* were sufficient to prevent a substantial loss of mtDNA diversity while 728 individuals resulted in a 41% decrease in haplotype richness.

Previous reports document that *L. kasmira* colonized from the inhabited Main Hawaiian Islands to the farthest north-western (NW) Hawaiian Islands, a distance of over 2000 km in just 34 years (Oda & Parrish 1982; Randall 1987). More recently, a range-wide genetic survey of *L. kasmira* demonstrated exceptional dispersal ability in this species (Gaither *et al*. 2010). Here we conclude that the rapid colonization across the NW Hawaiian Island was accompanied by maintenance of high levels of genetic diversity, indicating large numbers of colonists at every island along the way. The NW Hawaiian Islands now one of the largest marine protected areas in the world (Papahānaumokuākea Marine National Monument), and subject to large-scale efforts to prevent and eradicate alien introductions. In these circumstances, managers need to know whether the 350+ marine exotics in the inhabited Main Hawaiian Islands pose a threat to the nearly pristine habitats of the NW Hawaiian Islands. Our data indicate that highly dispersive species such as *L. kasmira* may prove to be the most effective invaders, and add a new layer to the findings of Oda & Parrish (1982) and Randall (1987); not only can exotic species jump to the NW Hawaiian Islands, they can do so in great numbers and with robust genetic diversity.

Acknowledgements

This study began with support from the National Geographic Society (grant No. NGS 7269-02 to DRR), and was further supported by research expeditions funded and staffed by the Papahānaumokuākea Marine National Monument. We thank Monument co-trustees NOAA Marine Sanctuaries, U.S. Fish and Wildlife Service (USFWS), and the State of Hawaii. This research was supported by NOAA National Marine Sanctuaries Program MOA No. 2005-008/66882 (BWB & RJT), Hawaii Sea Grant Nos. NA05OAR4171048 and NA09OAR4170060 (BWB), National Science Foundation (NSF) grants No. OIA0554657, OCE-0453167, OCE-0929031 (BWB), and OCE-0623678 (RJT), and the Evolution, Ecology, and Conservation Biology (EECB) specialization at the University of Hawaii. The views expressed herein are those of the authors and do not necessarily reflect the views of NSF, NOAA USFWS, the State of Hawaii, or any of their sub-agencies. Greg Concepcion, Matt Craig, Jonathan Dale, Toby Daly-Engel, Jeff Eble, Matt Iacchei, Stephen Karl, Randall Kosaki, Carl Meyer, Yannis Papastamatiou, Luiz Rocha, Zoli Szabo, Jill Zamzow, Joshua Reece, and the crew of the R.V. Hi'ialakai helped collect specimens. Aulani Wilhelm, Jo-Ann Leong, Hoku Johnson, Danielle Carter, Daniel Polhemus, Randall Kosaki, Ann Mooney, Elizabeth Keenen, and Kelly Gleason provided crucial logistic assistance to this project. We thank Marc Crepeau for valuable laboratory assistance and protocol development, Sarah Daley, Rajesh Shrestha and Mindy Mizobe of the HIMB EPSCoR core facility for their assistance with DNA sequencing, and Shelley Jones and all the members of the ToBo lab and staff at HIMB for their assistance, support and feedback throughout this project. This is contribution #1371 from the Hawaii Institute of Marine Biology, #7829 from the School of Ocean and Earth Science and Technology and #JC-06-30 from the University of Hawaii Sea Grant Program.

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Supporting Information

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

Table S1 Allele frequencies at three nuclear introns in the two source populations and across the introduced range.

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