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RESEARCH ARTICLE

Phylogeography, historical demography, and the role of post-settlement ecology in two Hawaiian damselfish species

Marina L. Ramon · Peter A. Nelson · Edward De Martini · William J. Walsh · Giacomo Bernardi

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Abstract Coral reef fish generally have relatively sedentary juvenile and adult phases and a presumed highly dispersive pelagic larval phase, yet previous studies that have tried to relate pelagic larval duration (PLD) to population structure have given inconsistent results. In the present study, the population structures of two damselfishes, *Stegastes fasciolatus* and *Dascyllus albisella*, were examined using mitochondrial control region sequences. The two species have similar PLDs (~25 and 27 days respectively), but consistently differ in their settlement preferences, habitat, and densities in populations throughout the Hawaiian Archipelago, from Hawaii north to Kure Atoll, and south to Johnston Atoll. Information on habitat preferences and population densities were collected between September 2000 and October 2002, and tissue samples for the genetic studies were collected between January and April 2004. Based on the differences in habitat and abundance of the two species, the expectation was that *S. fasciolatus* would have high genetic variability but little population structure compared to *D. albisella*, and this was largely confirmed. *Stegastes fasciolatus* had little population structure in most of the Hawaiian Islands, and *D. albisella* showed evidence of strong population structure throughout its range. An exception to this pattern was the large difference between the Kure Atoll population of *S. fasciolatus* and all others. These results suggest that the interaction of several biological factors (e.g. species-specific spawning habitat and season) with environmental factors (e.g. seasonal wind and current patterns) may have more influence on population structure than single life history characteristics, such as the PLD.

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M. L. Ramon · G. Bernardi (✉)
Department of Ecology and Evolutionary Biology,
University of California Santa Cruz, 100 Shaffer Road,
Santa Cruz, CA 95060, USA
e-mail: bernardi@biology.ucsc.edu

P. A. Nelson
H.T. Harvey and Associates,
983 University Ave, Los Gatos, CA 95032, USA

E. De Martini
National Oceanic and Atmospheric Administration,
National Marine Fisheries Service,
Southwest Fisheries Science Center,
Honolulu Laboratory, 2570 Dole Street,
Honolulu, HI 96822, USA

W. J. Walsh
Division of Aquatic Resources,
74-380B Kealakehe Parkway,
Kailua-Kona, HI 96740, USA

Introduction

Oceanic islands provide a unique opportunity to study speciation in near or complete isolation. Terrestrial examples, such as the radiation of Galapagos finches (Grant 1999), and the Canaries lizards (Thorpe et al. 1994) are considered among the best models to understand speciation resulting from island allopatry combined with adaptive radiation. The most isolated oceanic islands, the Hawaiian Archipelago, also harbor endemic groups of species of spiders, fruitfly, honeycreepers and silverswords, which have undergone adaptive radiations (Schluter 2000). In aquatic environments, however, the situation is more complex due to the high dispersal potential that usually characterizes

marine species. In the case of Hawaii, the freshwater gobies in the genus *Lentipes*, which have a marine larval phase, exhibit limited dispersal between islands (McDowall 2003). In contrast, the damselfish *Stegastes fasciolatus*, showed very high gene flow (based on allozymes) not only among the main islands, but also between the main islands and the very distant northwestern islands (Shaklee 1984). In fact, this classic study has long been an example of the effectiveness of larval dispersal in marine fishes (Baer 1999; Fauvelot and Planes 2002).

In marine species, dispersal potential has intuitively been correlated with gene flow (Roberts 1997). Indeed, species that lack a larval dispersal phase have been shown to exhibit restricted gene flow (Bernardi 2000; Planes et al. 2001; Taylor and Hellberg 2003; Bernardi and Vagelli 2004; Bay et al. 2006). However, when this hypothesis was specifically addressed using a panel of species with a range of dispersal potential (using the PLD as a proxy), results were mixed (Waples and Rosenblatt 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001; Bay et al. 2006; Purcell et al. 2006). In these studies, the choice of species focused on differences in pelagic larval duration, thus species with very different life histories and ecological characteristics were selected. Yet, in recent years, a new paradigm has emerged that may suggest an alternative approach.

While marine populations have long been considered open, with offspring recruiting to distant populations, empirical evidence showed that self-recruitment is not uncommon as once thought (Swearer et al. 2002; Jones et al. 2005; Levin 2006; Almany et al. 2007). In the case of fishes, larvae that were once regarded as passive propagules have been shown to exhibit swimming capabilities that allow them to capitalize on specific currents (Leis and McCormick 2002). The active use of eddies, for example, has been proposed as a possible mechanism for larval retention (Cowen 2002; Leis and McCormick 2002).

In this context, we have revisited Shaklee's (1984) original study by comparing two species of damselfishes: the Pacific Gregory, *Stegastes fasciolatus*, the original species used by Shaklee, and the domino damselfish, *Dascyllus albisella*. The use of two damselfishes allowed us to evaluate gene flow patterns in species that are phylogenetically related (Quenouille et al. 2004), thus avoiding several interpretative issues related to comparisons between dissimilar species (e.g. very different adult sizes and vagility). Indeed, the two species are similar in several ecological and evolutionary aspects, yet differ in key characteristics that may potentially influence population structure. Each species is a member of a widely distributed complex of four or five species (Bernardi et al. 2002; Randall 2005). *Stegastes fasciolatus* is a territorial herbivore, lives in colonies, and is broadly distributed across the Indo-Pacific; *D. albisella* is a Hawaiian Islands-Johnston Atoll endemic that is zooplanktivorous as juvenile-adult, occurs in juvenile social groups, and schools when adult (Hobson 1974; Booth 1992; Lobel 2003; K. Asoh pers. comm.).

Relative growth rates and longevities of these species are either unknown or poorly understood. Longevity data are available only for male *S. fasciolatus* (maximum longevity 10 years; McDonald 1981), *Dascyllus albisella* is presumed to be a moderately long-lived, late-maturing species (Hill and Radtke 1988). More importantly, their densities and relative abundances seem to be very different, a characteristic that may influence the overall population structure of the species.

The dispersal potential of the two species is similar when considering their pelagic larval duration, 25.0 days for *Stegastes fasciolatus*, and 26.8 days for *Dascyllus albisella* (based on total number of increments on newly settled fishes, ranges were 24–26 days, $n = 2$, and 25–29 days, $n = 7$, respectively, Wellington and Victor 1989). Swimming capabilities of newly hatched and settling larvae may be different but data are generally lacking. Both species hatch as comparably-sized larvae from nests of male-defended demersal eggs and settle as similar-sized recruits, which make it less likely that morphologically-based differences in larval behavior are important factors differentially affecting the genetic population structures of the two species.

A key difference between *Stegastes fasciolatus* and *Dascyllus albisella* is the habitat, occupied by reef-residential stage juveniles and adults. *Stegastes fasciolatus* settles generally in a wide range of rubble-rock habitats providing suitably small shelter holes, and occupies a broader variety of reef exposures and substrata as both juveniles and adults (E. DeMartini, unpubl.). The larvae of *Dascyllus albisella*, on the other hand, settle almost exclusively within highly structured coral substrata (almost exclusively on *Pocillopora* spp. corals) within wave-sheltered embayments and atoll lagoons, and remain in these habitats during the adult stage (Booth 1992; Danilowicz 1995a, b). These two habitats exhibit vastly different water circulation regimes. Indeed, water circulation is more and the propensity for larval dispersal is less constrained for *S. fasciolatus* on wave- and current-swept forereefs, where the majority of the latter species' populations must reside (inferred from the greater areal extent of fringing and barrier reef habitats in Hawaii), than it is for *D. albisella*, which is restricted to sheltered lagoons. These ecological differences should result in different genetic patterns. The characteristics of *S. fasciolatus*, being a predominantly forereef species predict a higher genetic diversity compared to *D. albisella* but with a lower level of population structure. In contrast, *D. albisella* should exhibit a lower dispersal potential, resulting in greater genetic structuring among geographically separated reef populations in the Hawaiian Archipelago.

For this study, we decided to test these predictions by evaluating the differences in density and abundance of the two species and using DNA sequences (as opposed to allozymes) that provide more power to uncover finer degrees of population structure. This study was performed over the entire extent of the Hawaiian Archipelago, from the Big Island of Hawaii in the main Hawaiian Islands (MHI) to the northernmost island of Kure in the northwestern Hawaiian Islands (NWHI) (Fig. 1), as well as Johnston Atoll for one species, thus providing the maximum potential for finding population structure, if any, within the Hawaiian Archipelago.

Materials and methods

Fish densities

Numerical densities of these and other fish species were estimated at 3–15 m depths at each of four atolls (French Frigate Shoals-FFS, Pearl and Hermes-PHR, Midway-MID, and Kure Atolls-KUR) during research expeditions on NOAA ships between August 2000 and October 2002. On each survey, two divers tallied all fish individuals from 1- to 20 cm Total Length (TL) encountered within three belt transects (each 25-m long by 4-m wide) centered within stations whose median areal extent was 3,000 m^2. From 37 to 74 stations comprising lagoonal patch reef, backreef, and forereef habitats were surveyed per cruise and atoll. A total of 74, 74, 37, and 59 stations were surveyed during the 25 months period at FFS, PHR, MID, and KUR, respectively (see DeMartini and Friedlander 2004 for additional details).

Collections, PCR amplifications, and DNA sequencing

Samples of *Stegastes fasciolatus* and *Dascyllus albisella* were collected from several islands (Tables 1, 2) by spear from January to April 2004. It is important to note that *S. fasciolatus* was not collected at Johnston Atoll because it does not occur there (Lobel 2003). *Dascyllus albisella* occurs at Kauai but not in great numbers, so it was not collected there due to logistical constraints. Whole Genomic DNA was extracted from muscle tissue, fin clippings or gill filaments that had been stored in 95% ethanol. Tissues were digested overnight at 55°C in 650 µl salt extraction buffer (400 mM NaCl, 10 mM Tris, 2 mM EDTA, 1% SDS, and 20 µg ml^{-1} Proteinase K). DNA was purified by standard chloroform extractions and isopropanol precipitation (Sambrook et al. 1989). Samples were processed using a standard PCR method with mitochondrial and nuclear primers.

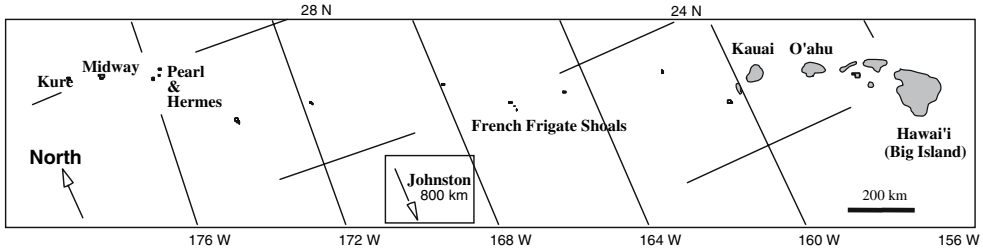

Fig. 1 Map of the Hawaiian Islands and Johnston Atoll where *Stegastes fasciolatus* and *Dascyllus albisella* individuals were collected

Table 1 Historical demography of *Stegastes fasciolatus*

	n	nH	HD	Theta (c)	Theta (v)	g
All samples	219	152	0.9858	0.377 (±0.018)	5.346 (±1.386)	634.6 (±72.9)
Hawaii (HAW)	27	24	0.989	0.044 (±0.002)	4.742 (±2.743)	1511.9 (±340.6)
Oahu (OAH)	42	36	0.988	0.069 (±0.004)	4.466 (±2.770)	1199.7 (±211.6)
Kauai (KAU)	49	42	0.992	0.073 (±0.004)	1.551 (±0.564)	982.7 (±87.0)
French Frigate Shoals (FFS)	24	21	0.978	0.034 (±0.001)	0.731 (±0.372)	1312.2 (±233.8)
Pearl and Hermes (PEH)	15	14	0.990	0.036 (±0.001)	5.699 (±2.948)	1463.5 (±384.6)
Midway (MWA)	20	19	0.995	0.042 (±0.002)	37.525 (±9.708)	2072.2 (±304.4)
Kure (KUR)	42	34	0.986	0.055 (±0.001)	1.605 (±1.209)	1144.9 (±315.2)
HAW, OAH, KAU	118	87	0.987	0.185 (±0.010)	2.548 (±0.856)	667.4 (±77.7)
FFS, PEH, MWA, KUR	101	81	0.987	0.142 (±0.009)	1.770 (±0.929)	696.8 (±99.0)
PEH, MWA, KUR	77	64	0.991	0.109 (±0.005)	2.428 (±0.654)	912.7 (±75.1)
MWA and KUR	62	51	0.990	0.084 (±0.003)	1.528 (±0.604)	969.3 (±155.3)

Rows represent the regions investigated, sample numbers (*n*), number of Haplotypes (nH), Haplotype diversity (HD), Theta with no growth (Theta c), Theta with growth (Theta v), and growth (g). The latter three columns are averages of 10 replicates between parentheses are their standard deviations

Table 2 Historical demography of *Dascyllus albisella*

	n	nH	HD	Theta (c)	Theta (v)	g
All samples	102	55	0.939	0.187 (±0.014)	0.790 (±0.098)	261.4 (±17.6)
Hawaii (HAW)	10	7	0.933	0.024 (±0.000)	0.032 (±0.001)	55.6 (±2.9)
Oahu (OAH)	8	8	1.00	0.033 (±0.001)	0.168 (±0.015)	331.6 (±37.9)
French Frigate Shoals (FFS)	25	19	0.973	0.047 (±0.002)	0.128 (±0.013)	243.4 (±37.4)
Pearl and Hermes (PEH)	28	15	0.876	0.052 (±0.002)	0.262 (±0.092)	396.1 (±53.3)
Midway (MWA)	7	6	0.952	0.059 (±0.001)	0.341 (±0.025)	189.5 (±9.4)
Kure (KUR)	14	11	0.956	0.051 (±0.001)	0.261 (±0.051)	294.2 (±45.7)
Johnston (JOH)	10	7	0.867	0.013 (±0.001)	0.416 (±0.025)	925.4 (±9.4)
HAW, OAH	18	15	0.980	0.048 (±0.001)	0.134 (±0.006)	237.0 (±12.0)
FFS, PEH, MWA, KUR	74	38	0.922	0.147 (±0.010)	0.563 (±0.090)	268.3 (±16.9)
PEH, MWA, KUR	49	29	0.921	0.128 (±0.004)	0.784 (±0.147)	327.5 (±33.6)
MWA, KUR	21	16	0.962	0.092 (±0.003)	0.445 (±0.084)	224.62 (±13.7)

Rows represent the regions investigated, sample numbers (*n*), number of Haplotypes (nH), Haplotype diversity (HD), Theta with no growth (Theta c), Theta with growth (Theta v), and growth (g). The latter three columns are averages of ten replicates, between parentheses are their standard deviations

Mitochondrial control region primers CR-A (5′-TTC CAC CTC TAA CTC CCA AAG CTAG-3′) (Lee et al. 1995) and StDloop.H (5′-CTG GAY AGA YRG CAC GGC ATG G-3′) were used for *S. fasciolatus* and DTPL (5′-TTT GTT ACA GCA AAT TAT TTAT–3′) (Bernardi et al. 2002) and CR-E (5′-CCT GAA GTA GGA ACC AGA TG–3′) (Lee et al. 1995) for *D. albisella*. Nuclear primers were used to amplify the first intron of the ribosomal protein S7 and the 4th intron of the Calmodulin gene. The two nuclear loci were sequenced for 15–30 individuals for each species and were found to be monomorphic. We therefore decided not to sequence the remaining individuals.

PCR amplifications were performed in a 25 µl volume with 1 unit AmpliTaq DNA Polymerase (Perkin Elmer), the manufacturer's buffer, 2.5 mM $MgCl_($, 0.25 mM each dNTP, and 1µM each primer. PCR products were gel purified in 1% agarose, excised from the gel, and then recovered using a QIAquick Gel Extraction Kit (QIAGEN). Double stranded PCR products were sequenced directly with the same primers used for the PCR amplifications using BigDye Chemistry (Applied Biosystems). The sequencing reaction products were cleaned of unincorporated dyes and salts and then run on an ABI3100 DNA Sequencer (Applied Biosystems). Sequencing was performed in one direction and was reconciled in Sequence Navigator (Applied Biosystems). All sequences have been deposited in GenBank (accession numbers EU126971-EU127291).

Phylogenetic analysis

Alignments of all gene regions were unambiguous and were aligned by eye using the program Se-Al (Rambaut 1996) and Clustal V as implemented in the software program Sequence Navigator (Applied Biostystem). Nucleotide sequence evolution models were evaluated using likelihood-ratio tests implemented by Modeltest v.3.6 (Posada and Crandall 1998). Neighbor-Joining reconstructions were based on substitution models obtained with Modeltest (K81uf + I + G for *Stegastes fasciolatus* and HKY + I + G for *Dascyllus albisella*) as implemented by the software package PAUP* (versin 4.0b10, Swofford 2002) based on mitochondrial control regions. As mentioned above, *Stegastes fasciolatus* and *Dascyllus albisella* are part of species complexes. The choice of representative outgroups was based on presumed sister "species" relationships. We therefore used the following outgroups: *Stegastes fasciolatus* from Guam (provided by H. Lessios) for *S. fasciolatus*, and *Dascyllus strasburgi* from the Marquesas Islands for *D. albisella* (following Bernardi et al. 2002).

Historical demography

Number of haplotypes and haplotype diversity was calculated using the software package DNAsp (Rozas and Rozas 1997). We estimated Θ ($\Theta = 2\,N\mu$, where μ is the mutation rate for mitochondrial control region), for *Stegastes fasciolatus* and *Dascyllus albisella* geographic regions. Regions were divided by islands, mainland islands and northwestern islands. The parameter Θ was estimated under two conditions: an unconstrained exponential growth parameter, and an assumption of constant growth N ($g = 0$). We used FLUCTUATE (Kuhner et al. 1998) to estimate the maximum likelihood of the parameters Θ and g (the exponential growth parameters in units of μ^{-1}). Seeds for all analyses were generated randomly and the default transition to

transversion ratio was used. Analyses were repeated ten times per island and island region to ensure stability of parameter estimates.

Population structure

Gene flow (F_{ST} and Nm) was estimated between the islands using ARLEQUIN (version 2.00; Schneider et al. 2000). We also performed an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) as implemented in ARLEQUIN to test for significant genetic structure between the island regions.

Results

Spatial patterns of species densities

Densities of the two species investigated differed among the four NWHI atolls of Kure, Pearl and Hermes, Midway and French Frigate Shoals (nested 2-way ANOVA; reef effect: $P < 0.01$) and overall between the two species (nested ANOVA; species effect: $P < 0.01$). *Stegastes fasciolatus* populations averaged 612.5 ind. ha^{-1}, over an order of magnitude denser than those of *Dascyllus albisella* (15.25 ind. Ha^{-1}). Both species were denser in discrete habitats within atoll lagoons compared to continuous habitats on forereefs (nested ANOVA; habitat-within-reef effect ($P < 0.0001$). Even so, greater proportions of *D. albisella* populations occupied lagoons versus forereefs, compared to *S. fasciolatus* (nested ANOVA; species by habitat-within-reef interaction effect: $P < 0.0001$). *Dascyllus albisella* populations averaged nearly 30% greater relative abundance in sheltered habitats over all four atolls surveyed.

DNA sequences

A total of 219 *Stegastes fasciolatus*, and 102 *Dascyllus albisella* individuals were collected for this study (Tables 1, 2). Control region primers amplified 378 bases of the 5′ end of the control region for *D. albisella* and 477 for *S. fasciolatus*. In the case of *D. albisella*, 71 of 378 aligned nucleotides were variable and 43 were phylogenetically informative. For *S. fasciolatus*, 96 of 477 aligned nucleotides were variable and 51 were phylogenetically informative. Insertions or deletions were not observed in either species. The high variability of the control region translated into high levels of haplotype diversity, 0.939 for *D. albisella* and 0.986 for *S. fasciolatus* (Tables 1, 2). This high haplotype diversity was not due to specific populations, all populations showed very high diversity and there was no significant difference in diversity between the main islands and northwestern islands (Tables 1, 2).

Phylogenetic reconstructions

Phylogenetic trees were evaluated for both the species, based on mitochondrial control regions (Figs. 2, 3). The sequences for *Stegastes fasciolatus* individuals did not partition into specific clades and no obvious geographic partitioning was observed (Fig. 2). This result was not a reflection of a diminution in genetic variability; in fact the control region was more variable in *S. fasciolatus* than in *D. albisella* (the scale bar in Figs. 2, 3 is the same 1% sequence divergence). In contrast to our findings for *S. fasciolatus*, the sequences of *Dascyllus albisella* grouped in two well-defined clades, confirming Bernardi et al.'s (2002) findings based on a smaller dataset (ten individuals). The presence of the two clades did not correspond to obvious geographic partitioning, with samples from any population being present in both the clades, except for the samples from Johnston Atoll, which were only found in one clade (Fig. 3). In addition, all Johnston atoll individuals but one (that is 9 out of 10), grouped in a small sub-clade. Besides the nine Johnston Atoll individuals, one individual from Midway Atoll also belonged to that sub-clade. The tenth individual from Johnston Atoll grouped with two individuals, both from Midway (Fig. 3).

Gene flow and population structure

Gene flow patterns were different in the two species investigated here. When analyzing classical measures of gene flow (F_{ST}), only 6 out of 21 population pairwise comparisons were found to be statistically significant in *Stegastes fasciolatus* (Table 3). The six values that were significant in *S. fasciolatus* corresponded to the comparisons of the island of Kure (the northernmost island) with the remaining six populations (Table 3). In contrast, 20 out of 21 population pairwise comparisons were statistically significant in *Dascyllus albisella* (Table 4). The single non-significant value was associated with Midway, a population with the smallest sample size (Tables 2, 4), suggesting that this may be a statistical artifact.

Besides being mostly not significant, F_{ST} values for *S. fasciolatus* were also very low, ranging from 0.00 to 0.102, which corresponds to a range of 4.4 migrants generation^{-1} up to panmixia. The six significant F_{ST} values that characterized the Kure population of *S. fasciolatus* ranged from 0.093 to 0.102 (N*m* from 6.4 to 4.9). While these were very high F_{ST} values, they were still lower than the values for the Kure population of *D. albisella* (Tables 3, 4). In fact, in all cases, F_{ST} values were higher in *Dascyllus albisella* than in *Stegastes fasciolatus*. Values of F_{ST} for *D. albisella*, ranged from 0.033 to 0.749, which corresponded to 14.6–0.17 migrants per generation (Table 3). When groups of islands, rather than population pairwise comparisons, were used to estimate gene

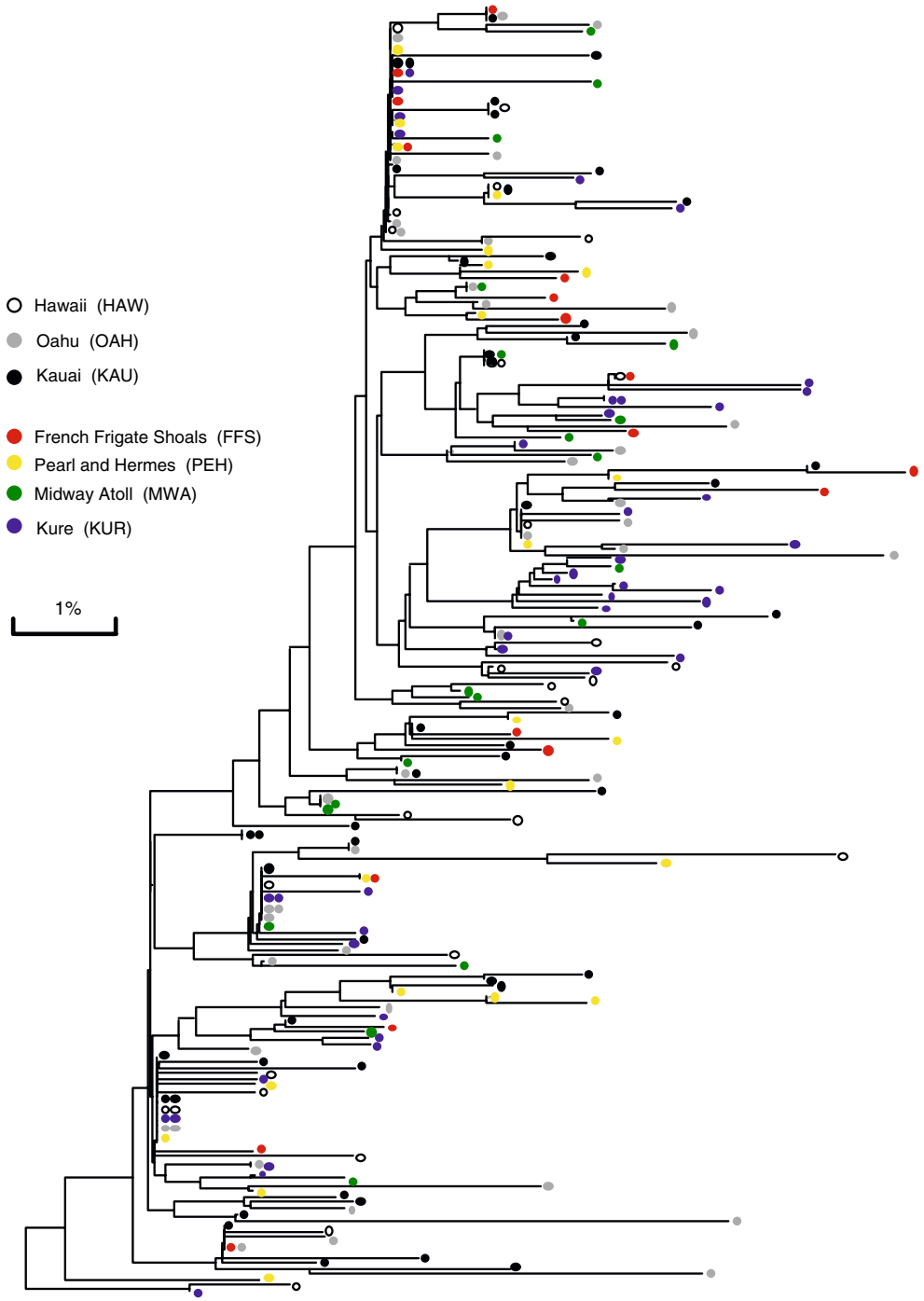

Fig. 2 Neighbor Joining tree of individuals, color-coded by collection site for *Stegastes fasciolatus*. *Scale bar* indicates 1% Kimura 2 sequence divergence

flow, trends were consistent for both the species (Table 5, first two left columns). For both species, F_{ST} values increased (and Nm values decreased) with distance. Indeed, when the main islands were compared with groups of northwestern islands that were farther and farther away, F_{ST} values increased from 0.02 to 0.102 for *S. fasciolatus* and from 0.232 to 0.759 in *D. albisella* (Table 5). Here too, F_{ST} values were always lower in *S. fasciolatus* than in *D. albisella*.

In order to further evaluate population structure, an analysis of molecular variance (AMOVA) was also performed, using alternative groupings. Results are summarized in Table 5. Similarly to what was presented above, the amount of variance attributed to the separation into groups increased with distance. When dividing the samples into two groups, the main islands in one group, and a subset of the northwest islands in the second group, the percent of the total variance

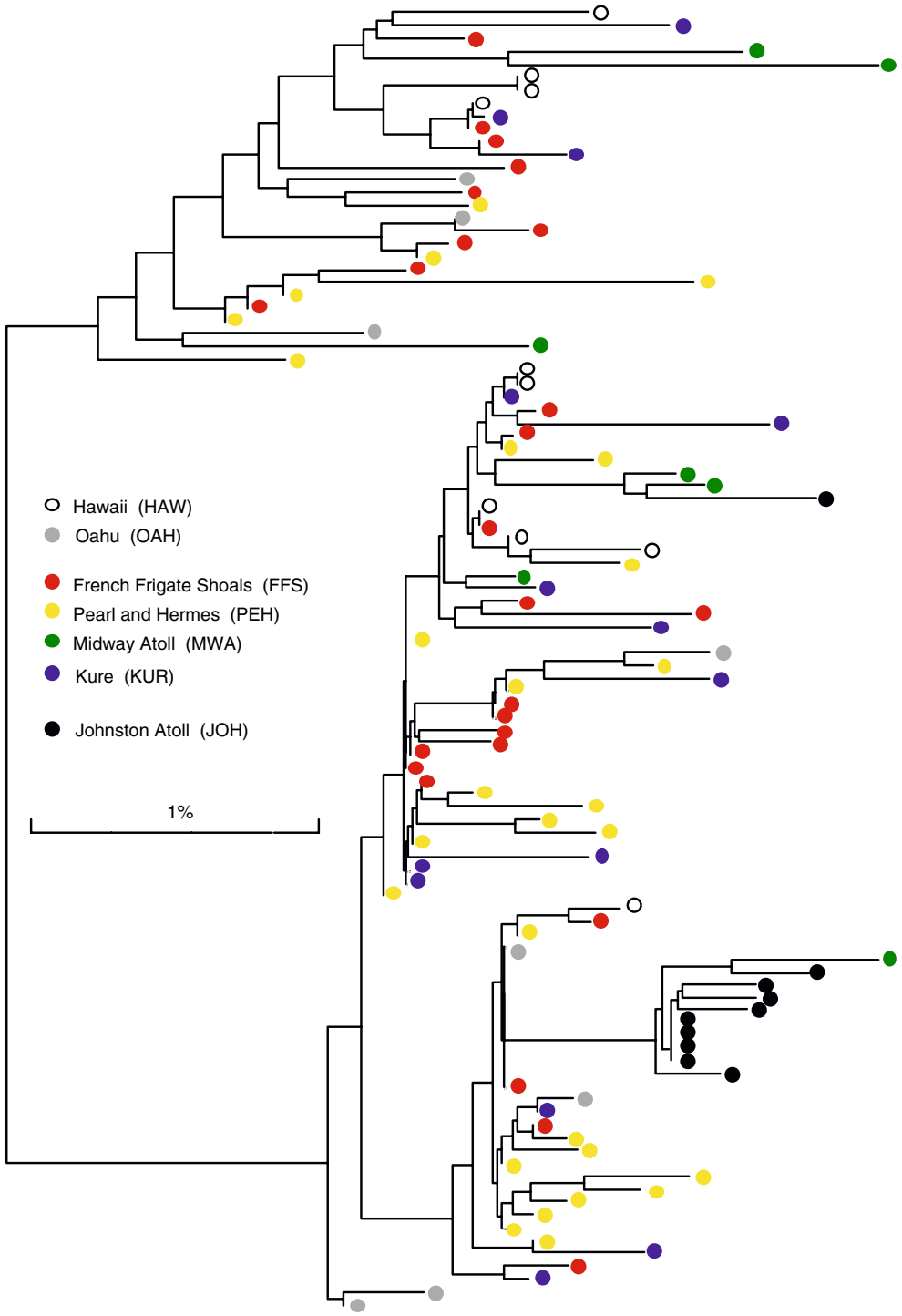

Fig. 3 Neighbor Joining tree of individuals, color-coded by collection site for *Dascyllus albisella*. *Scale bar* indicates 1% Kimura 2 sequence divergence

attributable to this separation increased with distance. In the case of *D. albisella*, this percentage varied from 11.7 to 31.4%, while the percentage was lower in *S. fasciolatus* but increased with distance as well (from 1 to 4.8%).

We checked for discrepancies in datasets that may have biased our results. In all the results presented so far, samples from Johnston Atoll (only obtained for *Dascyllus albisella*) were omitted. Thus the differences between the two species could not be attributed to the Johnston Atoll population. However, the main islands were represented by an additional island in *Stegastes fasciolatus* (the main island of Kauai, Fig. 1). When this island was removed from

Table 3 Population pairwise F_{st} (genetic structure) values for *Stegastes fasciolatus* below diagonal and Nm (number of migrants per generation) above

F_{st} values in *boldface* are significant at the 0.05 level

	HAW	OAH	KAU	FFS	PEH	MWA	KUR
Hawaii (HAW)	–	inf	929.272	inf	144.883	inf	**4.853**
Oahu (OAH)	0.000	–	inf	inf	289.917	209.268	**4.885**
Kauai (KAU)	0.001	0.000	---	inf	153.644	65.121	**4.527**
French Frigate Shoals (FFS)	0.000	0.000	0.000	–	457.829	41.036	**4.391**
Pearl and Hermes (PEH)	0.003	0.002	0.003	0.001	–	inf	**4.882**
Midway (MWA)	0.000	0.002	0.008	0.012	0.000	–	**6.409**
Kure (KUR)	**0.093**	**0.093**	**0.099**	**0.102**	**0.093**	**0.072**	–

Table 4 Population pairwise F_{st} (genetic structure) values for *Dascyllus albisella* below diagonal and Nm (number of migrants per generation) above

F_{st} values in *boldface* are significant at the 0.05 level

	HAW	OAH	FFS	PEH	MWA	KUR	JOH
Hawaii (HAW)	–	**6.768**	**14.041**	**0.973**	**4.369**	**0.196**	**0.616**
Oahu (OAH)	**0.069**	–	**14.597**	**1.025**	**4.510**	**0.205**	**0.479**
French Frigate Shoals (FFS)	**0.034**	**0.033**	–	**1.465**	12.28	**0.262**	**1.937**
Pearl and Hermes (PEH)	**0.339**	**0.328**	**0.254**	–	**2.345**	**1.657**	**0.831**
Midway (MWA)	**0.103**	**0.100**	0.039	**0.176**	–	**0.417**	**2.533**
Kure (KUR)	**0.718**	**0.709**	**0.656**	**0.232**	**0.545**	–	**0.167**
Johnston Atoll (JOH)	**0.448**	**0.511**	**0.205**	**0.376**	**0.164**	**0.749**	–

Table 5 Population pairwise F_{st} and Nm values for *Stegastes fasciolatus* and *Dascyllus albisella* between the main islands and selected populations

	F_{st} values	Nm	%Variance among groups	%Variance among populations w/in groups	%Variance within populations
	Main Islands	Main Islands	Main Islands	Main Islands	Main Islands
Dascyllus albisella/Stegastes fasciolatus					
NWI/JOH	NA/NA	NA/NA	NA/8.83	NA/34.38	NA/56.79
NWI + JOH	NA/**0.193**	NA/2.09	NA/6.98	NA/36.44	NA/56.58
FFS, PEH, MWA, KUR	**0.02/0.232**	24.66/1.65	1.73/0.981	3.20/31.18	95.82/57.09
PEH, MWA, KUR	**0.037/0.371**	12.85/0.85	2.81/28.67	2.52/19.16	94.67/52.17
MWA, KUR	**0.056/0.485**	8.45/0.53	4.83/31.42	1.68/33.74	93.49/34.83
KUR	**0.102/0.759**	4.42/0.16			
HAW + OAH v.s.FFS, PEH, MWA, KUR	**0.018/0.232**	27.38/1.65	0.00/11.73	4.63/31.18	95.42/56.79
HAW + OAH v.s. FFS, PEH, MWA	0.000/**0.167**	inf./2.49	0.00/8.44	0.00/19.20	100.00/72.36

F_{st} values in *boldface* are significant at the 0.05 level. For *S. fasciolatus*, main islands included Oahu, Hawaii and Kauai, and for *D. albisella*, main islands include Oahu and Hawaii. The last two lines correspond to comparisons between selected populations and the main islands of Hawaii and Oahu

analysis, results remained unchanged (Table 5, line 7). Since the only significant F_{ST} values were obtained in the Kure population in *S. fasciolatus*, we decided to determine if the Kure population was mostly responsible for driving the patterns obtained in *S. fasciolatus*. Indeed, when this population was removed, patterns obtained with *S. fasciolatus* tended to disappear while the results for *D. albisella* remained essentially unchanged (Table 5, Line 8).

Historical demography

Historical demographic parameters, Theta (Θ) and growth (g), are summarized in Tables 1 and 2. Values of Theta, which are correlated with population size, were always higher in *Stegastes fasciolatus* than in *Dascyllus albisella*. Population growth followed the same trend, with values at least threefold higher in *S. fasciolatus* than in *D. albisella*. Only one population of *D. albisella* exhibited a very high population growth, the population from Johnston Atoll (Table 1).

Discussion

Previous studies on allozyme markers showed no genetic structure in *Stegastes fasciolatus* from the main Hawaiian

Islands and three Northwestern Islands (French Frigate Shoals, Maro Reef, and Midway Atoll) (Shaklee 1984). Our results, based on DNA sequences, are consistent with Shaklee's analysis. Yet, the addition of the northernmost island of Kure in our study provided some unexpected information. While gene flow was very high between all other islands, Kure exhibited strong genetic isolation from the rest of the Archipelago. Thus, overall *Stegastes fasciolatus* was shown to be a species with little population structure in the majority of the Hawaiian islands except for the northernmost island of Kure. There are no known oceanographic factors that might help explain the genetic isolation of *Stegastes fasciolatus* at Kure. Also, except for its extreme geographic position in the archipelago, Kure is a typical atoll with a lagoon (like neighboring PHR and Midway) separated from the surrounding open ocean by a barrier reef interrupted by one or more passes. Unlike PHR and Midway, though, Kure has only a single, relatively narrow, natural pass, which might constrain the dispersal of propagules produced within its lagoon to greater extent than at the other two atolls.

In contrast, the other damselfish species, *Dascyllus albisella* exhibited very strong population structure throughout the range of the species. Ecological (biotic) and environmental (abiotic) factors can be invoked to explain the striking difference in population structure between thee two species.

In the absence of differences in pelagic larval duration, the higher genetic variability of *S. fasciolatus* compared to *D. albisella* was consistent with our predictions based on life-history attributes, including differences in population densities (which were, in turn, consistent with Theta values), adult and post-settlement habitat, and their associated different flow regimes. At an evolutionary scale, the differences in genetic variability of *S. fasciolatus* compared to *D. albisella* was further consistent with the general trend of higher diversity in forereef species compared to lagoonal ones (Fauvelot et al. 2003).

A likely important difference between the two species is spawning seasonality and how this interacts with tidal and ocean current flow patterns throughout the Hawaiian Archipelago and adjacent waters. Peak spawning in *Stegastes fasciolatus* occurs during February–April in Hawaii (MacDonald 1981; Walsh 1987). In contrast, *Dascyllus albisella* spawns mostly in June–August, even though some spawning occurs throughout the year (Danilowicz 1995a, b, 1997; Asoh 2003).

Spawning seasonalities likely interact strongly with seasonal differences in wind-related patterns of current speed and direction, eddy formation, and apparent pelagic larval transport for the Hawaiian Archipelago (Lobel and Robinson 1988; Cowen et al. 2000; Largier 2003; Cowen et al. 2006, Kobayashi in review) and also for Johnston Atoll (Kobayashi 2006). For *Stegastes fasciolatus*, the broadly distributed Indo-Pacific species (all *Stegastes* species are absent from Johnston Atoll for unknown reasons), the transport of pelagic larvae throughout the Hawaiian Archipelago is undoubtedly facilitated by spawning during the spring when tradewind generated geostrophic flow is greatest and eddy formation is at its peak (Lobel and Robinson 1988; Firing and Brainard 2006; Kobayashi and Polovina 2006). For *Dascyllus albisella*, the Hawaiian–Johnston endemic, later spawning during the summer when winds on average are lighter would likely limit the distances to which the larvae of this endemic species are transported. Using computer simulations in a Lagrangian, individual-based model inputting high resolution ocean current data and realistic combinations of spawning seasonality and PLDs ranging from 0.5 to 3–12 months, Kobayashi (in review) observed that, at shorter PLDs (more similar to those of these two damselfish species), retention of natal propagules was relatively high at particular reefs within the Hawaiian Archipelago even though the transport and reception of non-natal larvae and connectivity among reefs was fairly assured over multiple generations. Seasonal differences in species- and habitat-specific propagule production have not yet been incorporated in this model but were acknowledged as potentially important (Kobayashi, pers. comm.); and these could effect major differences in the connectivities among reef populations of species like *S. fasciolatus* and *D. albisella*. Advection by wind and currents can importantly influence not only transport but also recruitment success and year-class establishment in coral reef fishes (Milicich 1994; Kingsford and Finn 1997).

For species like *Dascyllus albisella*, the transport modeling results support the observed greater genetic differentiation of populations between Johnston Atoll and the Hawaiian Archipelago than among islands within Hawaii (Kobayashi (2006). Several potential larval transport corridors between Johnston Atoll and the Hawaiian Archipelago (one corridor to FFS in the mid-NWHI plus another corridor to Kauai in the MHI) have been identified, but only for dispersal involving PLDs of at least 40 days (Kobayashi 2006). Incidentally, while models are based on passive transport and larval swimming abilities may augment or curtail dispersal, there is little evidence of directional swimming in open water. Thus, one would expect that a species like *D. albisella* with a PLD of only 25 days should exhibit genetic differences between Johnston and Hawaii. Even for such a species, though, retention of larvae at Johnston might be extremely low, so recruitment success at Johnston likely would vary appreciably over time (e.g., among years) even though very few larvae ever disperse to Hawaii (Kobayashi 2006). The observed genetic distinctness between Johnston and Hawaii populations of *D. albisella* therefore conforms with expectations based on modeled dispersal and connectivity.

Several ecological and environmental factors likely interact to produce differing patterns of connectivity and genetic population structure in these two species, despite their similar PLDs. Clearly, these additional factors influence genetic population structuring in these species more than does planktonic larval duration. Habitat differences appear to influence dispersal consistently with expectations and species differences appear to be consistent with expectations and species differences in seasonality. Within-season lunar periodicity might also be influential. Other case studies elsewhere, and for other reef fish lineages, also illustrate the possible or even likely disconnect between genetic connectivity and PLDs if these are the only factors considered (Bowen et al. 2006). Although a number of studies indicate a positive relation between connectivity and PLD in reef fishes (Waples and Rosenblatt 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001; Bay et al. 2006; Purcell et al. 2006), or have argued for relationships based on theory (Kinlan et al. 2005), as many or more others have observed at best a weak relation when PLDs are evaluated without additional, modifying influences (Lester and Ruttenberg 2005; Macpherson and Raventos 2006; Bay et al. 2006).

This study contributes to the growing evidence that factors including habitat and seasonal current patterns that act as dispersal promoters and constraints, linked with species-specific reproductive natural history attributes like spawning seasonality, must be considered in addition to, not in lieu of, estimated PLDs when evaluating the phylogeographies of coral reef fishes. Our results are consistent with predictions based on ecological and demographic characteristics of our focal species, yet these results are based on two species only. The use of more species pairs, such as *Chromis vanderbilti* a broadly distributed Indo-Pacific species with a very protracted spawning season and *Chromis ovalis*, a Hawaiian endemic with a short spawning season in early spring, or other examples from Hawaii or elsewhere, would provide the replication necessary to generalize the patterns we observed.

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Erratum, below is the correct Table 5.

Table 5. Population pairwise Fst and Nm values for *Stegastes fasciolatus* and *Dascyllus albisella* between the main islands and selected populations. Fst values in boldface are significant at the .05 level. For for *S. fasciolatus*, main islands included Oahu, Hawaii and Kauai, and for *D. albisella*, main islands include Oahu and Hawaii. The last two lines correspond to comparisons between selected populations and the main islands of Hawaii and Oahu.

	Fst Values	Nm	%Variance Among Groups	%Variance Among Populations w/in Groups	%Variance Within Populations
	Main Islands	Main Islands	Main Islands	Main Islands	Main Islands
Stegastes fasciolatus / *Dascyllus albisella*					
NWI / JOH	NA / NA	NA/NA	NA / 8.83	NA/ 34.38	NA/ 56.79
NWI + JOH	NA / **0.193**	NA / 2.09	NA / 6.98	NA/ 36.44	NA/ 56.58
FFS, PEH, MWA, KUR	**0.02 / 0.232**	24.66 / 1.65	0.98 / 11. 73	3.20/ 31.18	95.82/ 57.09
PEH, MWA, KUR	**0.037 / 0.371**	12.85 / 0.85	2.81/ 28.67	2.52/ 19.16	94.67/ 52.17
MWA, KUR	**0.056 / 0.485**	8.45 / 0.53	4.83/ 31.42	1.68/ 33.74	93.49/ 34.83
KUR	**0.102 / 0.759**	4.42 / 0.16			
HAW+OAH v.s. FFS,PEH,MWA,KUR	**0.018/ 0.232**	27.38/ 1.65	0.00/ 11.73	4.63/ 31.18	95.42 / 56.79
HAW+OAH v.s. FFS,PEH,MWA	0.000/ **0.167**	inf./ 2.49	0.00/ 8.44	0.00/ 19.20	100.00 / 72.36