

Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories

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Abstract

The marine environment offers few obvious barriers to dispersal for broadcast-spawning species, yet population genetic structure can occur on a scale much smaller than the theoretical limits of larval dispersal. Comparative phylogeographical studies of sympatric sister species can illuminate how differences in life history, behaviour, and habitat affinity influence population partitioning. Here we use a mitochondrial DNA marker (612 bp of cytochrome *c* oxidase subunit I) to investigate population structure of three endemic Hawaiian broadcast-spawning limpets (*Cellana* spp.) with planktonic larvae that are competent to settle within 4 days. All three species exhibit significant population structure and isolation by distance, but the spatial scales of partitioning differ among the species. *Cellana talcosa* ($n = 105$) exhibits strong population structure between Kauai and the other main Hawaiian Islands (MHI) where the maximum channel width is 117 km, and no shared haplotypes were observed ($\Phi_{CT} = 0.30$, $P < 0.001$). In contrast, populations of *Cellana exarata* ($n = 149$) and *Cellana sandwicensis* ($n = 109$) exhibit weaker population structure within the MHI ($\Phi_{ST} = 0.03$ – 0.04 , $P < 0.05$), and between the MHI and the Northwestern Hawaiian Islands ($\Phi_{ST} = 0.03$ – 0.09 , $P < 0.01$), where the maximum channel width is 260 km. Biogeographical range and microhabitat use were correlated with estimates of dispersal, while phylogenetic affiliation and minimum pelagic larval duration were poor predictors of population partitioning. Despite similar life histories, these closely related limpets have contrasting patterns of population structure, illustrating the danger of relying on model species in management initiatives to predict population structure and dispersal in the context of marine protected area delineation.

Keywords: COI, fisheries management, marine protected areas, model taxa, mitochondrial DNA, pelagic larval dispersal

Received 23 November 2006; revision accepted 13 April 2007

Introduction

Recent meta-analyses support the long-standing view that dispersal potential of marine species is at least one to two orders of magnitude greater than terrestrial and freshwater organisms (Kinlan & Gaines 2003; Kinlan *et al.* 2005). Greater connectivity of sedentary marine organisms is routinely attributed to the dispersal potential of pelagic larvae and the scarcity of physical barriers among marine habitats (Mayr 1954; reviewed in Palumbi 1994; Shulman

1998). Biogeographical barriers between marine populations include obvious geographical features such as land masses, i.e. the Isthmus of Panama (Bermingham & Lessios 1993), but also more subtle factors such as currents and oceanographic regimes (Dawson 2001; Barber *et al.* 2002; Sotka *et al.* 2004). Indeed, there is a growing list of marine taxa that do not realize their apparent dispersal potential (Burton & Feldman 1981; Knowlton & Keller 1986; Shanks *et al.* 2003; Jones *et al.* 2005; Severance & Karl 2006).

Concordant genetic breaks in the distributions of broadcast-spawners have confirmed biogeographical barriers that were originally proposed based on species distributions. The demonstrated barriers include the

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California transition zone (Briggs 1974; Dawson 2001), the Florida transition zone between the Gulf of Mexico and the Atlantic Ocean (Reeb & Avise 1990; Avise 1992), the Indonesian channels between the Indian and the Pacific Oceans (Benzie 1999; Barber *et al.* 2000), the Mona Passage between the East and West Caribbean Sea (Colin 1975; Taylor & Hellberg 2003, 2006; Baums *et al.* 2006), and the Cook Strait between the North and South islands of New Zealand (Smith 1988; Apte & Gardner 2002; Goldstien *et al.* 2006a).

In each of the aforementioned cases, without detailed knowledge of currents, temperature gradients, and/or sea level change, the causes of these genetic breaks would not be predicted, *a priori*. Population partitions at the Florida transition zone and Indian/Pacific boundary are believed to be influenced by emergent land barriers during low sea level stands and subsequent vicariant divergence. The California transition zone, Florida transition zone, and Cook Strait are accompanied by latitudinal changes in the marine climate which may influence distributions via a myriad of pathways (Wares 2001; Sotka & Hay 2002; Sotka *et al.* 2004). All of these barriers are maintained, at least in part, by oceanic currents (Avise 1992; Benzie 1999; Dawson 2001; Sotka *et al.* 2004; Baums *et al.* 2006). The best example of this to date is the Mona Passage, where coupled biological-physical oceanographic models predict highly restricted larval dispersal across a channel that corresponds with a population genetic break (Baums *et al.* 2005, 2006; Galindo *et al.* 2006).

Despite the success of such bio-oceanographic models and the identification of concordant patterns of population structure, the geographical locations of genetic breaks often differ among taxa. Patterns of population structure have also been associated with habitat affiliation, phylogeny, and life history. Marko (2004) and Rocha *et al.* (2002) found that habitat affiliation was the best predictor of differential patterns of population structure in two species of thaid gastropods (*Nucella* spp.) and three species of Atlantic

surgeon fishes (*Acanthurus* spp.), respectively. The degree of genetic similarity is predictive of population structure in a group of three limpets in New Zealand, where the sister species *Cellana flava* and *Cellana radians* exhibit similar levels of partitioning relative to the more divergent *Cellana ornata* across Cook Strait (Goldstien *et al.* 2006a, b). Life history, specifically pelagic larval duration, is a predictor of realized dispersal and population genetic structuring (Shanks *et al.* 2003; Paulay & Meyer 2006), although there are notable exceptions (reviewed in Gooch 1975; Hedgecock 1986; Palumbi 1994; Bohonak 1999).

The ability to predict the location of genetic breaks across taxa applies directly to the implementation of marine protected areas (MPAs). Effective MPAs must not only sustain target species within their boundaries, but also serve as reproductive reservoirs that supply recruits to unprotected areas (Roberts *et al.* 2001). Thus, the positioning of MPAs is critical, and their success is maximized when guided by accurate information about the patterns of connectivity among populations (Crowder *et al.* 2000; Dawson *et al.* 2006; Steneck *et al.* 2006), which can be ascertained with molecular techniques (Grosberg & Cunningham 2001; Palumbi 2003). When direct tagging is infeasible, molecular genetic approaches are one of the few options for documenting patterns of connectivity and designing effective MPA networks (Swearer *et al.* 2002).

The Hawaiian Archipelago (Fig. 1) is recognized as an excellent system for the study of terrestrial speciation and evolution (Hillebrand 1888; Wagner & Funk 1995). Less appreciated are marine population processes which operate across a larger geographical expanse of high and low islands, atolls, and submerged reefs (e.g. Rivera *et al.* 2004; Andrews *et al.* 2006; Craig *et al.* 2007). The objectives of this study are to illuminate the traits that shape population structure, and ultimately to resolve and define management units for the three endemic Hawaiian limpets (*Cellana exarata*, *Cellana sandwicensis*, and *Cellana talcosa*), known locally as opihi. The opihi are closely related sister taxa

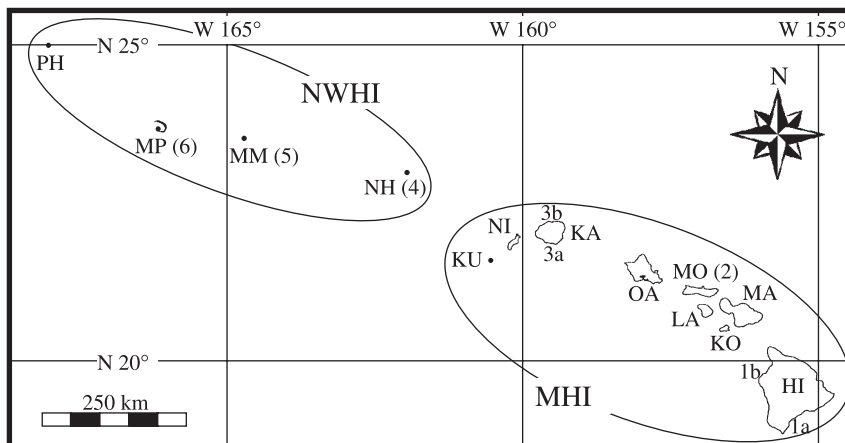


Fig. 1 Scaled map of the basaltic Hawaiian Islands. Island labels are as follows: Puhahonu (PH), Mokupapapa (MP), Mokumanamana (MM), Nihoa (NH), Kaula (KU), Niihau (NI), Kauai (KA), Oahu (OA), Molokai (MO), Lanai (LA), Kahoolawe (KO), Maui (MA), and Hawaii (HI). Collecting sites 1–6 are indicated.

(Reeb 1995, C.E.B. unpublished data), with similar larval duration (Corpuz 1981, 1983, C.E.B. unpublished data), but differing biogeographical ranges and habitat preferences (Kay & Magruder 1977).

Management concerns for the opihi are compelling. The opihi are a prominent component of Hawaiian culinary culture, and are harvested commercially, recreationally, and for subsistence. Between 1900 and 1944, the commercial harvest of opihi decreased by an order of magnitude, to a present day average of ~6300 kg annually (Jordan & Evermann 1902, 1905; Hawaii Department of Land & Natural Resources 2005). MPAs are integral to a management initiative intended to reverse the decline of opihi, as well as other marine taxa in Hawaii (Halpern & Warner 2002; Roberts *et al.* 2003).

The study of sympatric sister species provides an appropriate context for comparative phylogeographical studies (see Collin 2001; Dawson *et al.* 2002; Paulay & Meyer 2006). Here we conduct range-wide surveys of all three species using 612 bp of cytochrome *c* oxidase subunit I (COI) to evaluate migration rates and patterns of population partitioning. Given the approximately linear arrangement of islands (Fig. 1), genetic differentiation should be correlated with geographical distances among sampling sites. Modest genetic structure and clinal patterns are expected under an island-hopping model with recruitment patterns characterized by isolation by distance (IBD) (Wright 1943). Using the Hawaiian *Cellana*, we evaluate the utility of representative 'model' species in predicting connectivity within and among communities, an approach that is pertinent to the design of MPA networks. In particular, we investigate patterns of population genetic structure in the context of four criteria:

- 1 Habitat model: the intertidal *C. exarata* and *C. sandwicensis* will have concordant population structure relative to the subtidal *C. talcosa*. (See Materials and methods for details).
- 2 Biogeographical model: the wide-ranging *C. exarata* and *C. sandwicensis* will have less pronounced population structure than the range restricted *C. talcosa*.
- 3 Phylogenetic model: the sister species *C. sandwicensis* and *C. talcosa* will have concordant population structure relative to the basal *C. exarata*.
- 4 Larval life history model: all three species will have uniform levels of population structure based on their similar larval life histories.

Materials and methods

Sampling sites

All sampling took place within the Hawaiian Archipelago (Fig. 1), which is centrally located in the tropical Pacific

Ocean, ~3800 km from the nearest continent or high island. Within the main Hawaiian Islands (MHI), there are eight islands separated by oceanic channels of 11–160 km (Fig. 1). In the Northwestern Hawaiian Islands (NWHI), which is separated from the MHI by a 240-km channel, there are nine additional islands and a number of shallow, submerged reefs extending ~2400 km from the Big Island of Hawaii, with a maximum channel width of 380 km. Despite the broad geographical scale of the Hawaiian Archipelago, it spans less than 10° of latitude, which confers a more uniform climate than most continental margins. There are three primary currents in the Hawaiian Archipelago, the North Hawaiian Ridge Current which flows to the northwest along the island chain (Qiu *et al.* 1997), the Hawaiian Lee Countercurrent and the Subtropical Counter-current which both generally flow from west to east (Kobashi & Kawamura 2002). Theoretically, larvae should be able to get everywhere in archipelago on these currents. However, currents flow southwesterly through the channels, perpendicular to the island chain, and every channel between the islands holds the potential to constrict gene flow for species restricted to shallow coastal habitats. Based on Hawaiian geography, one would expect a simple population genetic pattern of IBD along the linear transect of island habitats.

Study organisms

Limpets in the genus *Cellana* are shallow water gastropods with moderate dispersal potential, although a number of species have broad distributions, occupying vast areas of the Asian, Indonesian, Indian, Australian, African coasts, as well as remote Indo-Pacific archipelagos (Powell 1973). Three endemic Hawaiian limpets (*C. exarata*, *C. sandwicensis*, and *C. talcosa*) inhabit the high intertidal, low intertidal, and shallow subtidal zones on wave-exposed rocky shores, respectively (Kay & Magruder 1977; C.E.B. unpublished data). Based on habitat affiliation, we expect the two intertidal species, *C. exarata* and *C. sandwicensis*, to have concordant population structure relative to the subtidal *C. talcosa*.

Cellana exarata and *C. sandwicensis* occur on every basaltic island, from the island of Hawaii (19°00'N, 155°40'W) to Puhahonu (25°01'N, 167°59'W), while *C. talcosa* is restricted to the MHI, from Hawaii to Niihau (22°00'N, 160°05'W) (Fig. 1). Based on biogeographical distribution, we expect that *C. exarata* and *C. sandwicensis* should exhibit similar patterns of population partitioning. If restricted range indicates restricted dispersal ability, *C. talcosa* should be characterized by the lowest dispersal and greatest corresponding population structure (Thorson 1950; Gilman 2006; Paulay & Meyer 2006).

In terms of larval behaviour, *Cellana exarata* and *C. sandwicensis* are competent to settle within 3 to 4 days of fertilization

in laboratory cultures (Corpuz 1981; 1983; C.E.B. unpublished data), but Corpuz (1983) noted that *C. exarata* could delay settlement and remain in the pelagic veliger stage for at least 18 days. We and others have cultured the third species (*C. talcosa*) under similar laboratory conditions, and its development mirrored that of the other two species (C.E.B. unpublished data, Sarver unpublished data). Based on our limited knowledge of larval duration and behaviour, all three species are expected to exhibit similar levels of dispersal and population structure.

Sequence divergence in 521 bp of the ribosomal subunit 16S is 1.2% between *C. sandwicensis* and *C. talcosa* and 2.2% between *C. exarata* and the aforementioned sister species (Reeb 1995; C.E.B. unpublished data). Because the three species of opihi are closely related, phylogenetically, they are predicted to exhibit similar levels of population partitioning. An alternate prediction, based on phylogenetic order of the taxa, is that *C. sandwicensis* and *C. talcosa* will exhibit concordant population structures relative to *C. exarata*.

Sampling

The three species of Hawaiian *Cellana* were sampled between 2003 and 2005 at eight sites on six islands (Fig. 1). To simplify sites labels, we have numbered the islands from one to six and sites within an island were designated as either a or b. Thus, the sampling sites in the MHI include (southeast to northwest): Halape, Hawaii (1a); Kalaemano, Hawaii (1b); Kalaupapa, Molokai (2); Poipu, Kauai (3a); and Princeville, Kauai (3b). The sampling sites in the NWHI include Nihoa (4), Mokumanamana (5), and the La Perouse Pinnacles at Mokupapapa (6).

Whole animals were collected, or a small piece of tissue (~10 mg) was removed from the mantle using a sterile razor blade. Tissue specimens were immediately frozen or preserved in 95% ethanol. The NWHI posed a particular challenge to collections because of their remoteness, the consequent inability of the authors to be present for sample collection, and the paucity of sampling opportunities. Opihi exist in the most hydrodynamically extreme habitat in the Hawaiian Archipelago, where the mean significant wave height is two to three-fold greater than the diurnal tidal range (C.E.B. unpublished data). As a result of its lower position on the shoreline, *C. sandwicensis* is more hazardous to collect than *C. exarata*, and we have fewer samples. Sample sizes per population ranged from $n = 11$ to $n = 36$ (Table 1).

PCR and sequencing

Genomic DNA was extracted from the tissue samples using QIAGEN DNeasy Animal Tissue Kits. One mitochondrial DNA locus, COI 612 bp, was amplified using polymerase chain reaction (PCR). We used the COI primers LCO1490:

Table 1 Sample size, total number of haplotypes, number of unique haplotypes to a sampling site (Locality haplotypes), haplotype diversity, and nucleotide diversity in COI for each species. Ce is *Cellana exarata*, Cs is *Cellana sandwicensis*, and Ct is *Cellana talcosa*

Region	Island	Site	Specimens (N)			Total no. of haplotypes			Locality haplotypes			Haplotype diversity (h)			Nucleotide diversity (π)			
			Ce	Cs	Ct	Ce	Cs	Ct	Ce	Cs	Ct	Ce	Cs	Ct	Ce	Cs	Ct	
MHI	HI	1a	20	22	20	18	16	18	11	11	8	11	0.98 ± 0.02	0.94 ± 0.02	0.98 ± 0.02	4 × 10 ⁻³ ± 3 × 10 ⁻³	4 × 10 ⁻³ ± 3 × 10 ⁻³	7 × 10 ⁻³ ± 4 × 10 ⁻³
		1b	21	20	23	14	15	17	9	11	9	11	0.97 ± 0.02	0.97 ± 0.03	0.97 ± 0.02	4 × 10 ⁻³ ± 3 × 10 ⁻³	5 × 10 ⁻³ ± 3 × 10 ⁻³	8 × 10 ⁻³ ± 5 × 10 ⁻³
	MO	18	21	24	14	14	18	9	10	14	10	14	0.96 ± 0.03	0.93 ± 0.04	0.97 ± 0.02	4 × 10 ⁻³ ± 3 × 10 ⁻³	5 × 10 ⁻³ ± 3 × 10 ⁻³	6 × 10 ⁻³ ± 3 × 10 ⁻³
NWHI	KA	3a	21	20	20	11	16	6	7	10	4	4	0.91 ± 0.04	0.97 ± 0.03	0.52 ± 0.13	4 × 10 ⁻³ ± 2 × 10 ⁻³	5 × 10 ⁻³ ± 3 × 10 ⁻³	1 × 10 ⁻³ ± 1 × 10 ⁻³
		3b	—	—	18	—	—	2	—	—	0	0	0.11 ± 0.10	—	—	—	—	2 × 10 ⁻⁴ ± 3 × 10 ⁻⁴
	NH	4	11	—	2	—	—	0	—	—	—	0.18 ± 0.14	—	—	3 × 10 ⁻⁴ ± 5 × 10 ⁻⁴	—	—	
Total	MM	23	18	—	1	13	—	0	9	—	—	0.96 ± 0.03	—	—	8 × 10 ⁻³ ± 4 × 10 ⁻³	—	—	
	MP	36	8	—	9	7	—	7	3	—	—	0.48 ± 0.10	0.96 ± 0.08	—	1 × 10 ⁻³ ± 1 × 10 ⁻³	5 × 10 ⁻³ ± 3 × 10 ⁻³	—	
			150	109	105	52	59	49	43	49	40	0.90 ± 0.02	0.96 ± 0.01	0.90 ± 0.02	3 × 10 ⁻³ ± 2 × 10 ⁻³	6 × 10 ⁻³ ± 3 × 10 ⁻³	6 × 10 ⁻³ ± 3 × 10 ⁻³	

5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994). Twenty microlitre PCRs were performed using 10 μ L of 2x Bioline (Bioline) Biomix Red, 2 μ L of 5 μ M primers, 2 μ L of template DNA, and 6 μ L of sterile deionized water. The PCRs were run for one cycle with a 4-min, 95 °C denaturation step, a 1-min 30 s, 48 °C annealing step, and a 2-min, 72 °C elongation step on a Bio-Rad (Bio-Rad Laboratories) Mycycler thermocycler. Thirty-four additional cycles were run with the following parameters: 30 s at 94 °C, 30 s at 48 °C, and 45 s at 72 °C, concluding with a final 10-min elongation step at 72 °C. PCR products were cleaned by adding 1.6 μ L of exonuclease 1 and 1.6 μ L of shrimp alkaline phosphatase (Exo-SAP) to 18 μ L of PCR product and incubating at 37 °C for 30 min and 80 °C for 10 min. Purified DNA fragments were sequenced by the commercial service Macrogen and the Hawaii Institute of Marine Biology EPSCoR Sequencing Facility. There were no indels or broken reading frames, and the DNA sequences were aligned by eye.

Data analysis

Analysis of molecular variance (AMOVA) was used for hierarchical analysis of partitioning of COI diversity within and among populations, as well as within and among groups of populations using ARLEQUIN 3.01 (Excoffier *et al.* 2005). Population pairwise Φ_{ST} values, Nei's average pairwise genetic distance (Nei & Li 1979), and exact tests of population differentiation (Raymond & Rousset 1995) were also computed with ARLEQUIN.

Tests based on the infinite-alleles (Ewens 1972; Watterson 1978; Slatkin 1996) and infinite-sites models (Tajima 1989; Fu 1997) were used to test for significant deviations in allele frequencies from neutral expectations, as implemented in ARLEQUIN. Because many population genetic estimates are relatively insensitive to weak selection (see Slatkin & Barton 1989), loci which do not fail these tests are expected to provide reliable inferences about population structure (Hutchison & Templeton 1999).

Coalescent-based calculations of migration rate among populations (N_eM) and the population mutation parameter (θ) were conducted with LAMARC 2.02 (Kuhner 2006). LAMARC was chosen over IM for this analysis because LAMARC is not limited by population number, while IM is restricted to two populations (Hey & Nielsen 2004). The calculations performed by ARLEQUIN are based on island model assumptions which are rarely met in natural populations (Whitlock & McCauley 1999). In contrast, LAMARC does not rely on the same population assumptions. A Bayesian Metropolis Hastings Markov chain Monte Carlo (MHMCMC) search strategy was implemented where one final Markov chain was executed with 60 000 samples, a final sampling interval of 40, and 2000 samples

to discard. Three simultaneous searches with heating were enabled at temperatures of 1, 1.1, and 1.3. The upper and lower Bayesian priors were set to their maximum and minimum possible values, respectively. MODELTEST 3.7 (Posada & Crandall 1998) was used to determine that a TrN base-substitution model (Tamura & Nei. 1993) was most appropriate for each of the three species investigated. The posterior probability distributions were examined to determine the validity of each estimated parameter.

SPSS 13.0 was used to conduct ordinary least squares regressions to assess the association between pairwise genetic and geographical distance matrices—IBD. In certain cases, data were transformed or quadratic explanatory terms were added to the model to satisfy all assumptions of ordinary least squares regression. For example, fitting a straight line through a curved set of data will produce a patterned, nonhomogenous spread of the residuals when plotted against the fits, thereby invalidating the least squares regression model and the associated statistics (Neter *et al.* 1996). We did not use reduced major axis regression (Hellberg 1994) because the population samples were collected at point locations (\ll 50 m of coastline) and populations were separated by a minimum of 63 km. Therefore, there is little variance in our measurements of distance relative to that of F -statistics (Nei *et al.* 1977), and ordinary least squares regression is the most appropriate analysis.

Two measures of geographical distance were used: the minimum travel distance between sites for pelagic larvae, and the cumulative minimum distance between islands. In the first geographical distance measure, no distinction is made between the distance travelled along a contiguous coastline and across the channels between islands. The second geographical distance measure assumes that islands are stepping-stones (*sensu* Kimura 1953), where distance along contiguous coastlines is ignored, and only the minimum cumulative open-water distance between islands is considered.

Results

Totals of 150 *Cellana exarata*, 109 *Cellana sandwicensis*, and 105 *Cellana talcosa* were sampled in this study (Table 1). Unique haplotypes were submitted to GenBank (Accession nos EF620934–EF621301).

Parsimony networks and population summary statistics

In all three species, we observed a large number of closely related haplotypes and high haplotype diversities (Table 1). Eighty-three per cent of the haplotypes were restricted to a single sampling site (hereafter referred to as locality haplotypes). In the total pooled samples, *C. sandwicensis* exhibited the highest haplotype diversity ($h = 0.96$),

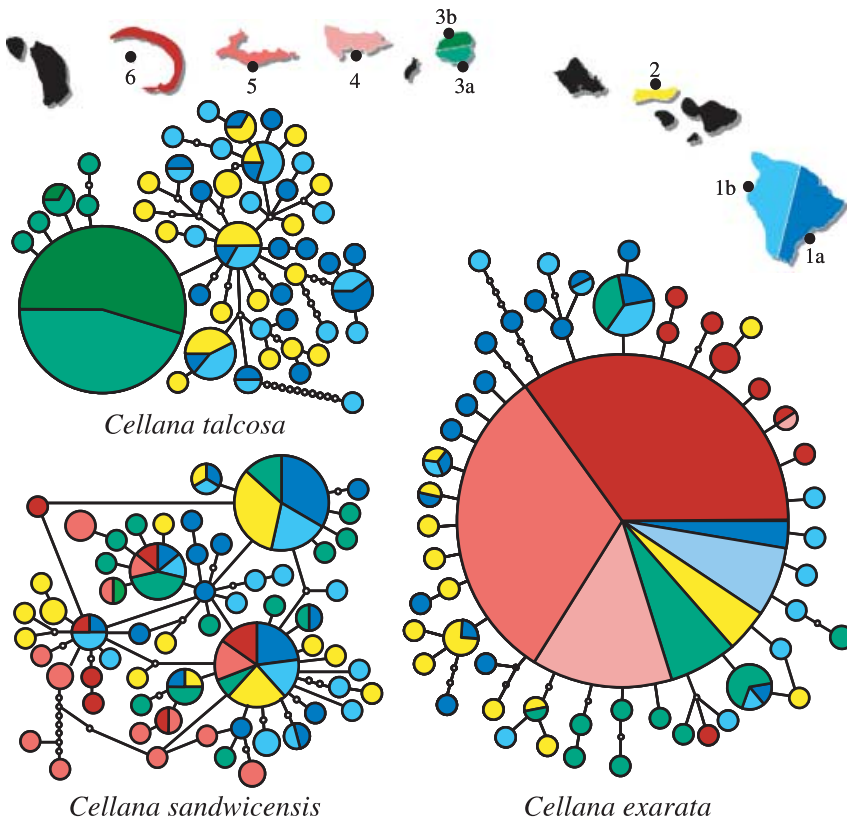


Fig. 2 Haplotype networks for each Hawaiian *Cellana* species. Each circle represents a unique haplotype connected by a line to those that differ by one base pair. Nodes on the lines indicate missing haplotypes. Each haplotype is colour-coded by site and size is proportional to frequency. The smallest coloured circles represent one occurrence of a haplotype. In the site key, the islands are arranged according to order in the archipelago and the NWHI were enlarged. MHI sampling site labels are as follows: Halape, Hawaii (1a); Kalaemano, Hawaii (1b); Kalauapapa, Molokai (2); Poipu, Kauai (3a); and Princeville, Kauai (3b). NWHI sampling sites are shaded red and labelled as follows: Nihoa (4); Mokumanamana (5); and Mokupapapa (6).

followed by *C. talcosa* ($h = 0.90$), and *C. exarata* ($h = 0.75$). Nucleotide diversities tended to be low ($\pi = 0-0.008$) and were positively correlated with haplotype diversity in the three species.

Parsimony networks for each Hawaiian *Cellana* species exhibited strikingly different patterns, despite having similar numbers of haplotypes (Fig. 2, Table 1). *C. sandwicensis* exhibited the lowest association between haplotype identity and geographical location. Three of the five most common *C. sandwicensis* haplotypes are shared between the NWHI (sites 4–6) and the MHI (sites 1–3). Even low frequency haplotypes were shared between islands and between the NWHI and MHI. Forty-nine of the 59 haplotypes were restricted to a locality, but 45 of these 49 were singletons and were only sampled at one site, by definition.

Cellana exarata exhibited an intermediate level of association between haplotype and geographical location (Fig. 2, Table 1). The parsimony network of *C. exarata* was defined by a single dominant haplotype ($n = 74$) observed at every site, and three haplotypes that were common in the MHI ($n = 4$). Most haplotypes diverge from the dominant haplotype by only one to three mutations. MHI sites exhibit higher diversity and a greater number of unique haplotypes than NWHI sites. Only one haplotype was shared between the NWHI (4–6) and MHI sites (1–3), indicating that successful migration events between these

regions are rare. The most striking spatial pattern is the detection of only one haplotype at site 5, while the flanking sites on Mokupapapa (6, northwest), Nihoa (4) and Kauai (3a, southeast) had nine, two, and 11 sampled haplotypes, respectively. Overall, 43 of 52 haplotypes were restricted to a locality (41 singletons).

Cellana talcosa had the strongest association between haplotype and geographical location, most notably with fixed differences among haplotypes sampled on Kauai (sites 3a, b) and the other MHI sampled (sites 1–2, Fig. 2, Table 1). Six haplotypes were observed on Kauai, which were each distinguished by one to four mutations from haplotypes sampled elsewhere in the MHI. In contrast, 18 haplotypes were identified at site 2 on Molokai, and 29 haplotypes were identified at sites 1a, b on the Island of Hawaii. Four haplotypes were observed on both islands, indicating greater connectivity between Molokai and Hawaii than between either of these sites and Kauai. Overall, 40 of 49 haplotypes were restricted to a locality (39 singletons).

Analysis of molecular variance

The sampling sites were grouped by region and site for an AMOVA of the COI gene (Table 2). The regions were defined as the NWHI (sites 4–6) and the MHI (sites 1–3)

Table 2 Analysis of molecular variance for each opihii species. The regions are NWHI vs. MHI for *Cellana exarata* and *Cellana sandwicensis*, and Kauai vs. Molokai and Hawaii for *Cellana talcosa*. The regional variance component relative to the total variance is Φ_{CT} . The between site within region variance component divided by the sum of itself and the within site variance is Φ_{SC} . Φ_{ST} is the sum of the variance due to region and site within region divided by the total variance. Statistical significance ($\alpha = 0.05$) is indicated by bold font

Source	d.f.	SS	MS	Var comp	% var	Φ_{CT} Φ_{SC}	Sig	Φ_{ST}	Sig
A. <i>C. exarata</i>									
Region	1	2.27	2.27	0.015	1.90	0.019	0.057	0.036	0.001
Site (region)	5	5.33	1.07	0.014	1.73	0.017	0.041		
Within site	143	111.65	0.78	0.781	96.37				
Total	149	119.25							
B. <i>C. sandwicensis</i>									
Region	1	6.90	6.90	0.125	6.86	0.069	0.001	0.078	0.001
Site (region)	4	7.99	2.00	0.018	0.97	0.010	0.179		
Within site	103	172.50	1.67	1.675	92.17				
Total	108	187.39							
C. <i>C. talcosa</i>									
Region	1	33.59	33.59	0.660	30.33	0.303	0.001	0.304	0.001
Site (region)	3	4.66	1.56	0.002	0.09	0.001	0.446		
Within site	100	151.55	1.52	1.515	69.58				
Total	104	189.8							

for *C. exarata* and *C. sandwicensis*, and the regions were defined as Kauai and the other MHI for *C. talcosa*, which is not known to occur in the NWHI.

In *C. exarata*, no significant effect of region was detected ($\Phi_{CT} = 0.02$, $P = 0.06$, Table 2a), but the effect of site nested within region was significant ($\Phi_{SC} = 0.02$, $P = 0.04$). The combined effect of site within region and region, i.e. the overall population genetic structuring, was highly significant ($\Phi_{ST} = 0.04$, $P < 0.01$).

In *C. sandwicensis*, genetic variation was significantly partitioned between regions ($\Phi_{CT} = 0.07$, $P < 0.01$, Table 2b) but not among the sites nested within the regions ($\Phi_{SC} = 0.01$, $P = 0.18$). The overall population genetic structuring was significant ($\Phi_{ST} = 0.08$, $P < 0.01$).

A large portion of the genetic variation in *C. talcosa* was partitioned between the regions ($\Phi_{CT} = 0.30$, $P < 0.01$, Table 2c), but there was no indication of partitioning among the sites nested within the regions ($\Phi_{SC} = 0.001$, $P = 0.45$). The overall population genetic structuring was highly significant ($\Phi_{ST} = 0.30$, $P < 0.01$).

Migration-gene flow estimates

In general, the lowest effective migration rates (N_eM) were associated with the largest oceanic gaps between sites. The migration rates of *C. exarata* and *C. sandwicensis* were lowest between the MHI and the NWHI, while the migration rate of *C. talcosa* was lowest between Kauai (sites 3a, b) and the other MHI (sites 1–2, Table 3). Given the haplotype frequency distribution (Table 1, Fig. 2), effective migration estimates should be less than one per generation (Slatkin

Table 3 Pairwise population migration rate estimates (N_eM) for each *Cellana* spp. based on Bayesian MHMCMC simulation. The estimates of migration are segregated by direction, where the columns are destinations and the rows are sources (below diagonal, east migration; above diagonal, west migration). The value of M calculated by LAMARC was multiplied by the θ of the destination populations, as calculated by LAMARC, and divided by four.

Region	Island	Site	1a	1b	2	3a	3b	5	6	
A. <i>C. exarata</i>										
MHI	HI	1a	—	21	9	0.07	—	0.02	0.53	
		1b	183	—	2.5	3.3	—	0.01	0.36	
	MO	2	48	7.3	—	0.05	—	0.01	0.34	
		3a	0.58	8.8	0.13	—	—	0.003	0.08	
	NWHI	MM	5	0.48	0.11	0.06	0.03	—	—	0.81
		MP	6	0.77	0.20	0.13	0.02	—	0.08	—
B. <i>C. sandwicensis</i>										
MHI	HI	1a	—	37	10	46	—	0.01	0.26	
		1b	58	—	2.3	0.81	—	0.09	0.58	
	MO	2	55	4	—	2.0	—	2.75	0.37	
		3a	55	0.39	0.71	—	—	6.5	0.36	
	NWHI	MM	5	0.05	0.63	11	9	—	—	4.3
		MP	6	0.35	0.66	0.45	0.49	—	1.6	—
C. <i>C. talcosa</i>										
MHI	HI	1a	—	21	6.3	0.03	0.01			
		1b	25	—	75	0.05	0			
	MO	2	3.8	19	—	0.08	0			
		KA	3a	0.06	0.09	0.16	—	1.0		
	3b		0.06	0.01	0.03	7.0	—			

MHI, Main Hawaiian Islands; NWHI, Northwestern Hawaiian Islands; HI, Island of Hawaii; MO, Molokai; KA, Kauai; MM, Mokumanamana; MP, Mokupapapa.

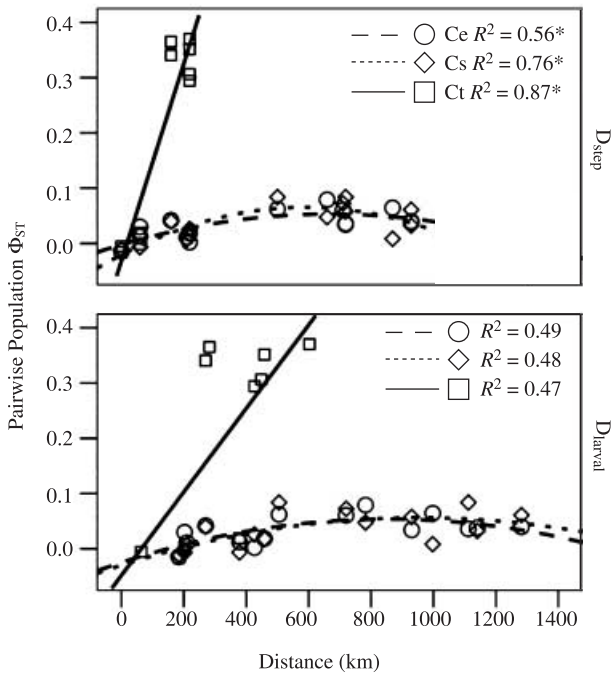


Fig. 3 Scatter plots of pairwise population Φ_{ST} vs. distance between sites for each Hawaiian *Cellana* species. Distances were measured as the absolute minimum travel distance between sites for a pelagic larva (D_{larval}) or the cumulative minimum stepping-stone distance between the islands that lie between the sites (D_{step}). Significant regressions ($\alpha = 0.05$) are denoted by an asterisk (*).

1987; Mills & Allendorf 1997) for *C. exarata* between the NWHI and the MHI and for *C. talcosa* between Kauai and the other MHI. The Bayesian estimates of migration rate are close to this expectation, where migration rates within the NWHI and between the NWHI and the MHI range from $N_{eM} = 0.03$ –1.30 for *C. exarata*. Similarly, migration rates ranged from $N_{eM} = 0.01$ –0.24 for *C. talcosa* between Kauai and the other MHI.

Within the MHI, estimated migration rates tended to be high ($N_{eM} > 5$) for all *Cellana* spp. among the Molokai and Hawaii sites (1, 2). However, estimates of migration rates between Kauai (site 3a) and the other MHI sites (1, 2) were inconsistent in both *C. exarata* and *C. sandwicensis*, where migration rate estimates ranged from $N_{eM} = 0.18$ –101. Specifically, there is a high migration rate between sites 3a and 1b for *C. exarata* ($N_{eM} = 12.1$), but not for *C. sandwicensis*, where there is a high migration rate between 3a and 1a ($N_{eM} = 101$). Interestingly, there is some agreement between pairwise Φ_{ST} values and the lowest estimated migration rates in the MHI, in that there is significant partitioning between sites 3a and 2 in *C. exarata* ($\Phi_{ST} = 0.04$, $P = 0.01$; $N_{eM} = 0.18$) and sites 3a and 1b in *C. sandwicensis* ($\Phi_{ST} = 0.03$, $P = 0.04$; $N_{eM} = 1.2$).

Within islands, estimates of dispersal were consistently high for both the big island of Hawaii (sites 1a, b) and

Table 4 Stepwise regression results for each species of *Cellana*. The dependent variable was the pairwise population Φ_{ST} and the independent factors were cumulative stepping-stone distance between sites (SSD), the number of steps between sites (S), and the minimum larval travel distance between sites (LTD). The independent factors were added in the order shown. In order to meet the assumptions of the model, independent factors were quadratic for *C. exarata* and *C. sandwicensis*, and linear for *C. talcosa*. Factors that explain a significant amount of variance ($\alpha = 0.05$), given the previous factors entered in the model are indicated by bold font.

Independent factors	R	R ²	ΔR^2	ΔF	df1	df2	Sig. ΔR^2
A. <i>C. exarata</i>							
SSD	0.75	0.56	0.56	7.74	1	4	0.050
S	0.90	0.81	0.25	6.43	1	3	0.085
LTD	0.91	0.83	0.02	0.43	1	2	0.580
B. <i>C. sandwicensis</i>							
SSD	0.87	0.76	0.76	19.13	1	4	0.012
S	0.87	0.76	0.00	0.01	1	3	0.927
LTD	0.91	0.82	0.06	3.32	1	2	0.210
C. <i>C. talcosa</i>							
SSD	0.93	0.87	0.87	52.27	1	3	0.005
S	0.99	0.98	0.11	40.77	1	2	0.024
LTD	0.99	0.99	0.00	1.84	1	1	0.404

Kauai (sites 3a, b), with all three species characterized by estimated migration rates $N_{eM} > 8$.

Isolation by distance

Pairwise Φ_{ST} values were regressed against the cumulative stepping-stone distance and the larval travel distance to assess conformation to an IBD model (Fig. 3, Table 4). Stepping-stone distance between sites explained a significant and substantial proportion of the population differentiation (Φ_{ST}) for *C. exarata* ($R^2 = 0.56$, $P = 0.05$), *C. sandwicensis* ($R^2 = 0.76$, $P = 0.01$), and *C. talcosa* ($R^2 = 0.87$, $P < 0.01$). The relationship between Φ_{ST} and stepping-stone distance was quadratic for *C. exarata* and *C. sandwicensis*, but linear for *C. talcosa*. Stepwise multiple regression indicated that the number of steps (channel crossings) between sites explained an additional 11% of the variance in Φ_{ST} , given the stepping-stone distance for *C. talcosa* ($\Delta R^2 = 0.11$, $P = 0.02$), but not for *C. exarata* or *C. sandwicensis*. Notably, larval travel distance (see Materials and methods) was not significantly related to pairwise Φ_{ST} .

The estimated migration rates from LAMARC for each *Cellana* spp. were plotted against the cumulative stepping-stone distance (Fig. 4). While most regressions of migration rate vs. distance are linear on a log-log scale, there was an asymptotic relationship between stepping-stone distance and the simulated migration rate of *C. talcosa*. Consequently,

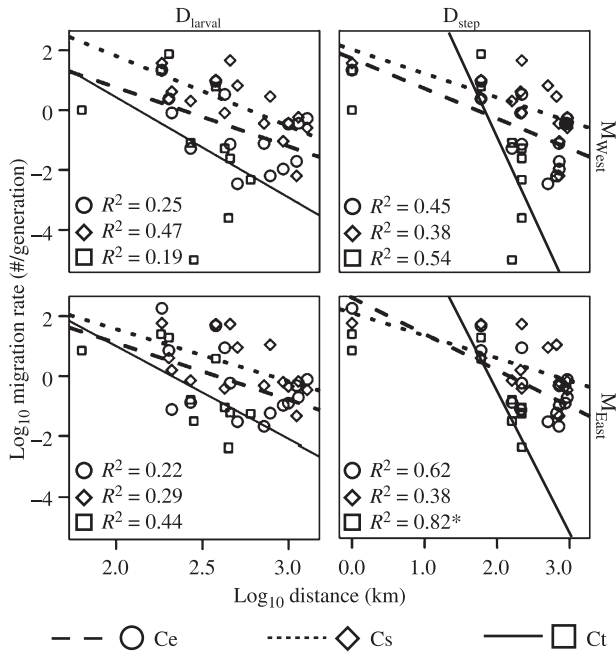


Fig. 4 Scatter plots of migration rate between pairs of sites vs. distance between sites for each Hawaiian *Cellana* species. Migration rates were calculated using Bayesian MHMCMC simulation (M_{East} & M_{West}). Distances were measured as the absolute minimum travel distance between sites for a pelagic larva (D_{larval}) or the cumulative minimum stepping-stone distance between the islands that lie between the sites (D_{step}). Significant regressions ($\alpha = 0.05$) are denoted by an asterisk (*).

we only analysed the linear section of the data points for *C. talcosa*. Variation in the simulated Bayesian estimates of migration rate was significantly explained by stepping-stone distance for *C. talcosa* in the eastward direction ($R^2 = 0.82$, $P = 0.03$) and a trend was detected for *C. exarata* in the eastward direction ($R^2 = 0.53$, $P = 0.06$). When considering only the linear section of the data for *C. talcosa*, the slope (-4.3) is significantly different than that of *C. exarata* (-1.2 , $P < 0.01$) and *C. sandwicensis* (-0.8 , $P < 0.01$) in the eastward direction. In the westward direction, there is an indication that the slope for *C. talcosa* (-6.6) is different than that for *C. sandwicensis* (-0.8 , $P < 0.06$), but not for *C. exarata* (-1.0 , $P = 0.06$).

While most regressions of migration rate vs. distance are linear on a log-log scale, there was an asymptotic relationship between stepping-stone distance and the simulated migration rate of *C. talcosa*. This may have been due to the arbitrary constraints placed on the upper limit of migration rate by LAMARC (10 000 migrants per generation), or may reflect a finite limit on the migration rate at short distances. An alternate interpretation based on data from the porcelain crab, *Petrolisthes cinctipedes*, is that the larvae are dispersing with greatest frequency at intermediate distances from the natal site (Toonen 2001).

Discussion

The endemic Hawaiian *Cellana* spp. are sympatric species in a monophyletic group, with similar life histories. In this range-wide survey, we were interested in illuminating aspects of marine phylogeography among these species for management purposes, in the context of the common assumption that a single representative or model species can be used as a proxy to estimate dispersal among marine communities. Would surveying one species give us the proper foundations for management of all three? With distinct differences in population structure, the ophi say 'no'.

Population genetic diversity and structure

All three species are characterized by high haplotype diversity and low nucleotide diversity. However, the distribution of genetic diversity differed significantly among sites for the three species. Haplotype diversity was high for all three species on Hawaii and Molokai (MHI), the areas of greatest population size, based on available habitat. Orienting along a southeast to northwest axis, the haplotype diversity of *Cellana talcosa* and *Cellana exarata* dropped significantly (although *C. exarata* diversity increased from site 5 to site 6). In contrast, *Cellana sandwicensis* exhibited no decline in genetic diversity throughout this range.

Significant population partitioning was detected in all three species of Hawaiian limpets with AMOVA, but regional patterns of differentiation were not consistent among species. Pairwise Φ_{ST} values did reveal one common feature: gene-flow restrictions between northwestern and southeastern populations. For *C. exarata* and *C. sandwicensis*, a minor restriction was identified between the NWHI and MHI, and for *C. talcosa* a major restriction was identified between Kauai (northwest end of distribution) and remaining MHI samples.

Channels appear to be significant barriers to gene flow. However, channel width did not uniformly predict dispersal restrictions. The 255-km channel separating the NWHI from the MHI seems to be a significant barrier to gene flow for all Hawaiian *Cellana*. However, NWHI sites separated by 210–260 km were not significantly partitioned. This may be due, in part, to limited statistical power in the NWHI samples: small sample sizes in *C. sandwicensis* and depauperate haplotype diversity in *C. exarata*. Alternatively, the currents between Kauai (MHI) and Nihoa (NWHI) may be more restrictive to larval transport than those between the NWHI sites.

Within the MHI, significant population partitioning was detected between *C. sandwicensis* populations separated by 426 km (sites 1b, 3a) and between *C. exarata* and *C. talcosa* populations separated by 270 km (sites 2, 3a). If we assume that the channels between the islands are the primary

barriers to gene flow, then *C. exarata* and *C. talcosa* exhibited significant population structure when separated by 159 km of water and one island (Molokai–Kauai), and *C. sandwicensis* exhibited significant partitioning when separated by 218 km of water and three islands (Hawaii–Kauai).

Geography and isolation by distance

Evidence of IBD was observed in all three opihi based on regressions of pairwise population Φ_{ST} and Bayesian MHMCMC migration rate against geographical distance. The slopes for the range-restricted *C. talcosa* were consistently much steeper than those for *C. exarata* or *C. sandwicensis*, indicating lower gene flow. In the regression of Φ_{ST} against distance, *C. exarata* and *C. sandwicensis* exhibited shallow but significant quadratic relationships while *C. talcosa* exhibited a steep linear relationship (Fig. 3) where isolation increases with distance. For *C. exarata* and *C. sandwicensis*, Φ_{ST} values may asymptote due to the dispersal potential of the organisms, the finite range of opihi habitats (~1300 km) and their spatial arrangement.

Previous studies of marine population structure have emphasized the importance of stepping-stone habitats in maintaining connectivity among marine populations (Riginos & Nachman 2001; Barber *et al.* 2002). IBD regressions indicated that the 'stepping-stone distance' among islands was a better predictor of isolation than the distance between collection sites, i.e. minimum larval travel distance. Additionally, significant structure was not detected between populations sampled on the same islands. These data indicate that there is high connectivity along coastlines within an island and comparatively low connectivity between adjacent islands. In Bayesian estimates of migration, the slopes were approximately -1 for *C. sandwicensis* and *C. exarata*, suggesting a one-dimensional stepping-stone arrangement of populations (Slatkin & Maddison 1989; Slatkin 1991; Hellberg 1994), as expected based on the generally linear arrangement of islands in the Hawaiian Archipelago. With a slope ranging from approximately -4 to -6 , *C. talcosa* exhibits a nonlinear IBD response and does not conform to theoretical expectations of either a one or two-dimensional stepping-stone model.

Evaluation of model species criteria

In the next four subsections, we evaluate hypotheses for predicting population structure in Hawaiian limpets. These correspond to habitat (intertidal-subtidal), biogeography (range size), phylogeny (sister species), and life history (larval duration).

Habitat. *C. exarata* and *C. sandwicensis* reside above the waterline, while *C. talcosa* resides subtidally. Based on habitat specialization and the fact that submergence is a

spawning cue for the two intertidal species (Corpuz 1983), we would expect concordant structure in the two intertidal species. The data are consistent with this prediction. *C. talcosa* larvae may behave differently to enhance recruitment into their specialized shallow subtidal habitat, perhaps leading to an increased degree of larval retention (see Paris & Cowen 2004) and greater genetic structure.

Biogeography and range size. If restricted range is indicative of limited dispersal, then *C. talcosa* should have the most pronounced population structure (Thorson 1950; Gilman 2006; Paulay & Meyer 2006). Indeed, many endemic Hawaiian fishes are believed to be the product of restricted gene flow (reviewed in Hourigan & Reese 1987), a somewhat counterintuitive notion given the vast geographical leap required to colonize Hawaii. Yet a growing body of evidence indicates that extensive dispersers can maintain contact between Hawaiian populations and conspecifics elsewhere in the central West Pacific (Lessios *et al.* 2003; Craig *et al.* 2007; Schultz *et al.* 2007), reducing opportunities for speciation in isolation. In contrast, when a species with limited dispersal makes the rare leap to Hawaii, it does not maintain genetic connectivity with other Pacific habitats, and the isolated Hawaiian population is set upon an independent evolutionary trajectory. In the Hawaiian limpets, restricted range seems to be a good predictor of gene flow. Clearly, the most abrupt partitioning was observed in *C. talcosa*, where all six haplotypes detected on Kauai were endemic (Fig. 2), thereby indicating no recent gene flow. Hence, the species with the most restricted range shows clear evidence of the most restricted gene flow in concordance with predictions of the biogeographical model.

Phylogeny. Based strictly on phylogenetic criteria, the sister taxa *C. sandwicensis* and *C. talcosa* should exhibit similar patterns of population partitioning relative to *C. exarata*. Phylogeny can be a powerful predictor of concordance in population structure and other organismal traits (Bowen *et al.* 2006). However, contrary evidence exists (Rocha *et al.* 2002). In opihi, similarities in population connectivity are not predicted by phylogenetic relationships. The two sister species are at opposite ends of the continuum, ranked from high to low gene flow: *C. sandwicensis* \geq *C. exarata* $>$ *C. talcosa*. Consequently, our data do not support the idea that phylogenetic similarity is correlated with population genetic patterns.

Larval life history. Based on the available data regarding the minimum pelagic larval duration of the Hawaiian *Cellana*, one would expect similar levels of population partitioning among these species. However, our data clearly do not support this model. The simplest explanation

for the observed difference in population structure between *C. talcosa* and the other opihi is a divergence in larval behaviour or duration (Scheltema 1988; Shulman 1998; Riginos & Victor 2001). Although the minimum larval duration is similar among the opihi, perhaps the maximum pelagic duration of *C. talcosa* larvae is shorter than the other two opihi species. Direct comparison of the larval biology of Hawaiian opihi should help to elucidate the root cause of the differential population structure observed in this monophyletic lineage.

Overall, two hypotheses were supported and two were rejected, but none of these hypotheses are mutually exclusive. For example, we believe there is a likely correlation between habitat type and larval behaviour that could be considered part of the larval life history. Additionally, the Hawaiian *Cellana* spp. do not allow for differentiation between the restricted range model and the habitat model because of the correlation between habitat preference and biogeographical range. Nonetheless, phylogeny and pelagic larval duration were not supported as predictors of population structure, while biogeographical range and habitat were supported. There are also clear signatures of recent range expansion (*C. talcosa*, Kauai) and genetic bottlenecks (*C. exarata*, NWHI) reflecting the role of historical events in shaping contemporary population structure.

Management implications

The resolution of connectivity among communities is imperative for the delineation of MPA networks (Dawson *et al.* 2006; Steneck *et al.* 2006). While Hawaiian *Cellana* exhibit a diversity of population genetic patterns, they nonetheless show significant structure in all three species. These findings, coupled with habitat differences (C.E.B. unpublished data), demonstrate that a species-specific treatment adds significantly to our understanding and management of these taxa. Specifically, current management practice, where all opihi species are pooled into a single management unit should be abandoned and replaced by strategies that incorporate the differences in population connectivity among species.

There is long-standing controversy regarding whether the uninhabited NWHI (a recently designated Marine National Monument and the world's largest MPA) is a source of larvae for the depleted fisheries of the MHI (Polovina *et al.* 1999; DeMartini & Friedlander 2004). In the case of opihi, the answer is no. There is significant partitioning between the NWHI and the MHI in both *C. exarata* (pairwise $\Phi_{ST} = 0.03-0.08$) and *C. sandwicensis* (pairwise $\Phi_{ST} = 0.01-0.08$) and estimates of dispersal (effective migrants per generation ≤ 2) are too low to augment the depleted MHI fishery. The patterns of population subdivision clearly indicate that the NWHI do not harbour source populations of opihi capable of seeding the MHI.

Even within the MHI, a single MPA is unlikely to function as a source for other islands because all three opihi exhibit significant structure on this geographical scale.

Conclusion

Population partitioning in the three species of Hawaiian *Cellana* was observed at finer spatial scales (< 200 km) than was expected for broadcast-spawning invertebrates with a pelagic larval phase. Barriers to gene flow do not include geologically ancient land masses (*sensu* Barber *et al.* 2002). Instead, deep open ocean channels and variable currents among islands (*sensu* Taylor & Hellberg 2003, 2006; Baums *et al.* 2006) act to restrict gene flow. Despite close phylogenetic affinity and similar life histories, the endemic Hawaiian *Cellana* exhibit distinctly different population structures. Among the four factors evaluated to explain patterns of connectivity, the habitat specificity and biogeography models best fit the data; while predictions based on phylogenetic affinity and larval life history did not match patterns of population partitioning. These contrasting population genetic signatures highlight the hazards of making sweeping predictions about population connectivity from alleged model organisms, even among closely related species with similar life histories.

Acknowledgements

We thank K. Andrews, M. Craig, I. Baums, R. Kosaki, N. Velasco, M. Tanaka, T. Nakano, J. Eble, R. Kawamoto, R. Okano, and the crew of the research vessel Hi'ialakai for assistance in obtaining specimens; G. Hughes, E. Brown, M. Carnevale, the Kalaupapa Kupuna and community, T. Tunison, S. Peck, V. Yamanaka, J. Kaluau, the Kaupulehu Kupuna, the Kaupulehu Developments Management Committee, L. Eldridge, and the Kona Village Resort for site access; R.H. Cowie, D. Carlon, B. Menge, and J. Lubchenco for the use of their laboratories; L. Basch for the use of equipment; C. Allen, M. Crepeau, J. Schultz, S. Daley, H. Ha, K. (Maddog) Hayes, and M. Dunford for laboratory assistance/guidance; Dale Sarver for sharing larval rearing results; G. Ochikubo for technical support; and C. Smith, K. Bridges, D. Duffy, L. Freed, H. M. Zaleski, G. Graw, G. Wallis, and two anonymous reviewers for comments and discussions. This study was supported by grants from the University of Hawaii Sea Grant College Program, the US Department of the Interior, National Park Service Pacific Cooperative Studies Unit, and the Northwestern Hawaiian Islands National Marine Sanctuary (MOA 2005-008/6882). Collections for this study were permitted by the State of Hawaii, Department of Land and Natural Resources, Division of Aquatic Resources, the US Fish & Wildlife Service, Kalaupapa National Historical Park, and Hawaii Volcanoes National Park. BSH was supported by US National Science Foundation grant DEB-0316308 and OCE-0453167. BWB was supported by US National Science Foundation grant OCE-0454873, and by the HIMB-NWHI Coral Reef Research Partnership (NMSP MOA 2005-008/6882). This is contribution number 1274 from HIMB, 7132 from SOEST, and JC-04-22 from the University of Hawaii Sea Grant College Program.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Pairwise population Φ_{ST} values, their significance, and the significance of exact tests of sample differentiation.

Table S2 Proportional pairwise population migration rate estimates (M) for each *Cellana* spp.

This material is available as part of the online article from:

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